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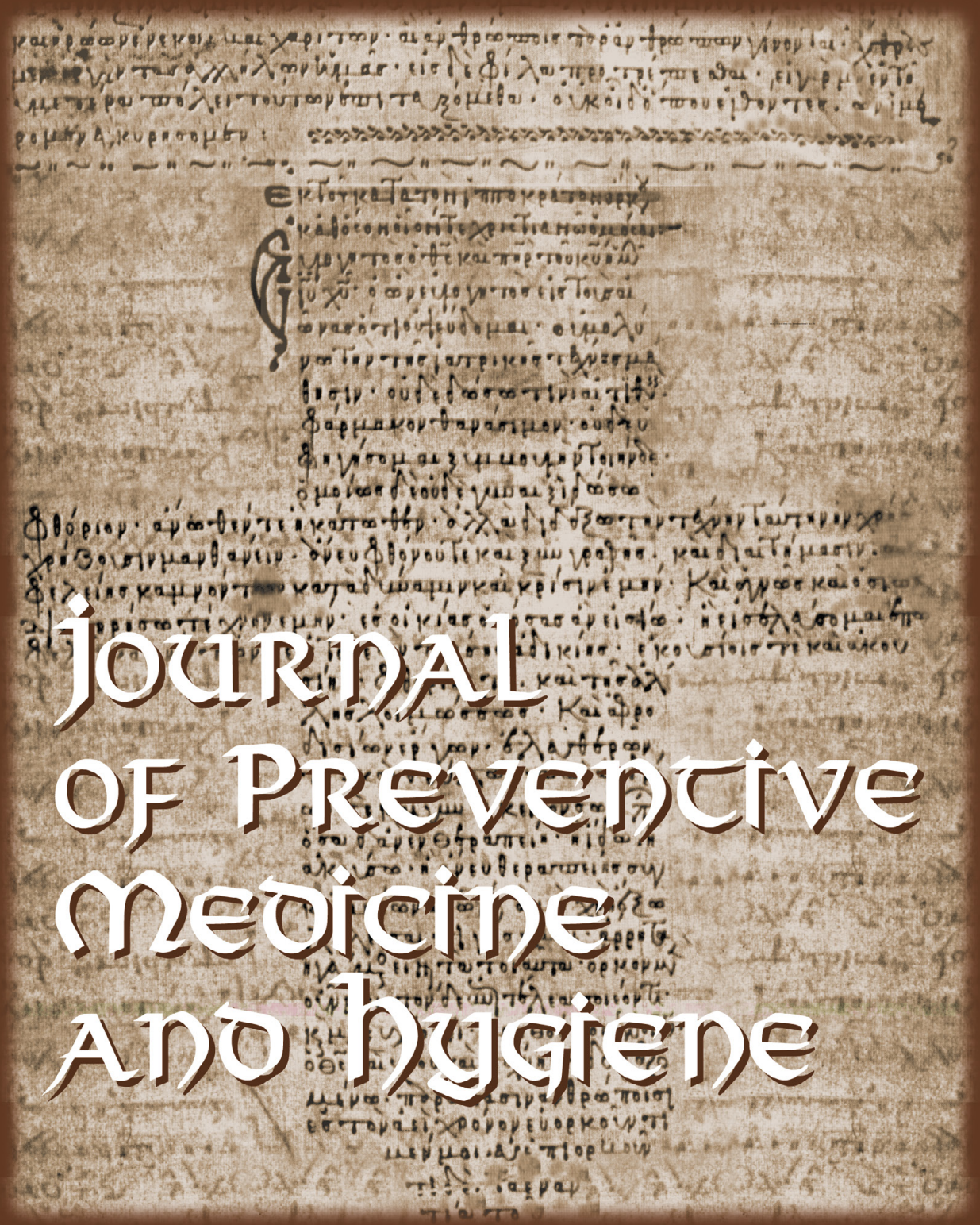

The original document of Hippocrates' oath

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CONTENTS

COVID-19

Overview

Could ozone be an effective disinfection measure against the novel coronavirus (SARS-CoV-2)?

LUIGI CRISTIANO
Aesthetic and Medical Biotechnologies Research Unit, Prestige, Loro Ciuffenna (AR), Italy

Keywords

Ozone • UV-light • Disinfection • Coronavirus • Work environment

Summary

The new SARS-CoV-2/COVID-19 emergency has imposed new disinfection and sanitation measures of work environments also to beauty and health professional workers and in this context ozone shows growing interest. Ozone has proven to be highly effective in killing bacteria, fungi, and molds and inactivating viruses both on the surfaces and suspended in the air. Ozone is proven to be effective also for the inactivation of the SARS virus, while for the novel SARS-CoV-2 it is supposed that it be equally effective but specific studies are needed.

Abbreviations

COVID-19: coronavirus infectious disease 2019.
SARS: severe acute respiratory syndrome.
SARS-CoV: severe acute respiratory syndrome coronavirus (2002).
SARS-CoV-2: severe acute respiratory syndrome coronavirus 2 (2019).

Main

Following the health emergency regarding the new SARS-CoV-2/COVID-19 to limit its spread, the sanitization of environments, devices, and objects has become imperative, in addition to individual protection and prevention measures. This applies not only to the clinical, industrial and public sector but also to beauty and health professionals and their work environments, i.e. beauty centers (beauticians, hairdressers, make-up artists), gyms (personal trainers, slimming professionals), professional ambulatories or functional rehabilitation centers (physiotherapists, physio-aesthetic specialists and non-invasive aesthetic medicine operators).

Numerous disinfection and sanitization methods, including heating sterilization [1], ultraviolet germicidal irradiation (UVGI) [2], and chemical disinfectants [1, 3, 4], have been proposed for work environments and surfaces but also for all kinds of fomites [5], including furniture, work devices, non-disposable work tools, and clothing to prevent and eradicate the spread of the new coronavirus responsible of the SARS-CoV-2 related-disease. Among these, in recent months, there has been particular attention to devices that generate ozone for this purpose [6] and so it is useful to ask if ozone can also be effective on the novel SARS-CoV-2.

Ozone is a gas formed by molecules made up of 3 oxygen atoms (O_3) and has the characteristic of being a powerful oxidizing agent. Thanks to this property it has proven to be highly effective in killing bacteria, fungi and molds [7, 8] and inactivating viruses [6, 9].

Ozone can, therefore, be used for the treatment of potentially contaminated surfaces, water, and ambient air thanks to its powerful germicidal effect on a wide spectrum of microorganisms, while it has been shown that on porous materials, such as textiles, it is less effective [10], unlike disinfection with UV lamps (UVGI) that are more effective on these materials [9] (Fig. 1). Recently, it was reported that ozone treatment can be also a widely accessible and effective method for the disinfection of personal protective equipment (PPE) for both healthcare workers and patients for safe re-use in times of shortage [11].

Ozone created by various kinds of devices, such as ozone generators or electrostatic air purifiers, can reach every corner of the environment of a single room or a larger space, depending on the device, without leaving any poisonous residue. The effectiveness of ozone in treating microorganisms, especially bacteria and viruses is related to various factors, i.e. ozone concentration, the temperature of the environment, humidity of the environment and exposure time [14] (Fig. 2).

It was shown that after 30 seconds of *in vitro* direct exposure to ozone, 99 percent of the viruses are inactivated [13]. Although this evidence is of considerable importance, outside of the laboratory models, there are various parameters that influence the time required to obtain the same result. First of all, it was seen that the inactivation of 99% of viruses by ozonation requires its spread at concentrations higher than those necessary for the bacteria [9, 14]. A longer exposure time, about 30 minutes, is necessary for the treatment of the surfaces of the environment (surface viruses), while

Fig. 1. Disinfection and sanitization methods: differences between ozone, UV light and chemical disinfectants. The figure shows the main differences between ozone and UV light sanitization [1, 3, 4, 6, 10, 14-16].

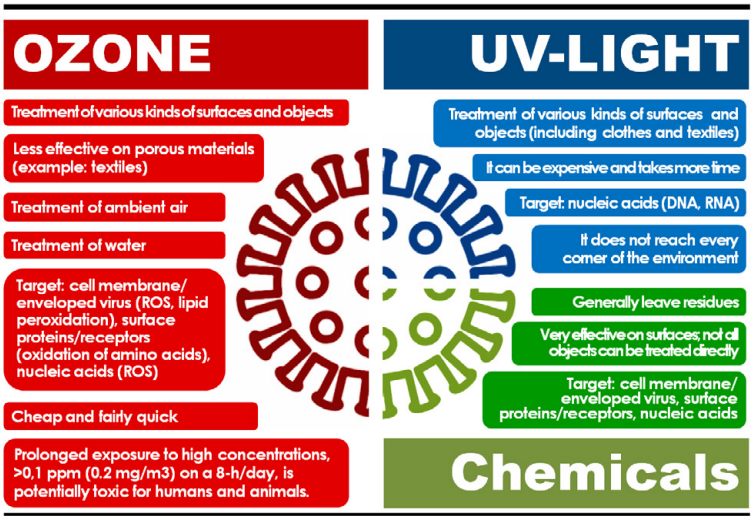
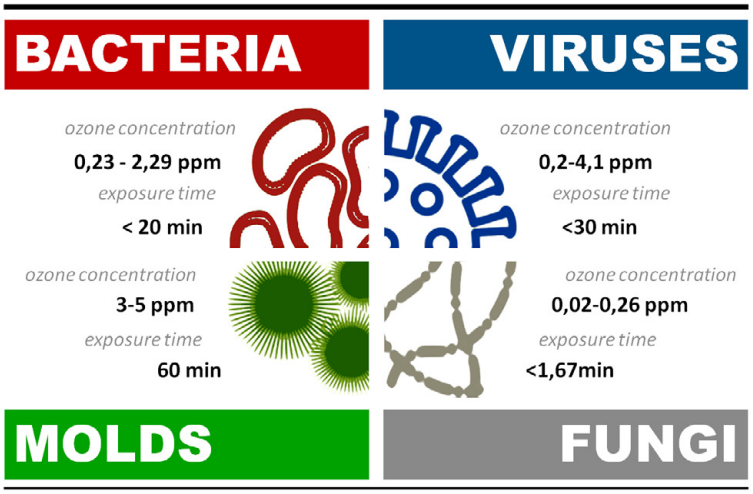


Fig. 2. Effective ozone parameters on the microorganisms. The figure shows the ozone concentration (ppm) and time exposure (minutes) necessary to reduce the microbial load by 99% [7, 14, 17].



for any viral particles suspended in the air (airborne viruses) 8-10 minutes are enough to remove 99.9% of them [17]. Viruses in water are more susceptible to ozone inactivation and short contact time, about 1 min or little more, are sufficient to inactivate 99% of them [9, 18].

Virus inactivation is complicated by two factors. The first is the presence or not of an envelope: in fact, it seems that enveloped viruses are more sensitive to ozone than naked viruses [1, 19]. The second factor is that highly related viruses may exhibit different response kinetics to the same biocide due to the variation of structural or genomic components [16]. However, ozone has been shown to cause the inactivation of viruses by affecting various molecular targets.

Capsid surface proteins, as well as membrane receptors present on enveloped viruses, are the first targets of ozone because it reacts directly with amino acids and functional groups of proteins. This leads to various consequences both on the structure of the virus, which is compromised, and on its infectious capacity because the specific viral receptors used by the virus to bind to host cells and invade them are altered. Furthermore, ozone damages the membranes of the enveloped viruses through peroxidation of phospholipids and generating numerous reactive oxygen species (ROS) capable of damaging also other viral macromolecules [9, 14, 20, 21]. In addition, ozone can inactivate viruses by causing damage to their genetic material, both DNA and RNA [14, 22]. The main mechanisms by which ozone acts on viruses, therefore, are due to the direct oxidation of various molecules and, indirectly, with the generation of ROS.

Ozone was shown to be highly effective to inactivate the SARS virus, in fact, it shows an inactivation rate not lesser than 99% [12]. The novel SARS-CoV2 (2019), an enveloped virus like all other coronaviruses, shows 80% of genome sequence similarity to SARS-CoV (2002)

and this suggests that ozone could be equally effective also on the novel coronavirus [12, 18].
In conclusion, although the existing scientific literature supports the effectiveness of ozone in the inactivation of viruses, there are very few studies about it on the SARS virus e not still a single study about its efficiency of inactivation on SARS-CoV-2. Therefore, in the absence of scientific literature, it is possible to assume that ozone is equally effective in inactivating SARS-CoV-2, however specific studies must be conducted to know also the ozone dose and effective exposure times.

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Conflict of interest statement

The author is employed in the Prestige company and has received a salary also for the research activities for the preparation of this paper

References

[1] Castaño N, Cordts SC, Jalil MK, Zhang K, Koppaka S, Bick AD, Paul R, Tang SK. Fomite transmission and disinfection strategies for SARS-CoV-2 and related viruses. 2020. https://arxiv.org/abs/2005.11443

[2] Cutler TD, Zimmerman JJ. Ultraviolet irradiation and the mechanisms underlying its inactivation of infectious agents. Anim Health Res Rev 2011;12:15-23. https://doi.org/10.1017/S1466252311000016

[3] Tseng C-C, Li C-S. Inactivation of viruses on surfaces by ultraviolet germicidal irradiation. J Occup Environ Hyg 2007;4:400-5. https://doi.org/10.1080/15459620701329012

[4] Rabenau HF, Kampf G, Cinatl J, Doerr HW. Efficacy of various disinfectants against SARS coronavirus. J Hosp Infect 2005;61:107-11. https://doi.org/10.1016/j.jhin.2004.12.023

[5] Vasickova P, Pavlik I, Verani M, Carducci A. Issues concerning survival of viruses on surfaces. Food Environ Virol 2010;2:24-34. https://doi.org/10.1007/s12560-010-9025-6

[6] Hudson JB, Sharma M, Vimalanathan S. Development of a practical method for using ozone gas as a virus decontaminating agent. Ozone: Sci Eng 2009;31:216-23. https://doi.org/10.1080/01919510902747969

[7] Najafi MBH, Khodaparast MHH. Efficacy of ozone to reduce microbial populations in date fruits. Food Control 2009;20:27-30. https://doi.org/10.1016/j.foodcont.2008.01.010

[8] Li C-S, Wang Y-C. Surface germicidal effects of ozone for microorganisms. AIHA Journal 2003;64:533-7. https://doi.org/10.1080/15428110308984851

[9] Tseng C, Li C. Inactivation of surface viruses by gaseous ozone. J Environ Health 2008;70:56-63.

[10] Menetrez MY, Foarde KK, Schwartz TD, Dean TR, Betancourt DA. An evaluation of the antimicrobial effects of gas-phase ozone. Ozone: Sci Eng 2009;31:316-25. https://doi.org/10.1080/01919510903043772

[11] Blanchard EL, Lawrence JD, Noble JA, Xu M, Joo T, Ng NL, Schmidt BE, Santangelo PJ, Finn MG. Enveloped virus inactivation on personal protective equipment by exposure to ozone. medRxiv 2020. https://doi.org/10.1101/2020.05.23.20111435

[12] Zhou M. Ozone: a powerful weapon to combat COVID-19 outbreak. 2020. http://www.china.org.cn/opinion/2020-02/26/content_75747237_4.htm (accessed 09.05.20).

[13] Roy D, Wong PK, Engelbrecht RS, Chian ES. Mechanism of enteroviral inactivation by ozone. Appl Environ Microbiol 1981;41:718-23.

[14] Kim JG, Yousef AE, Dave S. Application of ozone for enhancing the microbiological safety and quality of foods: a review. J Food Prot 1999;62:1071-87. https://doi.org/10.4315/0362-028x-62.9.1071

[15] Wickramatillake A, Kurukularatne C. SARS-CoV-2 human disinfection chambers: a critical analysis. Occup Med (Lond) 2020;kqaa078. https://doi.org/10.1093/occmed/kqaa078

[16] Wigginton KR, Pecson BM, Sigstam T, Bosshard F, Kohn T. Environ Sci Technol 2012;46:12069-78. https://doi.org/10.1021/es3029473

[17] Kowalski WJ, Bahnfleth WP, Whittam TS. Bactericidal effects of high airborne ozone concentrations on Escherichia coli and Staphylococcus aureus. Ozone: Sci Eng 1998;20:205-21. https://doi.org/10.1080/01919519808547272

[18] Shin GA, Sobsey, MD. Reduction of norwalk virus, poliovirus 1, and bacteriophage ms2 by ozone disinfection of water. Appl Environ Microbiol 2003;69:3975-8. https://doi.org/10.1128/aem.69.7.3975-3978.2003

[19] Dennis R, Cashion A, Emanuel S, Hubbard D. Ozone gas: scientific justification and practical guidelines for improvised disinfection using consumer-grade ozone generators and plastic storage boxes. J Sci Med 2020;2(1). https://doi.org/10.37714/JOSAM.V2I1.35

[20] Wigginton KR, Kohn T. Virus disinfection mechanisms: the role of virus composition, structure, and function. Curr Opin Virol 2012;2:84-9. https://doi.org/10.1016/j.coviro.2011.11.003

[21] Schentag JJ, Akers C, Campagna P, Chirayath P. Sars: clearing the air. In: Institute of Medicine (US) Forum on Microbial Threats; Knobler S, Mahmoud A, Lemon S, et al., editors. Learning from SARS: preparing for the next disease outbreak: workshop summary. Washington (DC): National Academies Press 2004. https://www.ncbi.nlm.nih.gov/books/NBK92445

[22] Victorin K. Review of the genotoxicity of ozone. Mutat Res 1992;277:221-38. https://doi.org/10.1016/0165-1110(92)90045-B

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Correspondence: Luigi Cristiano, Medical Biotechnology, Prestige – Aesthetic and Medical Biotechnologies Research Unit, Frazione Penna 62/M, 52028 Terranuova Bracciolini (AR), Italy - Tel. +39 0559705141 - E-mail: prestige.infomed@gmail.com / luigicristiano@libero.it

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Review

Determine the most common clinical symptoms in COVID-19 patients: a systematic review and meta-analysis

YOUSEF ALIMOHAMADI[1,2], MOJTABA SEPANDI[3,4], MARYAM TAGHDIR[3,5], HADISEH HOSAMIRUDSARI[6]
[1] Pars Advanced and Minimally Invasive Medical Manners Research Center, Pars Hospital, Iran University of Medical Sciences, Tehran, Iran; [2] Department of Epidemiology & Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; [3] Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran; [4] Department of Epidemiology & Biostatistics, Faculty of Health, Baqiyatallah University of Medical Sciences, Tehran, Iran; [5] Department of Nutrition & Food hygiene, faculty of Health, Baqiyatallah University of Medical Sciences, Tehran, Iran; [6] Department of Infectious Diseases, Baharloo Hospital, Railway Square, Tehran University of Medical Sciences, Tehran, Iran

Keywords

Clinical symptoms • COVID-19 • Meta-analysis

Summary

Introduction. *COVID-19 is an emerging infectious disease. The study about features of this infection could be very helpful in better knowledge about this infectious disease. The current systematic review and meta-analysis were aimed to estimate the prevalence of clinical symptoms of COVID-19 in a systematic review and meta-analysis.*

Methods. *A systematic review using Medline/PubMed, Scopus, and Google scholar has been conducted. In the current systematic review and meta-analysis, the articles published in the period January 1, 2020, to April 2, 2020, written in English and reporting clinical symptoms of COVID-19 was reviewed. To assess, the presence of heterogeneity, the Cochran's Q statistic, the I^2 index, and the tau-squared test were used. Because of significant heterogeneity between the studies the random-effects model with 95% CI was used to calculate the pooled estimation of each symptom prevalence.*

Results. *The most common symptoms in COVID-19 patients include: Fever 81.2% (95% CI: 77.9-84.4); Cough: 58.5% (95% CI: 54.2-62.8); Fatigue 38.5% (95% CI: 30.6-45.3); Dyspnea: 26.1% (95% CI: 20.4-31.8); and the Sputum: 25.8% (95% CI: 21.1-30.4). Based on the meta-regression results, the sample size used in different studies did not have a significant effect on the final estimate value ($P > 0.05$).*

Conclusions. *Considering the main symptoms of COVID-19 such as Fever, Cough, Fatigue, and Dyspnea can have a key role in early detection of this disease and prevent the transmission of the disease to other people.*

Introduction

The World Health Organization (WHO) described Coronavirus 2019 (COVID-19) as a public health emergency. The international concern of COVID-19 is more in comparison to Severe Acute Respiratory Syndrome (SARS), which previously was pandemic in 2003 [1]. Coronaviruses are important pathogens that can affect the lower respiratory tract in humans and can cause diseases ranging from a simple cold to severe infection with up to 50% lethality [2]. The COVID-19 is a highly contagious infectious disease and one infected person can infect an average of three other people [3] which is higher than that for SARS (1.7-1.9) and MERS (< 1), suggesting that SARS-CoV-2 has a greater potential for being outbreak. Evidence suggests that there are many similarities between COVID-19 and SARS. About 79.5% of the similarities in the genome sequence of these two viruses have been reported [4]. COVID-19 can spread in the community more easily than MERS and SARS because of the less severe clinical picture of it [5]. Although the disease is mild in most people, in some patients, especially those with other underlying diseases, there may be a respiratory failure, arrhythmias, shock, Kidney failure, cardiovascular damage, or liver failure [6, 7]. Currently, there is no effective antiviral treatment for the disease and only supportive care may be helpful [7] The case fatality rate (CFR) of COVID-19 was reported to be 3.8% but it can differ in patients who have comorbidities [8]. The CFR of COVID-19 is lower than that of SARS and that of MERS[5]. The most common symptoms are fever, cough, and myalgia or fatigue [9]. Although the clinical symptoms of the disease are nonspecific, understanding the symptoms is essential. Patient with fever and upper respiratory tract symptoms with lymphopenia or leukopenia should be considered as suspected [9] Patients may present with diarrhea a few days before the fever. A slight number of patients may report a headache [10]. Diarrhea is more common in SARS [5].

Combining the results of studies that have focused on the prevalence of COVID-19 related symptoms could be helpful in the best identification and diagnosis of infection. Because of the importance of symptoms in the identification of COVID-19 infection the current study was aimed to estimate the prevalence of Clinical Symptoms of COVID-19 in a systematic review and meta-analysis.

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Materials and methods

Eligibility criteria

All articles published in the period January 1, 2020, to April 2, 2020, written in English and reporting clinical symptoms of COVID-19 was reviewed. Review articles as well as articles that lacked original data or reported incomplete data were excluded.

Information sources and search strategy

We conducted a systematic review using Medline/PubMed, Scopus and Google scholar. The following search terms used: "Clinical features", "COVID-19", "coronavirus disease 2019", "coronavirus disease-19", "2019 novel coronavirus disease", "severe acute respiratory syndrome coronavirus", "clinical symptoms", "clinical characteristics" and "clinical manifestations". The searches were concluded by April 2, 2020, and two researchers independently assessed search results. References of related papers were also searched for other relevant articles to enhance the search strategy.

Study selection

After performing the search strategy some records were excluded because of Duplicates and unrelated. After that, the records screened based on abstracts and titles. The full text of related articles was then assessed according to the inclusion and exclusion criteria. Observational studies that reported clinical symptoms were included in the meta-analysis.

Data collection process and data items

Data including the type and date of publication, country, the sample size, age, and clinical symptoms of COVID-19 were extracted independently by two authors. A third person checked the article list and data extractions to ensure there were no duplicate articles and also resolved discrepancies about study inclusion.

Assessment of methodological quality

To assess the study quality of the case series studies the Institute of Health Economics (IHE) was used [11]. Also, the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist for observational studies was used assessment quality of the cross-sectional and cohort studies.

Meta-regression analysis

To assess the effect of sample size on pooled estimations the meta-regression analysis was used.

Statistical approach

To assessment, the presence of heterogeneity, the Cochran's Q statistic, the I^2 index, and the tau-squared test were used. Due to the difference in the age of patients, we perform subgroup analyzes in different age groups. Because of the presence, the significant heterogeneity between the studies the random-effects model with 95% CI was used to calculate the pooled estimation of symptoms prevalence. The data were analyzed using stata version 11.0.

Results

In the current systematic review and meta-analysis, 54 studies that estimated the symptoms related to COVID-19 were included in the final analysis (Tab. I). After searching PubMed and Google Scholar electronic databases, 1,498 possibly relevant articles were identified; 1,397 articles were removed due to unrelated to study purpose and duplication. Of the remaining 101 articles, 45 were excluded after screening based on abstract and title and 2 articles removed because of lack of needed information. Finally, 54 articles were included in the final meta-synthesis (Fig. 1).

Tab. I. Characteristics of the included studies on effective factors on mortality COVID-19, 2020.

Id	First author	Country	Design	Sample size
1	Dawei Wang [12]	China	Case series	138
2	Chaolin [13]	China	Cross-sectional	41
3	Chen [14]	China	Cross-sectional	99
4	Chung [15]	China	Cross-sectional	21
5	Chen [16]	China	Cross-sectional	29
6	Wang [12]	China	Cross-sectional	138
7	Kui [17]	China	Cross-sectional	137
8	Chang [18]	China	Cross-sectional	13
9	COVID-19 team Australia [19]	Australia	Cross-sectional	15
10	Li et al. [20]	China	Case series	24
11	Feng [21]	China	Case series	21
12	Zhang [22]	China	Case series	9
13	Feng [23]	China	Case series	15
14	Wang [24]	China	Cross-sectional	34
15	Xiaobo[25]	China	Cross-sectional	52
16	Jiong Wu et al. [26]	China	Cross-sectional	80
17	Zonghao Zhao [27]	China	Cross-sectional	77
18	Wen Zhao [28]	China	Cohort study	77
19	Wenjie Yang [29]	China	Cohort study	85
20	Matt Arentz [30]	USA	Case series	21
21	Ying Huang [31]	China	Retrospective	36
22	G Jian-ya Lei Liu [32]	China	Retrospective	51
23	Tao Chen [4]	China	Cohort	274
24	jin Zhang [33]	China	Cross-sectional	242
25	Shijiao Yan [34]	China	Retrospective	168
26	Jian Wu [35]	China	Retrospective	80
27	Yang Xu [36]	China	Retrospective	69
28	Fei Zhou [37]	China	Retrospective	191
29	Zenghui Cheng [38]	China	Retrospectively	11
30	Youbin Liu [39]	China	Retrospective	291
31	Yanli Liu [40]	China	Retrospective	109

continues

follows

Tab. I. Characteristics of the included studies on effective factors on mortality COVID-19, 2020.

32	Yonghao Xu [41]	China	Retrospective	45
33	Lang Wang [42]	China	Cohort	339
34	Zhichao Feng [43]	China	Cohort	141
35	Guo-Qing Qian [44]	China	Retrospective	91
36	BarnabyEdward Young [45]	Singapore	Case series	18
37	Ying Wen [46]	China	Retrospective	417
38	Jiaqiang Liao [47]	China	Retrospective	46
39	Xu Chen [48]	China	Cohort	291
40	Penghui Yang [49]	China	Cohort	55
41	Jie Liu [50]	China	Retrospective	64
42	Hang Fu [51]	China	Cross-sectional	52
43	Heshui Shi [52]	China	Cross-sectional	81
44	Wei Zhao [53]	China	Retrospective	101
45	Hua Fan [54]	China	Cohort	47
46	Ling Hu [55]	China	Retrospective	323
47	X. Zhao [56]	China	Cross-sectional	80
48	Zhaowei Chen [57]	China	Retrospective	89
49	Huijun Chen [58]	China	Retrospective	9
50	Rachael Pung [59]	Singapore	Retrospective	17
51	Wanbo Zhu [60]	China	Retrospective	116
52	Xiaoping Chen [61]	China	Retrospective	123
53	W. Guan [62]	China	Cross-sectional	1,099
54	Xi Xu[63]	China	Retrospective	90

According to the results of the analysis, the most common symptoms in patients with coronavirus include:

- Fever 81.2% (95% CI: 77.9-84.4);
- Cough: 58.5% (95% CI: 54.2-62.8);
- Fatigue: 38.5% (95% CI: 30.6-45.3);
- Dyspnea: 26.1% (95% CI: 20.4-31.8);
- and the presence of Sputum: 25.8% (95% CI: 21.1-30.4).

Other results are shown in Table II and Figure 2. Figure 2 presents the pooled estimation of some symptoms among COVID-19 patients.

Meta-regression analysis

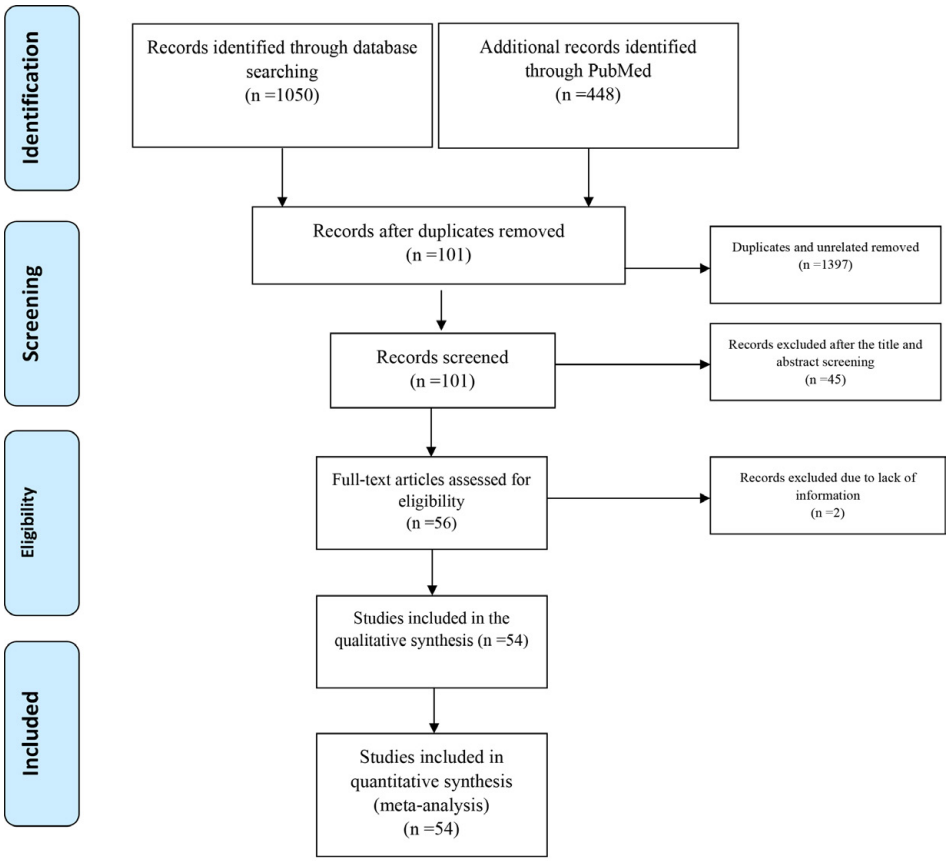
Based on the meta-regression results, the sample size used in different studies did not have a significant effect on the final estimate value ($P > 0.05$).

The distribution of the estimated prevalence of different symptoms according to sample sizes as shown in Figure 3.

The diagrams below show the percentage distribution of symptom estimation based on the volume of different samples.

Based on these charts, the estimated amount of chest pain, cough, dyspnea, hemoptysis, and fever with decreasing sample size showed a decreasing trend, while other symptoms showed an increasing trend with increasing sample size.

Fig. 1. PRISMA Flow Diagram for included studies in the current meta-analysis.



Tab. II. The prevalence of different symptoms among COVID-19 patients according to age groups.

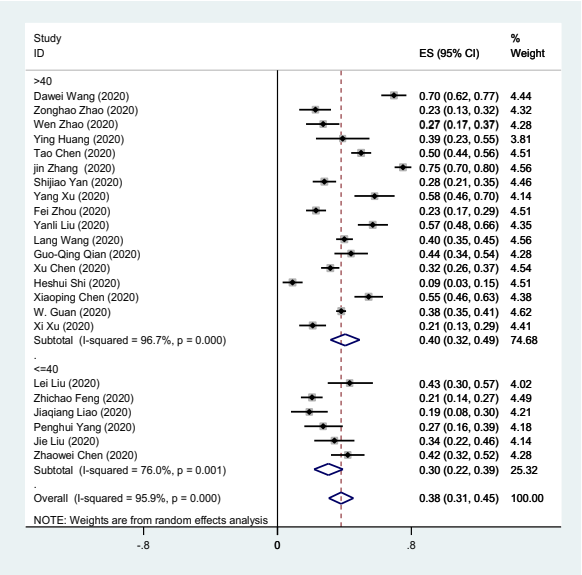
Symptom	Number of studies	Sample size	Pooled estimation			I^2 (%)	P	T^2
			< 40 years of old	> 40 years of old	Total			
Chest tightness	14	1,967	8.1 (3.7-12.6)	20.1 (9.6-30.6)	17 (13.1-25.4)	96.8	< 0.001	0.01
Cough	54	6,380	53.5 (44.3-62.7)	61.2 (56.3-66.1)	58.5 (54.2-62.8)	91.7	< 0.001	0.02
Diarrhea	36	4,995	3.5 (2.1-4.9)	8.6 (6.5-10.6)	7.6 (5.9-9.2)	83.9	< 0.001	0.001
Dyspnea	27	3,388	8.8 (2.6-15)	31.4 (24-38.7)	26.1 (20.4-31.8)	97.4	< 0.001	0.02
Fatigue	22	3,803	30.5 (21.9-39.1)	38.6 (29.9-47.2)	38.5 (30.6-45.3)	95.5	< 0.001	0.02
Fever	53	5,298	78.1 (73.3-82.8)	83 (79.1-86.9)	81.2 (77.9-84.4)	92.6	< 0.001	0.01
Hemoptysis	9	1,998	1.9 (0-4.6)	1.8 (0.008-2.9)	1.7 (0.008-2.6)	46.9	< 0.001	0.05
Headache	34	5,129	9.2 (5.4-13.1)	9.5 (7.1-12.0)	9.5 (7.5-11.6)	88.7	< 0.001	0.002
Myalgia	37	4,676	19 (14-23.9)	19.4 (14.9-24.0)	20.1 (16.5-23.7)	91.5	< 0.001	0.009
Shortness of breath	13	1,828	17.3 (3.6-30.1)	19.3 (11.2-27.5)	18.5 (12-24.9)	93.3	< 0.001	0.01
Sore throat	29	3,906	15 (9.6-20.4)	14.5 (10.9-18.2)	15 (12.1-18.0)	86	< 0.001	0.004
Sputum production	28	3,677	21 (15.4-26.7)	28 (22-34.1)	25.8 (21.1-30.4)	91	< 0.001	0.01

Discussion

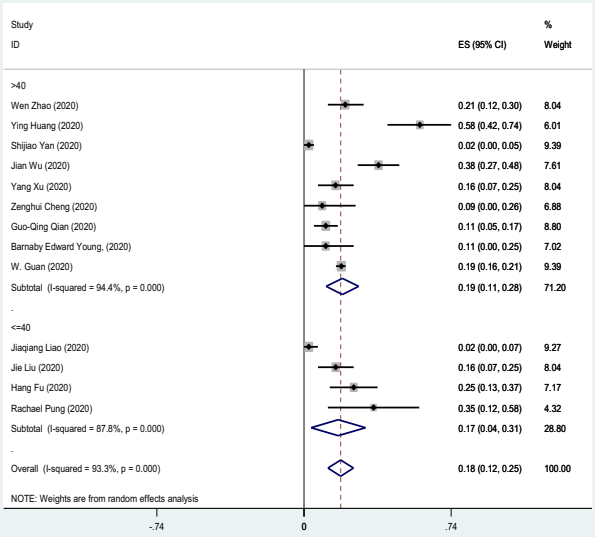
The COVID-19 is a new highly contagious infection that threatens people of all countries [64].

The clinical presentation of COVID-19 is wide, from asymptomatic infection to severe fatal diseases [14] Considering the shortage of diagnostic kits around the world this systematic review seems necessary, to find the clinical presentation of COVID-19 and using them in early diagnosis of this infection [13]. Unfortunately, there is no treatment for this virus, and patients' treatment is just focused on supportive care. On the other hand, the limited number of critical care centers and mechanical ventilation in the world culminates in high concern for the health care system [7]. To date, over 1,607,912 cases have been reported worldwide and from different countries [65]. To deal with such an emerging infectious disease, there an urgent need to identify and determine factors associated with the evolution of the disease and its outcomes. In this Systematic Review and Meta-Analysis study, we reported the clinical symptoms of COVID-19. Although the 2019-nCoV sequence is not the same as the other two viruses (SARS-CoV and MERS-CoV) that were pandemic in 2003 and 2012, respectively, they are somewhat similar in pathogenesis [66, 67]. Cytokines may play a role in human coronavirus infection. Indirect evidence suggests that in the second phase of 2019-nCoV infection: high fever, pneumonia, and hypoxemia occur despite a significant reduction in viral load [68]. In this systematic review and meta-analysis study, the clinical symptoms of COVID-19 were examined to provide a better understanding of the disease. The most common symptoms were fever and, cough, and fatigue that was consistent with the general symptoms of a viral infection and pneumonia. Similar to previous studies [25, 62], the present study showed that fever in 81.2% of cases, cough in 58.5% of cases, and fatigue in 38.5% of cases. Fever is the most common symptom in patients with COVID-19, but not all patients had fever [13, 69]. The fever is an alarming sign of the disease, vomiting, and fever (above 39 degrees) are usually associated with more severe illness and more length of stay in the hospital. Fever is less common in COVID-19 than in SARS and MERS [34, 70]. Therefore, more attention should be paid to COVID-19 patients who do not have fever as a source clue of infection, and if the surveillance system relies only on fever in patients, then some patients will be missed [71]. Diarrhea, myalgia, hemoptysis, and sore throats were less common symptoms in this review, these results were similar to those obtained for other viruses, such as SARS and MERS [26]. This may indicate that COVID-19 can also be classified as a similar infection to SARS and MERS infection, which targets the cells of the lower respiratory tract system. Although nasopharynx is theoretically the first organ infected with the COVID-19, a recent study [13] showed that infected individuals rarely show present upper respiratory symptoms at the onset of the infection. This suggests that the virus mostly targets the cells of the lower respiratory tract cells [72]. Research and clinical findings suggest that SARS-CoV-2 may be colonized in the nasopharynx but the immune system cannot identify COVID-19 in the early stages. Therefore, the virus can be removed from the body with its through natural reactions, including sneezing and runny nose. This demonstrates the importance of accurately

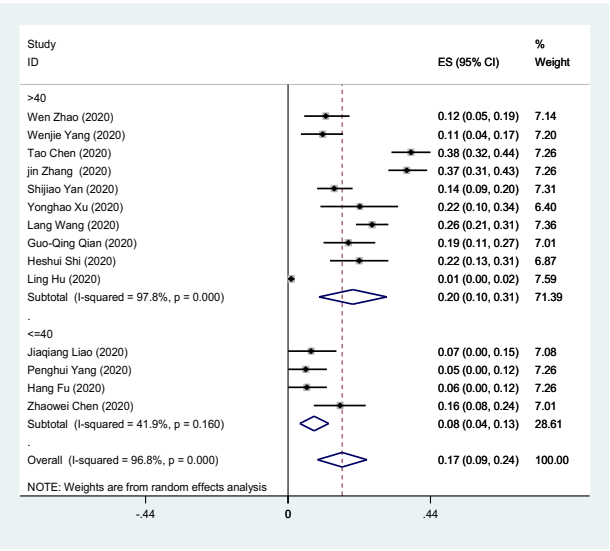
Fig. 2. The forest plots of some symptoms among COVID-19 patients.



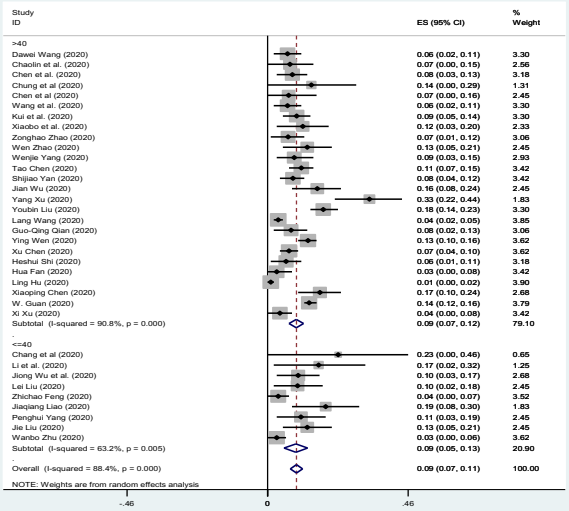
Fatigue



Shortness of breath



Chest tightness



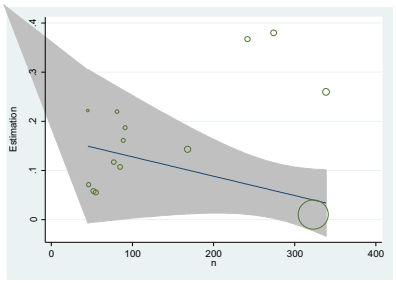
Headache

identifying COVID-19 symptoms at admission. Especially considering that studies have suggested the possibility of transmission of the disease by a healthy carrier [73]. This may be one of the reasons why COVID-19 is more contagious than SARS.

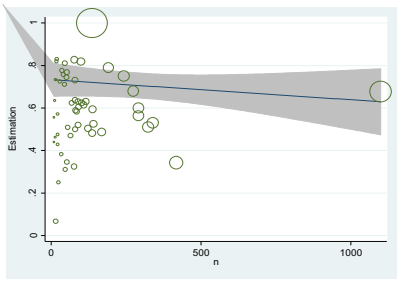
On the other hand, the lower incidence of early respiratory symptoms may be due to the presence of a pathogenic latency of SARS-CoV-2. Although gastrointestinal symptoms, especially diarrhea, were rare in the current study, the results of a study have shown that the SARS-CoV-2 virus can be isolated from the fecal samples of patients with gastrointestinal symptoms [74]. In another study, the SARS-CoV-2 virus has been isolated in a rectal swab of patients whose RT-PCR test results were negative with a throat swab sample [44]. Therefore, simultaneously sampling from throat and rectal may be useful, especially in patients with gastrointestinal symptoms. This review has some limitations which should be considered when interpreting the results. Most of the available studies for inclusion are from China. However the present study was done without any language restrictions and based on a comprehensive search strategy, only English electronic databases were searched; thus, it is likely that some related non-English papers have been missed.

Fig. 3. The distribution of estimated prevalence of symptoms according to different sample sizes (the X and Y axes are the sample size and estimated prevalence respectively).

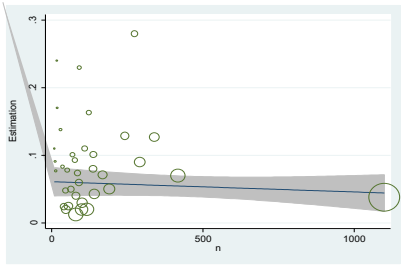
Chest tightness



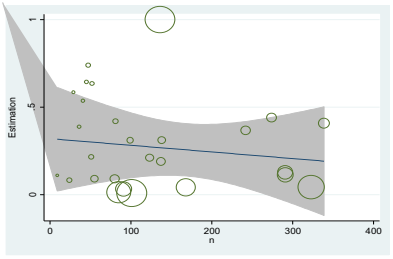
Cough



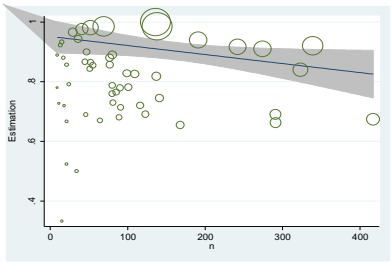
Diarrhea



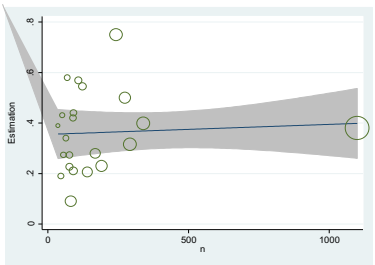
Dyspnea



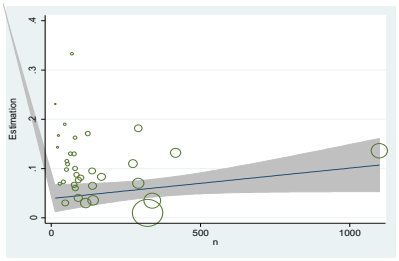
Fever



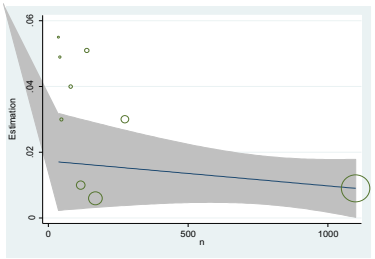
Fatigue



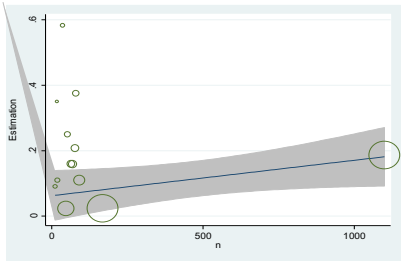
Headache



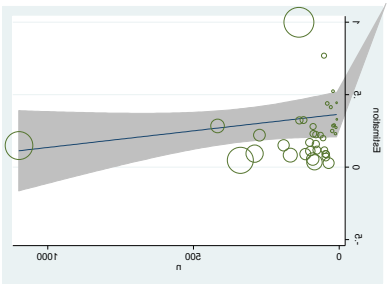
Hemoptysis



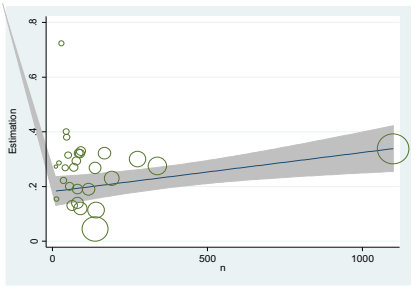
Shortness of breath



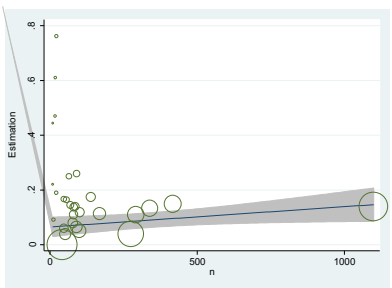
Myalgia



Sputum production



Sore throat



Conclusions

Due to the rapid spreading of this infection, the lack of diagnostic tools, and limited intensive care units in the world, the use of other factors such as the clinical features of COVID19 can serve to give early warning for the appropriate interventions and decrease the number of death of COVID-19. So considering the main symptoms of COVID-19 such as Fever, cough Fatigue and Dyspnea can have a key role in early detection of this disease.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

YA and MS formulated the research questions, designed the study, developed the preliminary search strategy, conducted the quality assessment, methodology, formal analysis, prepare drafts of the manuscript, review and editing, MT and HH refined the search strategy by conducting iterative database queries and incorporating new search terms, searched and collected the articles, re-viewed the manuscript content. Data extraction and prepare drafts of the manuscript. All authors critically reviewed the manuscript content. All authors have read and approved the final version of the manuscript.

References

[1] Bonilla-Aldana D, Dhama K, Rodriguez-Morales AJ. Revisiting the one health approach in the context of COVID-19: a look into the ecology of this emerging disease. Adv Anim Vet Sci 2020;8:234-7. https://doi.org/10.17582/journal.aavs/2020/8.3.234.237

[2] Drosten C, Günther S, Preiser W, Van Der Werf S, Brodt H-R, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med. 2003; 348:1967-76. https://doi.org10.1056/NEJMoa030747

[3] Alimohamadi Y, Taghdir M, Sepandi M. The estimate of the basic reproduction number for novel coronavirus disease (COVID-19): a systematic review and meta-analysis. J Prev Med Public Health 2020; 53(3):151-157. https://doi.org/10.3961/jpmph.20.076

[4] Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, Ma K, Xu D,Yu H, Wang HJB. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. bmj 2020;368. https://doi.org/10.1136/bmj.m1091

[5] Petrosillo N, Viceconte G, Ergonul O, Ippolito G, Petersen E. COVID-19, SARS and MERS: are they closely related? Clinical Microbiology and Infection. 2020;26:729-734. https://doi.org/10.1016/j.cmi.2020.03.026

[6] Anderson RM, Heesterbeek H, Klinkenberg D, Hollings worth TD. How will country-based mitigation measures influence the course of the COVID-19 epidemic? Lancet. 2020;395:931-4. https://doi.org/10.1016/S0140-6736(20)30567-5

[7] Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, Tan KS, Wang DY, Yan Y. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak–an update on the status. Mil Med Res.2020;7:1-10. https://doi.org/10.1186/s40779-020-00240-0

[8] Organization WH. Report of the WHO-China joint mission on coronavirus disease 2019 (COVID-19). 2020.

[9] Zu ZY, Jiang MD, Xu PP, Chen W, Ni QQ, Lu GM, Zhang LJ. Coronavirus disease 2019 (COVID-19): a perspective from China. Radiology. 2020: 296(2):E15-E25. https://doi.org/10.1148/radiol.2020200490

[10] Guan W-j, Ni Z-y, Hu Y, Liang W-h, Ou C-q, He J-x, Liu L, Shan H, Lei C-l, Hui DS. Clinical characteristics of 2019 novel coronavirus infection in China. MedRxiv 2020. https://doi.org/10.1056/NEJMoa2002032

[11] Case series studies quality appraisal checklist. https://www.ihe.ca/research-programs/rmd/cssqac/cssqac-about

[12] Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H,Cheng Z, Xiong Y. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020;17;323(11):1061-1069. https://doi.org/10.1001/jama.2020.1585

[13] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020 Feb 15;395(10223):497-506. https://doi.org/10.1016/S0140-6736(20)30183-5

[14] Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J,Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Feb 15;395(10223):507-513. https://doi.org/10.1016/S0140-6736(20)30211-7

[15] Chung M, Bernheim A, Mei X, Zhang N, Huang M, Zeng X, Cui J, Xu W, Yang Y, Fayad ZA, Jacobi A, Li K, Li S, Shan H. . CT imaging features of 2019 novel coronavirus (2019-nCoV). Radiology.2020;295:202:207. https://doi.org/10.1148/radiol.2020200230

[16] Chen L, Liu H, Liu W, Liu J, Liu K, Shang J, Deng Y, Wei S. Analysis of clinical features of 29 patients with 2019 novel coronavirus pneumonia. Zhonghua jie he he hu xi za zhi = Zhonghua Jiehe he Huxi Zazhi = Chinese Journal of Tuberculosis and Respiratory Diseases. 2020,43:E005-E005. https://doi.org/10.3760/cma.j.issn.1001-0939.2020.0005

[17] Kui L, Fang Y-Y, Deng Y, Liu W, Wang M-F, Ma J-P, Xiao W, Wang Y-N, Zhong M-H, Liu H-G. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. Chin Med J (Engl). 2020 May 5; 133(9): 1025-1031. https://doi.org/10.1097/CM9.0000000000000744

[18] Chang D, Lin M, Wei L, Xie L, Zhu G, Cruz CSD, Sharma LJJ. Epidemiologic and clinical characteristics of novel coronavirus infections involving 13 patients outside Wuhan, China. JAMA. 2020 Mar 17;323(11):1092-1093. https://doi.org/10.1001/jama.2020.1623

[19] COVID-19, Australia: Epidemiology Report 6 (Reporting week ending 19:00 AEDT 7 March 2020). Commun Dis Intell (2018) 2020 Mar 11;44. https://doi.org/10.33321/cdi.2020.44.21..

[20] Liu, Yi Shi Li and Zhang, Dongcui and Tang, Shigang and Chen, Haiou and Chen, Liang and He, Xinchun and Tong, Ming and Liu, Meng and Lin, Yihe and Zhu, Baining and Su, Xin, The Epidemiological and Clinical Characteristics of 2019 Novel Coronavirus Infection in Changsha, China (2/10/2020). Available at: SSRN: https://ssrn.com/abstract=3537093 - https://doi.org/10.2139/ssrn.3537093

[21] Pan F, Ye T, Sun P, Gui S, Liang B, Li L, Zheng D, Wang J, Hesketh RL, Yang L, Zheng C.. Time course of lung changes on chest CT during recovery from 2019 novel coronavirus (COVID-19) pneumonia. Radiology. 2020 Jun;295(3):715-721. https://doi.org/10.1148/radiol.2020200370

[22] Zhang M, Wang X, Chen Y, Zhao K, Cai Y, An C, Lin M, Mu. Clinical features of 2019 novel coronavirus pneumonia in the early stage from a fever clinic in Beijing. Zhonghua Jie He He Hu Xi Za Zhi. 2020 Feb 15;43(0):E013. https://doi.org/10.3760/cma.j.issn.1001-0939.2020.0013

[23] Feng K, Yun Y, Wang X, Yang G, Zheng Y, Lin C, Wang LF. Analysis of CT features of 15 children with 2019 novel coronavirus infection. Zhonghua Er Ke Za Zhi . 2020 Feb 16;58(0):E007. https://doi.org/10.3760/cma.j.issn.0578-1310.2020.0007

[24] X F Wang , J Yuan , Y J Zheng, J Chen , Y M Bao , Y R Wang, L F Wang , H Li , J X Zeng , Y H Zhang , Y X Liu , L Liu. Clinical and epidemiological characteristics of 34 children with 2019 novel coronavirus infection in Shenzhen. Zhonghua Er Ke Za Zhi . 2020 Feb 17;58(0):E008. https://doi.org/10.3760/cma.j.issn.0578-1310.2020.0008

[25] Yang X , Yu Y, Xu J , Shu H, Xia J , Liu H , Wu Y, Zhang L , Yu Z , Fang M , YuT , Wang T , Pan S , Zou X , Yuan S, Shang Y . Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med . 2020 May;8(5):475-481. https://doi.org/10.1016/S2213-2600(20)30079-5

[26] Wu J, Wu X, Zeng W, Guo D, Fang Z, Chen L, Huang H, Li C. Chest CT findings in patients with coronavirus disease 2019 and its relationship with clinical features. Invest Radiol. 2020 May;55(5):257-261. https://doi.org/10.1097/RLI.0000000000000670

[27] Zhao Z, Xie J, Yin M, Yang Y, He H, Jin T, Li W, Zhu X, Xu J, Zhao C , Li L, Li Y, Mengist HM, Zahid A, Yao Z, Ding C, Qi Y, Gao Y, Ma X. Clinical and Laboratory Profiles of 75 Hospitalized Patients with Novel Coronavirus Disease 2019 in Hefei, China. MedRxiv .2020. https://doi.org/10.1101/2020.03.01.20029785

[28] Zhao W, Yu S, Zha X, Wang N, Pang Q, Li T, Li A. Clinical characteristics and durations of hospitalized patients with COVID-19 in Beijing: a retrospective cohort study. MedRxiv .2020. https://doi.org/10.1101/2020.03.13.20035436

[29] Yang W, Cao Q, Qin L, Wang X, Cheng Z, Pan A, Dai J, Sun Q, Zhao F, Qu J, Yan F. Clinical characteristics and imaging manifestations of the 2019 novel coronavirus disease (COVID-19): A multi-center study in Wenzhou city, Zhejiang, China J Infect. 2020 Apr;80(4):388-393. https://doi.org/10.1016/j.jinf.2020.02.016

[30] Arentz M, Yim E, Klaff L, Lokhandwala S, Riedo FX, Chong M, Lee M. Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington State. JAMA. 2020 Apr 28;323(16):1612-1614. https://doi.org/10.1001/jama.2020.4326

[31] Huang Y, Zhou H, Yang R, Xu Y, Feng X, Gong P. Clinical characteristics of 36 non-survivors with COVID-19 in Wuhan, China. MedRxiv.2020. https://doi.org/10.1101/2020.02.27.20029009

[32] Jian-ya G. Clinical characteristics of 51 patients discharged from hospital with COVID-19 in Chongqing, China. MedRxiv. 2020. https://doi.org/10.1101/2020.02.20.20025536

[33] Zhang Jj, Dong X, Cao YY, Yuan Yd, Yang Yb, Yan Yq, Akdis CA, Gao Y. Clinical characteristics of 140 patients infected by SARS-CoV-2 in Wuhan, China. Allergy. 2020;75:1730-1741. https://doi.org/10.1111/all.14238

[34] Yan S, Song X, Lin F, Zhu H, Wang X, Li M, Ruan J, Lin C, Liu X, Wu Q, Luo Z, Fu W, Chen S, Yuan Y, Liu S, Yao J, Lv C. Clinical Characteristics of Coronavirus Disease 2019 in Hainan, China. MedRxiv. 2020. https://doi.org/10.1101/2020.03.19.20038539

[35] Wu J, Liu J, Zhao X, Liu C, Wang W, Wang D, Xu W, Zhang C, Yu J, Jiang B, Cao H, Li L. Clinical characteristics of imported cases of COVID-19 in Jiangsu province: a multicenter descriptive study. Clin Infect Dis. 2020 Jul 28;71(15):706-712. https://doi.org/10.1093/cid/ciaa199

[36] Xu Y, Li Y-r, Zeng Q, Lu Z-b, Li Y-z, Wu W, Dong S-y, Huang G, Wang X-h. Clinical characteristics of SARS-CoV-2 pneumonia compared to controls in Chinese Han population. MedRxiv. 2020. https://doi.org/10.1101/2020.03.08.20031658

[37] Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L,Wei Y,Hui Li H, Wu X, Xu J ,Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet . 2020, 395(10229):1054-1062. https://doi.org/10.1016/S0140-6736(20)30566-3

[38] Cheng Z, Lu Y, Cao Q, Qin L, Pan Z, Yan F, Yang W. Clinical Features and Chest CT Manifestations of Coronavirus Disease 2019 (COVID-19) in a Single-Center Study in Shanghai, China. AJR Am J Roentgenol. 2020 Jul;215(1):121-126. https://doi.org/10.2214/AJR.20.22959

[39] Liu Y, Li J, Liu D, Song H, Chen C,Pei X, Hu Z. Clinical features and outcomes of 2019 novel coronavirus-infected patients with cardiac injury . MedRxiv. 2020. https://doi.org/10.1101/2020.03.11.20030957

[40] Liu Y, Sun W, Li J, Chen L, Wang Y, Zhang L, Yu L.Clinical features and progression of acute respiratory distress syndrome in coronavirus disease 2019. MedRxiv. 2020.

[41] Xu Y, Xu Z, Liu X, Cai L, Zheng H, Huang Y, Zhou L, Huang L, Lin Y, Deng L, Li J , Chen S, Liu D, Lin Z, Zhou L, He W, Liu X, Li Y. Clinical findings in critical ill patients infected with SARS-Cov-2 in Guangdong Province, China: a multi-center, retrospective, observational study. MedRxiv. 2020. https://doi.org/10.1101/2020.03.03.2003066

[42] Wang L, He W, Yu X, Hu D, Bao M, Liu H, Zhou J, Jiang HJJoI:Clinical findings in critical ill patients infected with SARS-Cov-2 in Guangdong Province, China: a multi-center, retrospective, observational study. MedRxiv. 2020. https://doi.org/10.1016/j.jinf.2020.03.019

[43] Feng Z, Yu Q, Yao S, Luo L, Duan J, Yan Z, Yang M, Tan H, Ma M, Li TJm:Early prediction of disease progression in 2019 novel coronavirus pneumonia patients outside Wuhan with CT and clinical characteristics. MedRxiv. 2020. https://doi.org/10.1101/2020.02.19.20025296

[44] Qian GQ, Yang NB, Ding F, Ma AHY, Wang ZY, Shen YF, Shi CW, Lian X, Chu JG, Chen L, Wang ZY, Ren DW, Li GX, Chen XQ, Shen HJ, Chen XM. Epidemiologic and clinical characteristics of 91 hospitalized patients with COVID-19 in Zhejiang, China: a retrospective, multi-centre case series. QJM. 2020 Jul 1;113(7):474-481. https://doi.org/10.1093/qjmed/hcaa089

[45] Young BE, Ong SWX, Kalimuddin S, Low JG, Tan SY, Loh J, Ng O-T, Marimuthu K, Ang LW, Mak TMJJ:Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. JAMA. 2020; 323:1488-94. https://doi.org/10.1001/jama.2020.3204

[46] Wen Y, Wei L, Li Y, Tang X, Feng S, Leung K, Wu X, Pan X-F, Chen C, Xia JJm:Epidemiological and clinical characteristics of COVID-19 in Shenzhen, the largest migrant city of China. MedRxiv. 2020. https://doi.org/10.1101/2020.03.22.20035246

[47] Liao J, Fan S, Chen J, Wu J, Xu S, Guo Y, Li C, Zhang X, Wu C, Mou HJm:Epidemiological and clinical characteristics of COVID-19 in adolescents and young adults. TIJ. 2020; 1:100001. https://doi.org/10.1101/2020.03.10.20032136

[48] Chen X, Zheng F, Qing Y, Ding S, Yang D, Lei C, Yin Z, Zhou X, Jiang D, Zuo QJm: Epidemiological and clinical features of 291 cases with coronavirus disease 2019 in areas adjacent to Hubei, China: a double-center observational study. MedRxiv. 2020. https://doi.org/10.1101/2020.03.03.20030353

[49] Yang P, Ding Y, Xu Z, Pu R, Li P, Yan J, Liu J, Meng F, Huang L, Shi LJm:Epidemiological and clinical features of COVID-19 patients with and without pneumonia in Beijing, China. Medrxiv. 2020. https://doi.org/10.1101/2020.02.28.20028068

[50] Liu J, Ouyang L, Guo P, sheng Wu H, Fu P, liang Chen Y, Yang

D, yu Han X, kun Cao Y, Alwalid OJm:Epidemiological, clinical characteristics and outcome of medical staff infected with COVID-19 in Wuhan, China: A retrospective case series analysis. MedRxiv. 2020. https://doi.org/10.1101/2020.03.09.20033118

[51] Fu H, Xu H, Zhang N, Xu H, Li Z, Chen H, Xu R, Sun R, Wen L, Xie LJm: Association between Clinical, Laboratory and CT Characteristics and RT-PCR Results in the Follow-up of COVID-19 patients. MedRxiv. 2020. https://doi.org/10.1101/2020.03.19.20038315

[52] Shi H, Han X, Jiang N, Cao Y, Alwalid O, Gu J, Fan Y, Zheng CJTLID: Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. Lancet Infect Dis. 2020;20(4):425-434. https://doi.org/10.1016/S1473-3099(20)30086-4

[53] Zhao W, Zhong Z, Xie X, Yu Q, Liu J: Relation between chest CT findings and clinical conditions of coronavirus disease (COVID-19) pneumonia: a multicenter study. AJR Am J Roentgenol. 2020; 214:1072-7. https://doi.org/10.2214/AJR.20.22976

[54] Fan H, Zhang L, Huang B, Zhu M, Zhou Y, Zhang H, Tao X, Cheng S, Yu W, Zhu L:Retrospective analysis of clinical features in 101 death cases with COVID-19. MedRxiv. 2020. https://doi.org/10.1101/2020.03.09.20033068

[55] Hu L, Chen S, Fu Y, Gao Z, Long H, Ren H-w, Zuo Y, Li H, Wang J, Xv Q-bJm: Risk factors associated with clinical outcomes in 323 COVID-19 hospitalized patients in Wuhan, China. Clin Infect Dis. 2020;xx(xx):1-10. https://doi.org/10.1093/cid/ciaa539

[56] Zhao X, Liu B, Yu Y, Wang X, Du Y, Gu J, Wu XJCR: The characteristics and clinical value of chest CT images of novel coronavirus pneumonia. Clin Radio. 2020;75(5):335-340. https://doi.org/10.1016/j.crad.2020.03.002

[57] Chen Z, Hu J, Zhang Z, Jiang S, Wang T, Shi Z, Zhang ZJm:Caution: clinical characteristics of COVID-19 patients are changing at admission. Available at SSRN 3546044. 2020. https://doi.org/10.1101/2020.03.03.20030833

[58] Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, Li J, Zhao D, Xu D, Gong QJTL: Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet. 2020; 395:809-15. https://doi.org/10.1016/S0140-6736(20)30360-3

[59] Pung R, Chiew CJ, Young BE, Chin S, Chen MI, Clapham HE, Cook AR, Maurer-Stroh S, Toh MP, Poh CJTL:Investigation of three clusters of COVID-19 in Singapore: implications for surveillance and response measures. Lancet. 2020; 395(10229):1039-1046. https://doi.org/10.1016/S0140-6736(20)30528-6

[60] Zhu W, Xie K, Lu H, Xu L, Zhou S, Fang SJJoMV:Initial clinical features of suspected coronavirus disease 2019 in two emergency departments outside of Hubei, China. J Med Virol. 2020; 92: 1525–1532. https://doi.org/10.1002/jmv.25763

[61] Chen X, Jiang Q, Ma Z, Ling J, Hu W, Cao Q, Mo P, Yang R, Gao S, Gui XJm: Clinical characteristics of hospitalized patients with SARS-CoV-2 and hepatitis B virus co-infection. Virol Sin. 2020:1-4. https://doi.org/10.1101/2020.03.23.20040733

[62] Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC et al: Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020; 382:1708-20. https://doi.org/10.1056/NEJMoa2002032

[63] Xu X, Yu C, Qu J, Zhang L, Jiang S, Huang D, Chen B, Zhang Z, Guan W, Ling ZJEJoNM, et al:Imaging and clinical features of patients with 2019 novel coronavirus SARS-CoV-2. Eur J Nucl Med Mol Imaging. 2020:1-6. https://doi.org/10.1007/s00259-020-04735-9

[64] Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Song Z-G, Hu Y, Tao Z-W, Tian J-H, Pei Y-YJN:A new coronavirus associated with human respiratory disease in China. Nature. 2020;579:265-9. https://doi.org/10.1038/s41586-020-2008-3

[65] World meters, availabale from: https://www.worldometers.info/coronavirus/#countries

[66] Hu B, Zeng L-P, Yang X-L, Ge X-Y, Zhang W, Li B, Xie J-Z, Shen X-R, Zhang Y-Z, Wang NJPp:Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. PLoS Pathog. 2017;13:e1006698. https://doi.org/10.1371/journal.ppat.1006698

[67] Haagmans BL, Al Dhahiry SH, Reusken CB, Raj VS, Galiano M, Myers R, Godeke G-J, Jonges M, Farag E, Diab AJTLid:Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. Lancet Infect Dis. 2014; 14:140-5. https://doi.org/10.1016/S1473-3099(13)70690-X

[68] Peiris JSM, Chu C-M, Cheng VC-C, Chan K, Hung I, Poon LL, Law K-I, Tang B, Hon T, Chan CJTL: Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet. 2003; 361:1767-72. https://doi.org/10.1016/S0140-6736(03)13412-5

[69] Yu N, Li W, Kang Q, Xiong Z, Wang S, Lin X, Liu Y, Xiao J, Liu H, Deng D, et al:Clinical features and obstetric and neonatal outcomes of pregnant patients with COVID-19 in Wuhan, China: a retrospective, single-centre, descriptive study. Lancet Infect Dis. 2020;20(5):559-564. https://doi.org/10.1016/S1473-3099(20)30176-6

[70] Zumla A, Hui DS, Perlman S: Middle East respiratory syndrome. Lancet. 2015; 386:995-1007. https://doi.org/10.1016/S1473-3099(20)30176-6

[71] World Health Organization, Clinical management of severe acute respiratory infection when novel coronavirus (2019-nCoV) infection is suspected: interim guidance, 28 January 2020. available from: https://apps.who.int/iris/handle/10665/330893

[72] Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, Zhong W, Hao PJSCLS:Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci. China Life Sci. 2020; 63:457-60. https://doi.org/10.1007/s11427-020-1637-5

[73] Bai Y, Yao L, Wei T, Tian F, Jin D-Y, Chen L, Wang MJJ:Presumed asymptomatic carrier transmission of COVID-19. JAMA. 2020; 323:1406-7. https://doi.org/10.1001/jama.2020.2565

[74] Zhang H, Kang Z, Gong H, Xu D, Wang J, Li Z, Cui X, Xiao J, Meng T, Zhou WJB:The digestive system is a potential route of 2019-nCov infection: a bioinformatics analysis based on single-cell transcriptomes. BioRxiv. 2020. https://doi.org/10.1101/2020.01.30.927806

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Correspondence: Mojtaba Sepandi, Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran, - E-mail: msepandi@gmail.com

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ORIGINAL ARTICLE

An original logigramme to make safe discharge and community reintegration for COVID-19 patients

FRANCESCA DONNO, ALBERTO FEDELE
Department of Preventive Medicine and Public Health, Local Health Agency of Lecce (Apulia), Italy

Keywords

COVID-19 • Safe discharge • Community reintegration criteria

Summary

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the microorganism responsible for the aggressive Coronavirus Disease (COVID-19) pandemic. During the such pandemic, discharge and community reintegration of patients are critical phases in guaranteeing public health. A review of the international and Italian experiences that represent the best available evidence was carried out, mainly focusing on the precise allocation of tasks and related responsibilities. The report provides a proposal for a systematic management pathway dedicated to COVID-19 patients. The original result is a logigramme to guide health practitioners on discharge and community reintegration of COVID-19 patients. To standardize clinical attitudes helps in ensuring quality of care and patient safety, should be a core element even during a public health emergency. The logigramme suggests, after discharge, 14 days of further isolation with regular health monitoring and, finally, the execution of a nasopharyngeal swab for identification of SARS-CoV-2 viral RNA. Home-cared patients should be placed on 7 days of further isolation after at least 2 negative RT-PCR tests for respiratory tract samples (nasopharyngeal swab). The logigramme is already used in the Department of Prevention - Local Health Agency of Lecce (Apulia) but it will be updated according to the latest research findings.

Introduction

On January 30th 2020, the WHO declared the international outbreak of the new coronavirus SARS-CoV-2 as a public health emergency of international concern, as provided for in the international health regulation of 2005 [1].
The Severe Acute Respiratory Syndrome (SARS) is known to be a "High Consequence Infectious Disease (HCID)" also called Infectious Disease of High Consequence (IDHC), i.e. a disease which poses a significant threat to human health. Patients affected by such diseases usually develop severe symptoms and require a high level of assistance with death rates that may be quite high [2].
The Apulia Region, starting from January 31st, under a decree from the Ministry of Health, established a regional Task Force for the management of the infectious outbreak; the Task Force arranged the "Operational protocol for the management of suspected COVID-19 cases in Apulia" and, after the cases reported in Lombardy and Veneto, the "COVID-19 Operational plan, Apulia Region". By the Order of the Regional Government No 172, dated 6 March 2020, within the meaning of Article 11 of D. Lgs. No 1/2018 and to transpose the Measures provided for in the Document *Civil protection operational measures for the management of the epidemiological outbreak of COVID-19* as referred to in the note of the Head of Civil Protection Department – Coordinator of OCDPC Interventions No. 630/2020 prot. n. COVID/001656 of 3 March 2020, the regional Crisis Unit is established to take actions and measures, as specifically identified in the above-mentioned Document [3].

The scenario of the clinical condition of patients at national level is influenced by the fact that it has not yet been classified in all Regions and Autonomous Provinces in a standardised manner in accordance with the provisions of COVID-19 Surveillance, since the structured processing of such data is still under preparation. The assessment provided by the Italian Institute of Health updated to April 2nd reported that the clinical condition was available only for 39,884 cases, of which 2,360 (5.9%) asymptomatic, 5,587 (14.0%) paucisymptomatic, 5,155 (12.9%) with symptoms whose severity level was not specified, 17,085 (42.9%) with mild symptoms, 8,494 (21.3%) with severe symptoms requiring hospitalisation, 1,203 (3%) with clinical picture of critical illness requiring hospitalisation in Intensive Care Units [4].
From Apulia epidemiological bulletin, updated to 7 April 2020 [5], it was found that out of 2,514 confirmed cases, 938 were in isolation (known in 1,646 cases), 708 hospitalized (known in 1,646 cases), 209 deceased (known in 1,779 cases) and 168 recovered (known in 1,779 cases). According to the recent literature, the clinical process evolution of hospitalized patients has shown that 10-20% of patients are hospitalized in a RICU (Respiratory Intensive Care Unit), 3-10% are intubated, 2-5% died [6].
The number of hospitalized patients in Apulia (which "in large percentage" are the potential patients to be discharged) is the reason because of we have developed a dedicated project-pathway. It is an original organizational model for improving patient safety and quality care inside and outside the hospital.

Actually, as recommended by ECDC (European Center for Disease Prevention and Control), we answered to a strategic clinical governance requirement: define the "state of discharge" of patients according to the "*checklist for the designated treatment facility for HCID case (s)*" [2].

Purpose and objectives

The mission of the National – and therefore Regional – Healthcare System is to guarantee a "fair and appropriate" quality of healthcare. The review of the discharge letters of COVID-19 patients from hospital units showed the presence of a widely diversified de-hospitalization attitude: in case of discharge as a consequence of two negative swabs as recommended by Italian Ministry of Health [7] or with one negative swab and one unknown, it is recommended a 14-days long fiduciary isolation, whose follow-up is entrusted to general medicine doctors; instead, in other cases, it is simply recommended observing common precautionary measures.
It is necessary to standardize the COVID-19 patients discharge from the hospital, in order to have both a systemic (shared at a regional level) and a systematic perspective and to ensure the healthcare safety.
The final objective of this work is to assess, promote and spread guidelines dedicated to the management of COVID-19 patients, home-cared or hospitalized ones (from hospital discharge to community reintegration).
These guidelines have been developed and calibrated based on currently available national and international experiences and best evidences. We have developed a procedure to be used as a decision support for all Healthcare Professionals involved in the COVID-19 care system (home cared patients and hospitalized ones).
This procedure could be reviewed in accordance with progressive scientific updates.

Focus on RT-PCR test performed using swabs

The most commonly used and reliable test for diagnosis of COVID-19 has been the RT-PCR test performed using nasopharyngeal swabs or other upper respiratory tract specimens, including throat swab or, more recently, saliva.
In most individuals with symptomatic COVID-19 infection, viral RNA in the nasopharyngeal swab as measured by the cycle threshold (Ct) becomes detectable as early as day 1 of symptoms and peaks within the first week of symptoms onset. Positivity starts to decline by week 3 and subsequently becomes undetectable.
The virus persists longer with higher load and peaks later in the respiratory tissue of patients with severe disease and the duration of the virus was significantly longer in men than in women, and significantly longer in patients older than 60 years than younger ones [8].
In some cases, viral RNA has been detected by RT-PCR even beyond week 6 after the first positive test. A few cases have also been reported positive after 2 consecutive negative PCR tests performed 24 hours apart [9].
The evidence of the extrapulmonary detection of viral RNA in both symptomatic and asymptomatic subjects is an interesting element from a clinical and epidemiological point of view.
Zou et al. 2020 report that the viral load of asymptomatic patients was similar to symptomatic ones, indicating a transmission potential of asymptomatic or pre-symptomatic patients. The study reports that patients with few or no symptoms had modest levels of detectable viral RNA in the oropharynx for at least 5 days [10].
However, we know that a positive PCR outcome reflects only the detection of viral RNA and does not necessarily indicate presence of viable virus [11].
Further studies, such as culture of SARS-CoV-2, should be carried out to investigate the actual potential infectiousness.
It has been reported that several cases recovered from COVID-19 tested positive for SARS-CoV-2 after discharge (re-detectable positive, RP) but the clinical characteristics, significance and potential cause of RP patients remained unknown [12].
Four patients with COVID-19 who met criteria for hospital discharge or discontinuation of quarantine in China (absence of clinical symptoms and radiological abnormalities and 2 negative RT-PCR test results) had positive RT-PCR test results 5 to 13 days later [13].
In literature there is also a study regarding the case of an asymptomatic discharged patient with SARS-CoV-2 nucleic acid retested positive. Although no scientific evidence demonstrated that a discharged patient who had repeated SARS-CoV-2 nucleic acid positive could be infectious to others, these clinical experiences after discharge arouse concern regarding the present discharge standard of COVID-19 [14].
All these evidences justify the need for particular attention in management during the community reintegration of COVID-19 patients to protect public health.

International review of COVID-19 patients discharge

In order to discharge clinically recovered patients, the WHO, CDC and ECDC recommend the collecting of two respiratory swabs at least 24 hours apart from each other [15-17].
The ECDC reports that, current criteria for discharge COVID-19 patient include both resolution of symptoms and laboratory evidence of SARS-CoV-2 clearance from the upper respiratory tract. Criteria should be reviewed according to the local context [18]. The current international criteria for discharge are summarised in the table written by ECDC [17].
According to some scientifically tested references, under unspecified conditions ("if suitable") home care could be performed in symptomatic patients who no longer require hospitalization [19].

On March 24th the CDC clarified that, when clinically indicated, COVID-19 patient can be discharged from a health care facility. For such patients, a discharge does not require that they meet CDC criteria for the discontinuation of COVID-19 transmission-based precautions (TBP) [20].
CDC guidelines provide a possible dual "destination" for discharged COVID-19 patients. For the patient discharged home the discharging facility should consider the home's suitability for assuring patient's isolation and patient's ability to adhere to home isolation recommendations. Isolation should be maintained at home if the patient returns home before discontinuation of TBP.
For the patients discharged to Long-term Care (LTC) or Assisted Living Facilities there are three possible scenarios:

1. when TBPs are still required transferred COVID-19 patients should go to a facility with adequate personal protective equipment and an ability to adhere to infection prevention and control recommendations for the care of COVID-19 patients. Preferably, the patient would be placed at a facility that has already cared for COVID-19 cases, in a specific unit designated to care for COVID-19 residents;
2. transferred COVID-19 patients for whom TBPs have been discontinued but continue to have persistent symptoms of COVID-19 (e.g., a persistent cough) should be placed in a single room and be restricted to their room;
3. transferred COVID-19 patients for whom TBPs have been discontinued and the symptoms have resolved do not require further restrictions.

ECDC technical report suggests criteria to be considered when deciding whether a confirmed COVID-19 case can be safely discharged from hospital or released from home isolation: the most important factors are the existing capacity of the healthcare system, laboratory diagnostic resources, and the ongoing epidemiological situation.
"COVID-19 patients may be discharged from hospital and moved to home care (or other types of non-hospital care and isolation structures) based on:

- clinical criteria: e.g. no fever for > 3 days, improved respiratory symptoms, pulmonary imaging showing obvious absorption of inflammation, no hospital care needed for other pathology, clinician assessment;
- laboratory evidence of SARS-CoV-2 clearance in respiratory samples;
- 2 to 4 negative RT-PCR tests for respiratory tract samples: nasopharynx and throat swabs with sampling interval ≥ 24 hours. Testing at a minimum of 7 days after the first positive RT-PCR test is recommended for patients that clinically improve earlier;
- serology: appearance of specific IgG when an appropriate serological test is available".

The scientific report suggests, after discharge, 14 further days of isolation with regular health monitoring (e.g. follow-up visits, phone calls) if, and only if, the patient's home is equipped for patient isolation and the patient takes all necessary precautions that we call "self-control" (e.g. single room with good ventilation, face-mask, reduced close contact with family members, separate meals, good hand sanitation, no outdoor activities) in order to prevent further spread of SARS-CoV-2 and to guarantee public health [17].
The Ministry of Health and Family Welfare, Government of the People's Republic of Bangladesh proposed a "Revised Discharge Policy for COVID-19" and the classification of the patients based on clinical severity: mild/very mild/pre-symptomatic cases; moderate cases admitted to dedicated COVID Health Centre (Oxygen beds); severe cases including immunocompromised (HIV patients, transplant recipients, malignancy). Only for the last two categories RT-PCR test is required before discharge [21].

National and regional contextualization: Lecce's clinical pathway for hospital discharge and community reintegration

By now, in the existing procedures for the management of hospital discharge in Italy (in particular in Veneto [22] and Liguria [23]) the presence or absence of symptoms or clinical recovery is considered a critical element in the decision-making procedure.
Tuscany [24] has developed some guidelines to manage the path of the COVID-19 patients inside and outside the hospital. These guidelines are considered valid and shareable since the positivity/negativity of the nasopharyngeal swab is considered fundamental.
Unless there are conditions of absolute unavailability to adopt laboratory support, it is considered essential to use a "test-based strategy" focused on the execution of the pre-discharge Nose-Pharyngeal swab. SARS-CoV-2 RNA is isolated in only 32% of Oropharyngeal Swabs (OP), which is significantly lower if compared to 63% of Nasopharyngeal Swabs (NP) [25].
The positive RNA amount reaches the target peak 7-10 days after the onset of symptoms in the upper respiratory tract specimens and subsequently it decreases constantly, while in the lower respiratory tract specimen it remains higher for 3 weeks after the onset of the disease [26].
WHO [27] and CTS (Italian scientific and technical committee) [28] recommend using NP as standard diagnostic approach.
Based on the research of international and national sources, the following step-by-step pathway dedicated to the COVID-19 patient during hospital de-escalation and discharge is proposed.
For hospital de-escalation of patients who no longer need (clinical criterion) assistance in Intensive Care, it is expected that:

1. if positivity to SARS-CoV-2 persists (ascertained with a positive result for the presence of SARS-CoV-2 in NP swabs) but clinical stabilization exists (ordinary definition criteria), it is expected transferring to a COVID-19 normal block hospitalization;
2. if negative for SARS-CoV-2 (ascertained with a negative result for the presence of SARS-CoV-2 in 2 NP swabs collected at least 24 hours apart) but in

need of medical assistance, it is required transferring to a NO COVID-19 ordinary hospitalization block;
3. if prolonged respiratory assistance is needed, it is required: respiratory rehabilitation in a structure with a dedicated COVID-19 area in case of persistence of SARS-CoV-2 positivity (ascertained with a positive result for the presence of SARS-CoV-2 in NP-swabs); or respiratory rehabilitation in a NON COVID-19 structure in case of persistence of SARS-CoV-2 negativity (ascertained with a negative result due to the presence of SARS-CoV-2 in 2 NP-swabs collected at least 24 hours apart).

The development of a set of clinical indicators of "clinical stabilization" is based on the statistical evaluation of the frequency of evidence of the signs related to the symptoms presented by COVID-19 patients:

1. anosmia and ageusia seem to be part of important symptoms and clues for the diagnosis of COVID-19, mostly in the early stage of the disease [29, 30];
2. fever often present with non-constant frequency in different cohorts of patients. Despite this COVID-19 cannot be excluded, even if fever is not present;
3. gastrointestinal symptoms, about 10% of patients develop gastrointestinal symptoms (nausea and/or diarrhoea), before the insurgence of fever and dyspnoea [31];
4. silent hypoxia. In particular elderly can develop hypoxia even in absence of dyspnoea symptoms [32].

Recent studies report that COVID-19 could present a broader clinical spectrum characterized by the absence of any symptoms to heart, digestive tract or Ear-Nose-Throat (including anosmia and ageusia) manifestations and by the presence of peculiar skin manifestations [33]. Physical examination is usually non-specific [6].

Discharge criteria (clinical + test-based) proposed by Toscana Region [24] that are considered as sharable are the following:

1. at least 48 hours of apyrexia;
2. saturation level ≥ 94% (≥ 90% for chronic patients) in ambient air from at least 48 hours or P/F (PaO_2/FiO_2) in ambient air > 300 from at least 48 hours RR (Respiratory Rate) < 22 at rest;
3. two negative nasopharyngeal swabs collected 24 hours apart and negative test for SARS-CoV-2 before hospital discharge.

Such criteria have to be fulfilled in order to proceed with the next steps of the clinical path dedicated to the patient who required hospitalization for COVID-19 treatment.

The logigramme: a community reintegration protocol for COVID-19 patients

We reported the protocol we have developed and already applied (Figs. 1, 2):

- *If the patient's discharge to his own home is possible:*
  1. hospital doctor suggests home isolation for 14 days;
  2. hospital doctor sends the discharge letter to DPHCM (Department of Public Health and Community Medicine). The letter specifies that home isolation is suggested and also clarify that the follow-up will be managed by the GP (General Practitioner);
  3. the DPHCM requires that COVID-19 patients have to self-isolate at home, following the already existing operating modes prior to evaluation with NP swab in case of symptomatic patients, at the end of the isolation period.

  During the follow-up the communication between GP e DPHCM is constantly guaranteed.
- *In case of patient discharge at long-term care facility:*
  1. the hospital doctor suggests home isolation for further 14 days under the control of the long-term care facility. As specified in a local document Apulia's document [34] it's necessary to provide that the facility satisfies technical-logistic-structural requirements;
  2. hospital doctor sends the discharge letter at DPHCM; the letter explicit that home isolation is required and that the DPHCM is responsible for the follow-up phase (also specifies the fields of competence and clinical management dependent on the GP);
  3. the DPHCM requires that COVID-19 patients have to self-isolate at home, following the already existing operating modes prior to evaluation with NP swab in case of symptomatic patients, at the end of the isolation period.

  During the follow-up the communication between GP, long-term care facility and DPHCM is constantly guaranteed.
- *Lastly, for the patient that did not need hospitalization and that has been managed at his own home, community reintegration follows the process as below:*
  1. DPHCM makes the decision of the home isolation up to clinical resolution, following the already existing operating modes;
  2. DPHCM is responsible for the follow-up during the home isolation period, as indicated by ministry guidelines;
  3. the patients, during the follow-up communicate to the GP and DPHCM the insurgence of symptoms for the fulfilments of respective competences;
  4. DPHCM orders the execution of NP swab following the pre-set criteria (14 days after the first day of isolation and 7 days after the first day in absence of symptoms);
  5. DPHCM activates 7 further days of home isolation after at least 2 negative RT-PCR tests for respiratory tract sample (NP swab);
  6. DPHCM requires that COVID-19 patients have to self-isolate at home, following the already existing operating modes, after 7 days from the result of two consecutive negative SARS-CoV-2 NP swabs (collected 24 hours apart).

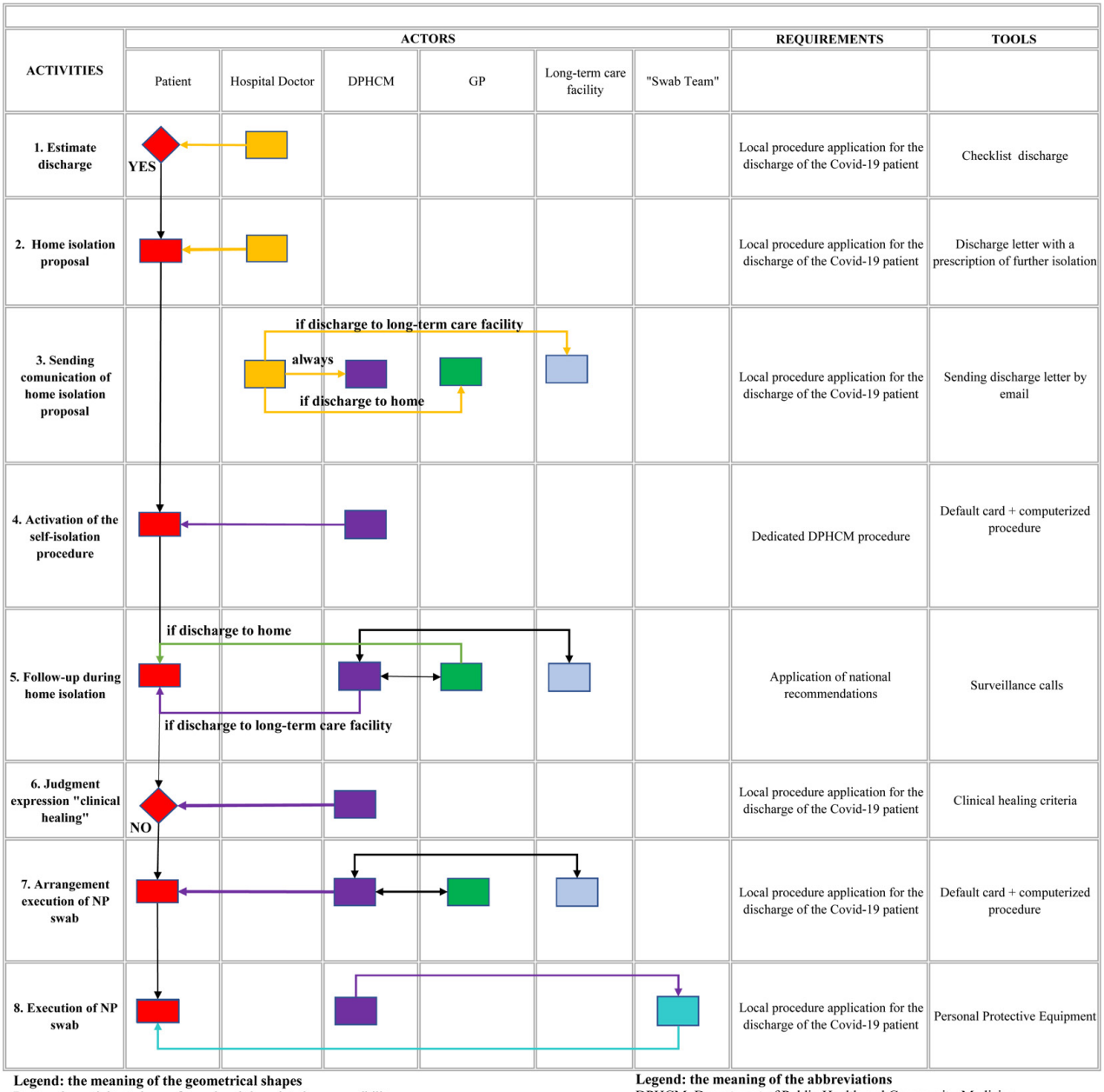
Fig. 1. Logigramme to safe community reintegration for home cared COVID-19 patient.

ACTIVITIES	ACTORS				REQUIREMENTS	TOOLS
	Patient	DPHCM	GP	"Swab team"		
1.Evaluation of home isolation	YES				Application of local procedure	Predefined criteria
2. Activation of the self-isolation procedure	reporting signs and/or symptoms				Application of local procedure	Default card + computerized procedure
2. Follow-up during home isolation	reporting signs and/or symptoms				Applicationn of national indications	Surveillance calls
3. Swab execution					Application of local procedure	Predefined criteria
4. Evaluation at least 2 negative NP SWAB at the end of home isolation	YES				Application of local procedure	Predefined criteria
5. Activation 7 days of further isolation					Application of local procedure	Default card + computerized procedure
6. Formal closure of home isolation					Application of local procedure	Default card + computerized procedure

Legend: the meaning of the geometrical shapes
Rectangle: activity to be performed and the related rensponsibility
Rhombus: choice to make/decision to be taken
Arrow: direction of the activity

Legend: the meaning of the abbreviations
DPHCM: Department of Public Health and Community Medicine
GP: General Practitioner
NP: nasopharyngeal

Fig. 2. Logigramme to safe discharge and community reintegration for hospitalized COVID-19 patient.



The present pathway could be implemented by integrating the serological tests that are now available: they are fundamental in finding the virus and for conducting the epidemiologic evaluation of viral diffusion, but as OMS recommends [35] further evidence is needed about their performances and operational utility before their use for the SARS-CoV-2 diagnosis [36].

Conclusions

Since COVID-19 is a novel disease, guidance by scientific and globally shared evidence is often unavailable: it is necessary to urge and promote standardized behavior.

We potentially provide a practical and feasible solution to satisfy the need for an optimal community reintegration for COVID-19 patients.

The leading idea is sharing a tool of COVID-19 patients management, overcoming certain limitations that have conditioned the present care system.

The logigramme based on a systematic review is already used in our local area: step by step it explains what we do and how we do it.

The punctual and rigorous description of tasks and related responsibilities should have significant impact on single patient's outcome and public health too.

The real effects of logigramme implementation will be studied and analyzed in the second phase of our study

according to the outcome of ongoing studies about viral clearance.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

FD and AF conceived the study and drafted the manuscript. All authors revised the manuscript and performed a search of the literature. All authors have read and approved the latest version of the paper for publication.

References

[1] Federazione Nazionale degli Ordini dei Medici Chirurghi e degli Odontoiatri (FNOMCEO). COVID-19, la malattia da nuovo coronavirus (SARS-CoV-2) - III edizione (27-02-2020). Available at: https://portale.fnomceo.it/wp-content/uploads/2020/03/dossier_coronavirus_def_27-02-2020-compresso.pdf

[2] European Centre for Disease Prevention and Control (ECDC). Technical Report. Health emergency preparedness for imported cases of high-consequence infectious diseases - Operational checklist for country preparedness planning in the EU/EEA countries. p. 1. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/Health-emergency-preparedness-imported-cases-of-high-consequence-infectious-diseases.pdf

[3] Bollettino Ufficiale della Regione Puglia (BURP) - n. 33 del 12-3-2020. ORDINANZA DEL PRESIDENTE DELLA GIUNTA REGIONALE 6 marzo 2020, n. 172 Misure operative di protezione civile inerenti "la definizione della catena di comando e controllo del flusso delle comunicazioni e delle procedure da attivare in relazione allo stato emergenziale determinato dal diffondersi del virus COVID-19". Atto di recepimento. pp. 17722. Available at: http://burp.regione.puglia.it/documents/10192/48247592/Bollettino+numero+33+-+Ordinario+-+anno+2020/5d508aa8-afbd-4734-bbe8-a5991bdcf5d1

[4] Istituto Superiore di Sanità (ISS). Epidemia COVID-19 - Aggiornamento nazionale 2 aprile 2020 - ore 16:00. p. 5. Available at: https://www.epicentro.iss.it/coronavirus/bollettino/Bollettino-sorveglianza-integrata-COVID-19_2-aprile-2020.pdf

[5] Press Regione Puglia. Epidemia COVID-19. Bollettino Epidemiologico Regione Puglia - 7 aprile 2020. Aggiornamento delle 16.30. Available at: https://press.regione.puglia.it/documents/65725/216593/Bollettino+Covid+07-04.pdf/e3f49b0d-78a9-26b3-318d-f0fab8e17a68?t=1586330074151

[6] Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, Liu L, Shan H, Lei C, Hui DSC, Du B, Li L, Zeng G, Yuen K-Y, Chen R., Tang C, Wang T, Chen P, Xiang J, Li S, Wang J, Liang Z, Peng Y, Wei L, Liu Y, Hu Y, Peng P, Wang J, Liu J, Chen Z, Li G, Zheng Z, Qiu S, Luo J, Ye C, Zhu S, Zhong N; for the China Medical Treatment Expert Group for Covid-19. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382:1708-20. https://doi.org/10.1056/NEJMoa2002032

[7] Ministero della Salute. Parere del Consiglio Superiore di Sanità: definizione di Paziente guarito da Covid-19 e di paziente che ha eliminato il virus SARS-CoV-2. p. 3. Available at: http://www.trovanorme.salute.gov.it/norme/renderNormsanPdf?anno=2020&codLeg=73458&parte=1%20&serie=null

[8] Zheng S, Fan J, Fei Y, Baihuan F, Bin L, Qianda Z, Guoliang X, Sha L, Ruonan W, Xianzhi Y, Weizhen C, Qi W, Dan Z, Yanchao L, Renjie G, Zhaohui M, Siming L, Yanyan X, Yaxi G, Jinming Z, Hangping Y, Kaijin X, Xiaoyang L, Guoqing W, Jianying Z, Qiang F, Hongliu C, Yunqing Q, Jifang S, Yu C, Tingbo L. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. BMJ 2020;369:m1443. Published online April 21, 2020. https://doi.org/10.1136/bmj.m1443

[9] Nandini S, Sundararaj SJ, Akihide R. Interpreting diagnostic tests for SARS-CoV-2. JAMA 2020. https://doi.org/10.1001/jama.2020.8259

[10] Lirong Z, Feng R, Mingxing H, Lijun L, Huitao H, Zhongsi H, Jianxiang Y, Min K, Yingchao S, Jinyu X, Qianfang G, Tie S, Jianfeng H, Hui-Ling Y, Malik P, Jie W. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med 2020;382:1177-9. https://doi.org/10.1056/NEJMc2001737

[11] Wölfel R, M Corman V, Guggemos W, Seilmaier M, Zange S, Müller M, Niemeyer D, Jones T, Vollmar P, Rothe C, Hoelscher M, Bleicker T, Brünink S, Schneider J, Ehmann R, Zwirglmaier K, Drosten C, Wendtner C. Virological assessment of hospitalized patients with COVID-2019. Nature 2020;581:465-9. https://doi.org/10.1038/s41586-020-2196-x

[12] Jianghong A, Xuejiao L, Tongyang X, Shen Q, Jing Y, Haocheng Y, Furong Q, Chengguang S, Yang L, Lifei W, Xiaoya C, Na L, Qingxian C, Fang W, Jun C, Yingxia L, Yunfang W, Feng Z, Yang F, Xiaohua T, Lei L, Zheng Z. Clinical characteristics of the recovered COVID-19 patients with re-detectable positive RNA test. medRxiv preprint. 2020. https://doi.org/https://doi.org/10.1101/2020.03.26.20044222

[13] Lan L, Dan X, Guangming Y, Chen X, Shaokang W, Yirong L, Haibo X. Positive RT-PCR test results in patients recovered from COVID-19. JAMA 2020;323:1502-3. https://doi.org/10.1001/jama.2020.2783

[14] Zhang JF, Yan K, Ye HH, Lin J, Zheng JJ, Cai T. SARS-CoV-2 turned positive in a discharged patient with COVID-19 arouses concern regarding the present standard for discharge. Int J Infect Dis 2020;97:212-4. https://doi.org/10.1016/j.ijid.2020.03.007

[15] World Health Organization (WHO). Clinical management of severe acute respiratory infection when novel coronavirus (2019-nCoV) infection is suspected. Interim guidance. Geneva: WHO 2020. Available at: https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected

[16] Centers for Disease Control and Prevention (CDC). Interim guidance for discontinuation of transmission-based precautions and disposition of hospitalized patients with COVID-19. Atlanta: CDC. Available at: https://www.cdc.gov/coronavirus/2019-ncov/hcp/disposition-hospitalized-patients.html

[17] European Centre for Disease Prevention and Control (ECDC). Novel coronavirus (SARS-CoV-2). Technical report. Discharge criteria for confirmed COVID-19 cases – when is it safe

to discharge COVID-19 cases from the hospital or end home isolation? Stockholm: ECDC. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-Discharge-criteria.pdf

[18] European Centre for Disease Prevention and Control (ECDC). Coronavirus disease 2019 (COVID-19) pandemic: increased transmission in the EU/EEA and the UK – seventh update 25 March 2020. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/RRA-seventh-update-Outbreak-of-coronavirus-disease-COVID-19.pdf

[19] The British Medical Journal (BMJ). BMJ Best Practice. Covid-19, the right clinical information, right where it's needed (last update: 12.03.2020; p. 32). Available at: https://snlg.iss.it/wp-content/uploads/2020/03/BMJ-BEST-PRACTICE_COVID-19_BMJ-2020.pdf

[20] American Hospital Association. Coronavirus update. March 24, 2020, CDC Updates guidance on discharging patients with COVID-19. Available at: https://www.aha.org/system/files/media/file/2020/03/coronavirus-update-cdc-updates-guidance-on-discharging-patients-with-covid-19-3-24-2020.pdf

[21] Ministry of Health and Family Welfare, Government of India. Revised Discharge Policy for COVID-19. Available at: https://www.mohfw.gov.in/pdf/ReviseddischargePolicyforCOVID19.pdf

[22] Regione del Veneto. Procedura regionale. Nuovo coronavirus (SARS-CoV-2). Rev. 02 del 6.03.2020. p.46. Available at: https://ospedalevillasalus.it/wp-content/uploads/2020/03/Procedura-regionale-Covid-19-rev._2-6.3.2020.pdf

[23] Sistema Sanitario Regione Liguria - ASL4. Gestione clinica del paziente Covid19+ (versione 30.03.2020), ASL 4, Sistema Sanitario Regione Liguria. Available at: http://www.asl4.liguria.it/wp-content/uploads/2020/04/gestione-clinica-del-paziente-covid19.pdf

[24] Regione Toscana. Linee di indirizzo per la gestione del percorso COVID-19 in ambito ospedaliero e peri-ospedaliero. Available at: http://www301.regione.toscana.it/bancadati/atti/Contenuto.xml?id=5247300&nomeFile=Ordinanza_del_Presidente_n.14_del_17-03-2020-Allegato-A

[25] Wenling W, Yanli X, Ruqin G, Roujian L, Kai H, Guizhen W, Wenjie T. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA 2020;323:1843-4. https://doi.org/10.1001/jama.2020.3786

[26] Cheng PKC, Wong ADA, Tong LKL, Ip SM, Lo ACT, Lau CS, Yeung EYH, Lim WWL.Viral shedding patterns of coronavirus in patients with probable severe acute respiratory syndrome. Lancet 2004;363:1699-700. https://doi.org/10.1016/S0140-6736(04)16255-7

[27] World Health Organization. Global Surveillance for human infection with novel coronavirus (2019-nCoV) – Interim guidance 21 January 2020. p. 2. Available at: https://www.ephi.gov.et/images/20200121-global-surveillance-for-2019-ncov.pdf

[28] Ministero della Salute. Nuovo Coronavirus. FAQ - Covid-19, domande e risposte. Available at: http://www.salute.gov.it/portale/nuovocoronavirus/dettaglioFaqNuovoCoronavirus.jsp?lingua=italiano&id=228

[29] Lee Y, Min P, Lee S, Kim SW. Prevalence and duration of acute loss of smell or taste in COVID-19 patients. J Korean Med Sci 2020;35:e174. https://doi.org/10.3346/jkms.2020.35. e174

[30] Tong JY, Wong A, Zhu D, Fastenberg JH, Tham T. The Prevalence of olfactory and gustatory dysfunction in COVID-19 patients: a systematic review and meta-analysis. Otolaryngol Head Neck Surg 2020:194599820926473. https://doi.org/10.1177/0194599820926473

[31] Wang D. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. 7.02.2020. JAMA 2020;323:1061-9. https://doi.org/10.1001/jama.2020.1585

[32] Xie J, Tong Z, Guan X, Du B, Qiu H, Slutsky AS. Critical care crisis and some recommendations during the COVID-19 epidemic in China. Intensive Care Med 2020;46:837-40. https://doi.org/10.1007/s00134-020-05979-7

[33] Van Damme C, Berlingin E, Saussez S, Accaputo O. Acute urticaria with pyrexia as the first manifestations of a COVID-19 infection. J Eur Acad Dermatol Venereol 2020;34:e300-1. https://doi.org/10.1111/jdv.16523

[34] Regione Puglia. Dipartimento promozione della salute, del benessere sociale e dello sport per tutti. "Prot. N. AOO/005/251 del 25/03/2020 – Urgente. Oggetto: misure per la prevenzione, il contrasto e il contenimento dell'emergenza epidemiologica da Covid-19 – Indicazioni operative per la gestione dell'emergenza nelle Rsa anziani". Available at: https://www.sanita.puglia.it/documents/20182/99101921/Misure+prevenzioni+e+contrasto+Rsa+Anziani+25032020/e3035dc2-645b-4843-8acf-91cd4e38b61e

[35] World Health Organization. Laboratory biosafety guidance related to coronavirus disease 2019 (COVID-19) - Interim Guidance 12 February 2020. Available at: https://apps.who.int/iris/bitstream/handle/10665/331138/WHO-WPE-GIH-2020.1-eng.pdf

[36] Ministero della Salute. Direzione Generale della Prevenzione sanitaria, Ufficio 5 Prevenzione delle malattie trasmissibili e profilassi internazionale. 0011715-03/04/2020-DGPRE-DGPRE-P - Pandemia di COVID-19 - Aggiornamento delle indicazioni sui test diagnostici e sui criteri da adottare nella determinazione delle priorità. Aggiornamento delle indicazioni relative alla diagnosi di laboratorio. p. 3. Available at: http://www.trovanorme.salute.gov.it/norme/renderNormsanPdf?anno=2020&codLeg=73799&parte=1%20&serie=null

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Correspondence: Francesca Donno, Department of Preventive Medicine and Public Health - Local Health Agency of Lecce, viale Don Giovanni Minzoni 8, 73100 Lecce (Apulia), Italy - E-mail: francescadonno@gmail.com

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ORIGINAL ARTICLE

All's not well with the "worried well": understanding health anxiety due to COVID-19

GANESH KINI[1], RAVICHANDRA KARKAL[1], MADHAVI BHARGAVA[2,3]
[1] Department of Psychiatry, Yenepoya Medical College, Yenepoya (Deemed to be University), Mangalore, Karnataka, India;
[2] Department of Community Medicine, Yenepoya Medical College, Yenepoya (Deemed to be University), Mangalore, Karnataka, India;
[3] Center for Nutrition Studies, Yenepoya (Deemed to be University), Mangalore, Karnataka, India

Keywords

Health anxiety • Worried well • COVID-19 pandemic • Quarantine • Novel coronavirus

Summary

The novel corona virus (SARS- CoV2) pandemic has created an unprecedented public health problem and a mental health crisis looms ahead. The isolation, socio-economic disruption, uncertainty and fear of contagion have led to a spike of health anxiety in the general public. Individuals with health anxiety may get dismissed as the "worried well" in this pandemic due to disruption of mental health services and inability of healthcare systems to understand the psychosocial factors in the background. Education of general public, training of healthcare workers in cognitive behavioural model of health anxiety and timely referral to mental health professionals in severe cases is need of the hour.

Introduction

The novel corona virus (SARS-CoV2) which was first reported in Wuhan, China has engulfed the world in fear [1] World Health Organization declared the COVID-19 as a pandemic on March 11, 2020 [2]. Many countries had nation-wide lockdown including India, halting most economic activity leading to unemployment, displacement of migrant workers and loss of livelihood. The pandemic has created an unprecedented public health problem and has overwhelmed healthcare systems globally [3]. Medical and research communities are still discovering the enigmatic COVID-19 which manifests not only as Acute Respiratory Illness but also with a wide variety of dermatological, neurological, cardiac, gastrointestinal, and ocular symptoms; and guidelines for prevention and treatment are evolving each day [4-9]. The isolation, socio-economic disruption, uncertainty and fear of contagion have led to a mental health crisis which is being acknowledged worldwide [10]. Individuals who are worried about infection with the virus may not get adequate care due to disruption of mental health services during the pandemic.

The "worried well" are unwell too

A term 'worried well' is often used for persons who are relatively healthy but consider themselves as affected or likely to be affected. In present pandemic these are the persons who test negative or may not fit into the definition of a 'suspect'. In addition to these individuals, patients with illness anxiety disorder, panic disorder, generalised anxiety disorder, depression, somatic symptom disorder, and obsessive compulsive disorder may not get access to mental health services. Our medical training and biomedical model of disease approach is compounded by the burden of keeping abreast with newer technical guidelines. This leads to suboptimal attention to the psycho-social factors and patients being told, "it's all in your head". As a result vicious cycle consisting of poor patient satisfaction and doctor shopping is set-off and this itself be detrimental in the present pandemic situation.
People in quarantine who are not equipped with self-care and coping strategies feel lonely, socially isolated and may find it difficult to handle the parallel 'infodemic' [11]. This leads to poor sleep and nutrition, lack of exercise, substance abuse, excessive usage of internet, and social media [12-14]. Moreover, the images of body bags piling up in hospitals or news of death in their own district/ state are threatening stimuli which give rise to unpleasant emotions of fear and anxiety. The precariousness of the current scenario, isolation, unhealthy lifestyle and overload of ambiguous information leads to chronic stress. This may act as a trigger for health anxiety in susceptible individuals or may worsen pre-existing mental health conditions. In severe conditions extreme health anxiety can even drive a patient towards committing suicide.

Understanding health anxiety: cognitive behavioural model

Threat cues can activate the dysfunctional schemas which are irrational assumptions and beliefs from an earlier experience. Once these schemas are activated every event and stimuli in the environment get coloured by these. Some have schemas of being painfully aware, especially grief, even after death; and that they can tempt fate by

thinking too positively. Individuals may see 'worrying' in a positive light and believe it will prevent negative events from happening or ward off danger and end up evoking more negative scenarios in their mind. These schemas once activated can in turn lead to misinterpretations of bodily symptoms, negative thoughts, and anxiety [15]. Individuals may pay undue attention to ever growing list of symptoms of COVID-19, become hyper-vigilant to internal and external body processes and any benign bodily sensation may be perceived as a symptom. Moreover, symptoms arising from stress, insomnia, withdrawal from alcohol, etc. may get ignored. The uncertainty surrounding the pathophysiology, incubation period, mode of infection, testing, and treatment of this novel illness is intolerable for such individuals [9, 16, 17]. Repeated worrying about how to not get infected and being more preoccupied with worries can reduce actual vigilance from the threat. Patients may even start doubting the competency of the doctor and endanger the fragile therapeutic relationship brought on by repeated reassurance seeking behaviour. Anxiety in turn leads to specific behaviours which are unhelpful and maintain the vicious cycle of health anxiety [15]. They may even repeatedly search internet for information, examine bodily fluids such as sputum and faeces; measure temperature, pulse rate and blood pressure; or go for repeated medical consultations. Some may develop avoidance behaviour and isolate themselves from family members, avoid revealing their symptoms to others, or even skip medical appointments fearing they might test positive for COVID-19 or be put in quarantine. This can lead to safety behaviours of self-medication which can even harm if not in appropriate dose [18].

Pre-existing mental illness, poor experience with healthcare systems in past, childhood sexual abuse, familial conflicts, marital discord, and other environmental factors can make an individual susceptible to develop these cognitive distortions. Certain personality factors such as perfectionism ("My body should not have any symptoms"), rigidity in thinking, neuroticism (predominant negative emotions), high harm avoidance (leading to unhelpful safety behaviours), and anxiety sensitivity (tendency to interpret anxiety symptoms as signals of catastrophic physical illness) may increase vulnerability of individuals [15].

Recommendations to address health anxiety and the 'worried well'

A fine balance is required between communicating public health response and stigma mitigation; and our experience in HIV has a lot to teach. Education and awareness campaigns targeting general public using all possible channels of communication can break the chain of misinformation. Stigma reduction strategies to reduce the clout of dread around individuals infected with the virus are need of the hour [19]. All healthcare workers especially in triage areas and fever clinics should be trained about health anxiety. Empathetic listening and attention to psycho-social factors may allay fears. People who are in quarantine should be given access to tele-counseling services and timely referral to psychiatrists in case of severe health anxiety or suicidal ideation. Self-care strategies in general public, relaxation techniques such as breathing and muscle relaxation exercises should be offered to all people in quarantine. Mindfulness meditation can help individuals to be more self-aware and accept their bodily sensations in a non-judgmental fashion. Cognitive behavioural therapy (CBT) is useful psychotherapeutic option in which individuals are encouraged to journal their behaviours of checking for bodily sensations, searching health related information on internet and reassurance seeking behaviours. They are also trained to challenge their thoughts related to their health and made to generate alternative thoughts. With increased penetration of the internet, even in rural areas, internet based CBT for Health Anxiety can be rolled out on an accelerated basis with significant cost benefits [20].

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

GK wrote the Cognitive behavioural model section, RK wrote introduction and edited the manuscript, MB gave suggestions for recommendations to manage health anxiety from public health perspective.

References

[1] WHO. Pneumonia of unknown cause - China [Internet]. WHO 2020 [cited 2020 May 24]. Available from: http://www.who.int/csr/don/05-january-2020-pneumonia-of-unkown-cause-china/en

[2] WHO Timeline - COVID-19. WHO 2020 [cited 2020 May 24]. Available from: https://www.who.int/news-room/detail/27-04-2020-who-timeline-covid-19

[3] WHO releases guidelines to help countries maintain essential health services during the COVID-19 pandemic [cited 2020 May 24]. Available from: https://www.who.int/news-room/detail/30-03-2020-who-releases-guidelines-to-help-countries-maintain-essential-health-services-during-the-covid-19-pandemic

[4] Darlenski R, Tsankov N. Covid-19 pandemic and the skin - What should dermatologists know? Clin Dermatol 2020 [cited 2020 May 24]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7102542 - https://doi.org/10.1016/j.clindermatol.2020.03.012

[5] Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, Chang J, Hong C, Zhou Y, Wang D, Miao X, Li Y, Hu B. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurol 2020;77:683-90 [cited 2020

May 24]. Available from: https://jamanetwork.com/journals/jamaneurology/fullarticle/2764549 - https://doi.org/10.1001/jamaneurol.2020.1127

[6] Zheng Y-Y, Ma Y-T, Zhang J-Y, Xie X. COVID-19 and the cardiovascular system. Nat Rev Cardiol 2020;17:259-60. https://doi.org/10.1038/s41569-020-0360-5

[7] Gu J, Han B, Wang J. COVID-19: gastrointestinal manifestations and potential fecal-oral transmission. Gastroenterology 2020;158:1518-9. https://doi.org/10.1053/j.gastro.2020.02.054

[8] Wu P, Duan F, Luo C, Liu Q, Qu X, Liang L, Wu K. Characteristics of ocular findings of patients with coronavirus disease 2019 (COVID-19) in Hubei Province, China. JAMA Ophthalmol 2020;138:575-8 [cited 2020 May 24]. Available from: https://jamanetwork.com/journals/jamaophthalmology/fullarticle/2764083. https://doi.org/10.1001/jamaophthalmol.2020.1291

[9] Sanders JM, Monogue ML, Jodlowski TZ, Cutrell JB. Pharmacologic treatments for coronavirus disease 2019 (COVID-19): a review. JAMA 2020;323:1824-36 [cited 2020 May 24]. Available from: https://jamanetwork.com/journals/jama/fullarticle/2764727 - https://doi.org/10.1001/jama.2020.6019

[10] Brooks SK, Webster RK, Smith LE, Woodland L, Wessely S, Greenberg N, Rubin GJ. The psychological impact of quarantine and how to reduce it: rapid review of the evidence. Lancet 2020;395:912-20. https://doi.org/10.1016/S0140-6736(20)30460-8

[11] Hua J, Shaw R. Corona Virus (COVID-19) "infodemic" and emerging issues through a data lens: the case of China. Int J Environ Res Public Health 2020;17(7). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7177854 - https://doi.org/10.3390/ijerph17072309

[12] Balanzá-Martínez V, Atienza-Carbonell B, Kapczinski F, Boni RBD. Lifestyle behaviours during the COVID-19 - time to connect. Acta Psychiatr Scand 2020;141:399-400. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/acps.13177 - https://doi.org/10.1111/acps.13177

[13] Shettar M, Karkal R, Kakunje A, Mendonsa RD, Chandran VM. Facebook addiction and loneliness in the post-graduate students of a university in southern India. Int J Soc Psychiatry 2017;63:325-9. https://doi.org/10.1177/0020764017705895

[14] Gao J, Zheng P, Jia Y, Chen H, Mao Y, Chen S, Wang Y, Fu H, Dai J. Mental health problems and social media exposure during COVID-19 outbreak. PLoS ONE 2020;15:e0231924. https://doi.org/10.1371/journal.pone.0231924

[15] Taylor S. Understanding and treating health anxiety: A cognitive-behavioral approach. Cogn Behav Pract 2004;11:112-23. https://doi.org/10.1016/S1077-7229(04)80015-4

[16] Cevik M, Bamford C, Ho A. COVID-19 pandemic - a focused review for clinicians. Clin Microbiol Infect 2020;26:842-7 [cited 2020 May 24]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7182753 - https://doi.org/10.1016/j.cmi.2020.04.023

[17] Li Y, Yao L, Li J, Chen L, Song Y, Cai Z, Yang C. Stability issues of RT-PCR testing of SARS-CoV-2 for hospitalized patients clinically diagnosed with COVID-19 [published online ahead of print, 2020 Mar 26]. J Med Virol 2020;92:903-8. https://doi.org/10.1002/jmv.25786. https://doi.org/10.1002/jmv.25786

[18] Wong YK, Yang J, He Y. Caution and clarity required in the use of chloroquine for COVID-19. Lancet Rheumatol 2020;2:e255. https://doi.org/10.1016/S2665-9913(20)30093-X

[19] Sathyanath MS, Sathyanath S. Stigma reduction and provision of mental health services in the public health response to COVID-19. Indian J Community Health 2020;32:269-72.

[20] Hedman E, Andersson G, Andersson E, Ljótsson B, Rück C, Asmundson GJG, Lindefors N. Internet-based cognitive–behavioural therapy for severe health anxiety: randomised controlled trial. Br J Psychiatry 2011;198:230-6. https://doi.org/10.1192/bjp.bp.110.086843

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Correspondence: Ravichandra Karkal, Department of Psychiatry, Yenepoya Medical College, Deralakatte, Mangaluru - Tel.: +91 7259459470 - E-mail: minddocravi@gmail.com

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Overview

The seedbeds of Tuberculosis: is it time to target congregate settings and workplaces?

ALFREDO MONTECUCCO[1,2], GUGLIELMO DINI[1,2], ALBORZ RAHMANI[1,2], LAURA STICCHI[1,2], PAOLO DURANDO[1,2]
[1] Department of Health Sciences (DISSAL), University of Genoa, Italy; [2] IRCCS Polyclinic San Martino Hospital, Genoa, Italy

Keywords

Tuberculosis • TB • Latent tuberculosis infection • LTBI • Occupational medicine • Public health • Congregate settings and workplaces • Elimination

Summary

Countries where the incidence of Tuberculosis (TB) is low display a low transmission rate in the general population, and this rate has progressively declined in recent decades; however, TB epidemiology has shown a shift of the disease burden from the general population to specific populations at higher risk, such as vulnerable individuals and hard-to-reach groups. In low-incidence countries, preventive and therapeutic strategies must therefore be geared towards targeted interventions in these populations, with the priority being to promptly identify and treat latent tuberculosis infection (LTBI) rather than manage infectious cases. One of the most complex challenges in this area is to identify population subgroups with increased incidence/prevalence of LTBI/TB.

The aim of this study was to provide a concise overview of the main studies and available evidence concerning the epidemiology of TB and LTBI in non-healthcare congregate settings, with specific emphasis on studies conducted in occupational settings and workplaces.

Recognizing settings at increased risk might contribute to eliminating TB in low-incidence countries, a challenge which requires tailored responses.

Occupational and preventive medicine has a major role to play in directing ad hoc policies and programs of LTBI surveillance. If TB is to be eradicated, it is essential to contain the seedbeds of infection: indeed, as long as a large reservoir of infected subjects exists, new active TB cases may arise at any time.

Background

Tuberculosis (TB) remains the leading cause of death due to an infectious disease among adults worldwide. Currently, tuberculosis causes more than 10 million cases globally, resulting in approximately 1.5 million deaths each year [1]. The global distribution of the disease is widely heterogeneous. The lowest rates are mostly registered in high-income countries, including most Western European countries, Canada, the United States of America, Australia and New Zealand.

The epidemiology of TB in low-incidence countries (< 10 cases per 100,000 inhabitants per year) is characterized by a low transmission rate in the population at large, and this has progressively declined over recent decades.

Europe's TB burden is among the lowest in the world, and overall notifications in most countries have been decreasing over the last five years [2].

Many efforts have been made in recent years to eradicate TB, particularly through the detection and active management of TB cases. This strategy alone, however, is not enough to eliminate TB. In order to successfully break the chain of infection and disease, an integrated strategy [3, 4] that includes LTBI management is required.

In addition, it is estimated that approximately 1.7 billion people globally (a fourth of the world's population) are infected with *Mycobacterium tuberculosis* (MT) [5].

In this regard, as most new TB cases are the result of reactivation of LTBI rather than a recent primary infection, the enhancement of LTBI screening and treatment strategies is well recognized as a key driver of TB elimination, especially in areas of low TB prevalence.

The incidence of active cases is concentrated among vulnerable groups, hard-to-reach populations and cross-border migrants. One of the most complex challenges in this area is to identify population subgroups with a high incidence/prevalence of TB. Today, prioritized strategies in low-incidence countries must therefore be geared towards targeted interventions in these populations, with the aim of identifying and treating infections promptly rather than managing cases of TB disease.

It is well known that the transmission of MT is more likely in confined environments where population density is high, such as healthcare facilities, shelters for the homeless, long-term care facilities, and community settings such as schools and workplaces. The concentration of active TB cases in congregate settings engenders a greater risk of transmission of MT among those who frequent these settings. The presence of vulnerable populations in high congregate settings might constitute one of the worst scenarios, especially in non-healthcare settings. As defined by the CDC, a congregate setting is an environment where a number of people reside, meet or gather in close proximity for either a limited or extended period of time [6]. The aim of the present study was to provide an overview of the literature on the epidemiol-

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ogy of TB and LTBI in non-healthcare congregate settings, with specific emphasis on studies conducted in occupational settings or studies comparing the prevalence/incidence rates of LTBI/TB in such settings with those recorded in the general population.

Methods

An analysis of the literature by means of a method that simplifies the components of a systematic search [7] formed the basis of the rapid overview of evidence presented in this study. Medline and Embase electronic databases were searched for articles published between January 2000 and December 2019 that reported epidemiological data on TB/LTBI in congregate settings. The search was restricted to countries and territories with a low incidence of TB, as per the latest WHO data [1], and to systematic reviews and meta-analyses of observational studies. The language was restricted to English and Italian. Our search contained the following terms: tuberculosis, LTBI, prison, congregate and occupational setting (tuberculosis OR TB OR latent tuberculosis infection OR LTBI) AND ((congregate OR highly populated) OR (prisons OR correctional OR shelters)) OR (workplace OR occupational). Approval from the Ethics Committee was not required.

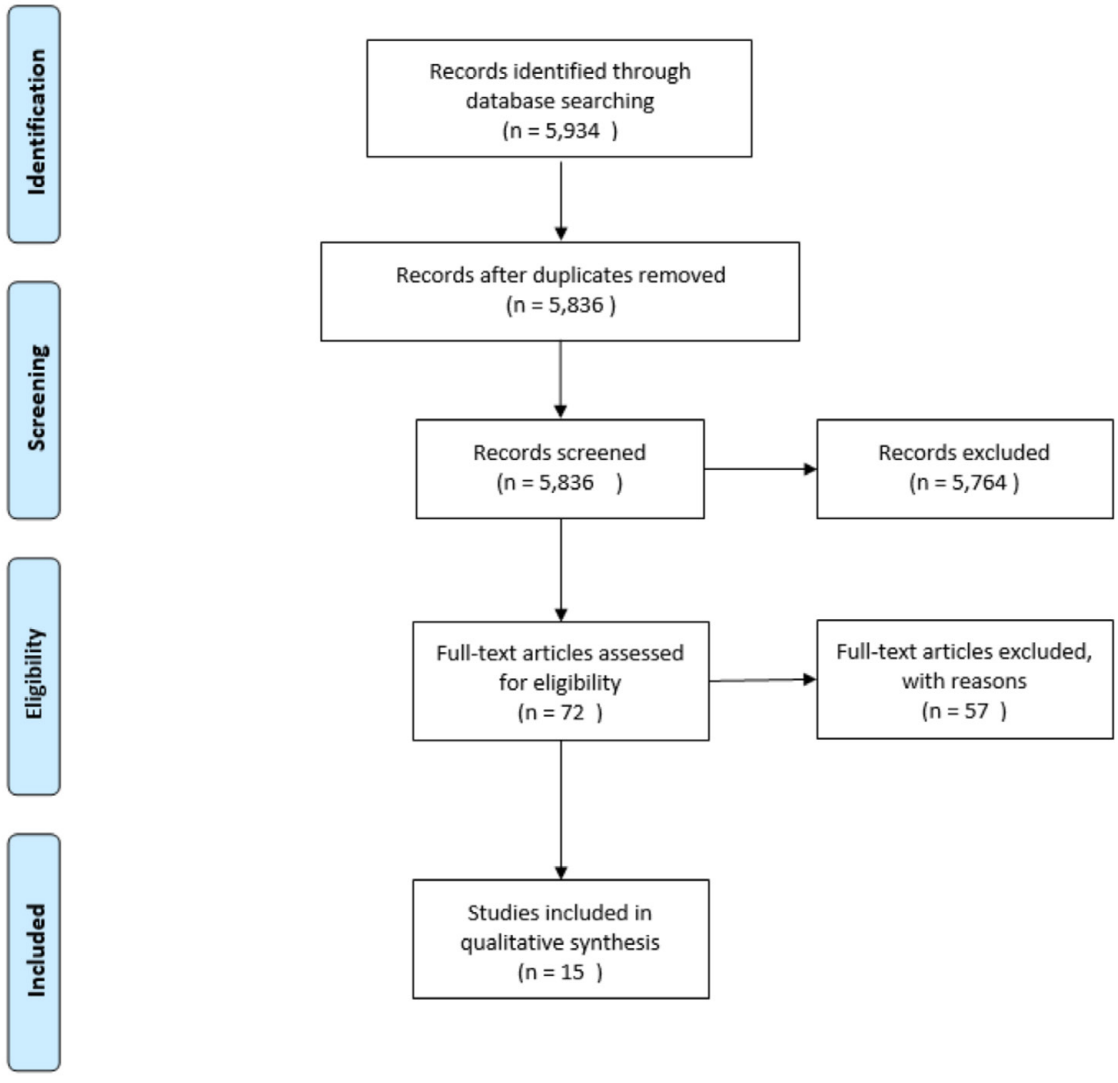
Results

A total of 5,934 citations were screened. Subsequently, 15 articles [8-22] fulfilled the eligibility criteria and were included (Fig. 1).

Summary of studies included

A briefly summary of the studies included is reported in Table I.

Fig. 1. PRISMA 2009 flow chart of the literature search.



Tab. I. Characteristics of studies included

	Setting	Study population	LTBI epidemiology	TB epidemiology	Determinants	Strategies to pursue
Baussano et al. [8]	Correctional facilities	N=31,336 inmates	Incidence 26.4 (IQR: 13.0-61.8)	Incidence 23.0 (IQR: 11.7-36.1)	Inadequate nutrition, HIV prevalence, overcrowding	Education on early identification of TB and early case management, screening of inmates on arrival, isolation of TB cases are potentially effective measures
Kawatsu et al. [9]	Correctional facilities	NA	Average prevalence 40.3%	NA	Duration of incarceration, history of previous incarcerations were identified as risk factors for high LTBI prevalence	NA
Moreira et al. [10]	Correctional facilities	NA	NA	Prevalence <1%	Transmission within the prison	Education on early identification of TB, early case management and appropriate treatment, screening of inmates on arrival, isolation of cases with positive smears
Lambert et al. [11]	Correctional facilities	n = 5878 correctional employees and inmates	NA	From 8 to 29 every 100,000	Local jails, recent arrival from non-low-incidence country	Systematic screening and treatment of LTBI and TB among correctional employees and inmates remain essential to TB prevention and control
Binswanger et al. [12]	Correctional facilities	n=81,610 correctional officers	5.5% (range 3.8-8.3%) of correctional facilities monitored reported at least one episode of cuti-conversion among employees	NA	NA	NA
Grenzel et al. [13]	Correctional facilities	n=110.393 correctional facility workers	16% ([95% IC 10-22%], I2=93,3%, p<0,001)	Incidence of active TB ranged from 0.61 to 450/10,000	LTBI-associated risk factors included duration of employment, older age, country of birth, current tobacco smoking, reported contact with prisoners, and BCG vaccination.	Systematic surveillance and infection control measures are necessary to protect these highly vulnerable workers. Need for infection control measures in such high-risk settings
Kunst et al. [14]	Reception centres for asylum seekers - Europe	Migrants and asylum seekers	Prevalence of TST positivity ranged between 27.8% and 44.9%, IGRA positivity ranged between 17.4% and 29%.	Incidence ranged between 26 and 671 TB cases every 100,000	Country of birth, the reason for migration (e.g., asylum seekers), date of entry into the host country, factors favouring progression from LTBI to active TB	Recommend harmonising case definitions, reporting standards and policies for TB/LTBI screening.
Lönnroth et al. [15]	Reception centres for asylum seekers - Europe	Migrants and asylum seekers	LTBI prevalence, with a pooled positivity of 45% on TST and 24% on IGRA.	NA	Overcrowded settings, in both the country of origin and of destination, together with factors such as malnutrition, exposure to infectious cases and increased incidence of HIV.	Integrated strategies of early diagnosis and treatment, together with active inclusion in the social and cultural fabric

continues

follows

Tab. I. Characteristics of studies included

	Setting	Study population	LTBI epidemiology	TB epidemiology	Determinants	Strategies to pursue
Bozorgmehr et al. [16]	Reception centres for asylum seekers - Germany	N=89,294 asylum seekers	NA	3,47 (95% CI: 1.78-5.73; I2 = 94.9%; p <0.0001) every 1,000	Country of origin, post-migration factors such as duration of stay in host country	Establish factors during migration and initial accommodation which may lead to higher transmission rates or re-activation of LTBI
Scotto et al. [17]	Reception centres for asylum seekers - Italy	NA	Prevalence of positivity to the TST varied between 30 and50%, of these, IGRA test positivity ranged from 26.5% to 29.6%	Immigrants accounted for 66% of new TB cases occurring in Italy	Country of birth, poor living conditions, poor nutrition	Emphasis on social protection and poverty-alleviation programmes
Bamrah et al. [18]	Homeless sheltlers - USA	N=270,948 TB cases among homeless	NA	Incidence ranged from 36 to 47 every 100,000	Difficulties regarding access to medical care, duration of contagiousness, delayed diagnosis	Identification and treatment of homeless persons with LTBI
Nava-Aguilera et al. [19]	Homeless sheltlers	NA	NA	NA	Belonging to an ethnic minority, being a native of the country, residing in an urban area, drug use, excessive alcohol consumption, previous incarceration, HIV, young age, male gender	Improvement of prevention and control strategies
Isler et al. [20]	Homeless sheltlers - Canada	N = 841	Prevalence of 12.9%. The incidence of cuti-conversion ranged from 2.3 to 3.5 per 100 people per year.	NA	Neither demographics nor workplace character-istics were associated with the incidence of con-version	Improvement of TST screening and medical surveillance of shelter workers in a low-incidence setting
Grenfell et al. [21]	Drug rehabilitation communities – Europe & North America	NA	Prevalence ranged from 17% to 52% in Europe; from 12% to 39 in US	Prevalence ranged from 0,5% to 66% with broad heterogeneity among studies	Male gender, long periods of injected substance abuse, HIV-negative status and TCD4+ lymphocyte values within normal range	Improve surveillance of TB and co-infections among people who inject drugs
Deiss et al. [22]	Drug rehabilitation communities	NA	Prevalence ranged from 10% to 59%.	NA	Age and duration of drug abuse, homelessness, alcohol abuse and history of detention	Prompt identification of LTBI, successful treatment of LTBI and TB disease

NA, not applicable

Correctional facilities

Baussano and colleagues [8] conducted a systematic review to investigate the incidence and risk of LTBI and TB in prisons in various countries in comparison with the general population. The review included 23 original studies from the 1990s to 2010. Five studies from the US and one from Brazil assessed the incidence of LTBI in penitentiaries; n = 19 investigated the incidence of TB (n = 13 in low-incidence settings); n = 2 studies investigated both the impact of infection and the disease. The estimated average annual incidence rate ratio (IRR) for LTBI was 26.4 (interquartile range [IQR]: 13.0-61.8); the IRR for TB was 23.0 (IQR: 11.7-36.1). The estimated median fraction (PAF%) of TB in the general population attributable to exposure in prison settings was 8.5% (IQR: 1.9% -17.9%) and 6.3% (IQR: 2.7% -17.2%) in low-/middle-income countries.

Kawatsu and colleagues [9] carried out a systematic review to investigate the incidence of LTBI among prison

inmates. They reported an LTBI incidence of 40.3% in countries with a low incidence of TB and 73.0% in countries with a medium/high incidence. The incidence of LTBI ranged from 5.9 to 6.3 per 100 inmates in countries with a low incidence of TB, while it was 61.8 per 100 inmates in countries with a high incidence.

Moreira and colleagues [10] conducted a systematic meta-analysis review that investigated the prevalence of TB among prison inmates between 1997 and 2016. Their study included n = 29 original articles regarding 2163 cases of TB among inmates. The combined prevalence of TB among inmates was 2%. The prevalence among detainees from countries with a TB incidence between 0 and 24 cases per 100,000 inhabitants was less than 1%. In countries with a TB prevalence of 25-99/100,000, the prevalence of TB among prisoners was reported to be 3%; in countries with an incidence ≥ 300/100,000 inhabitant, the prevalence was reported to be 8%.

A study conducted in the USA by Lambert et al. [11] found 299 cases of active TB among prison employees in 35 US states; 49% of the diagnoses were made at the onset of clinical symptoms, 31% of the diagnoses were incidental, 11% were made through occupational health surveillance, and 9% were made through contact investigations of contagious cases.

The study by Binswanger et al. [12] was conducted in the USA on a population of 81,610 prison employees. Of the correctional facilities included in the study, 5.5% reported at least one episode of cuti-conversion to TST among employees.

The systematic meta-analysis review by Grenzel et al. [13] reported a prevalence of LTBI among prison workers in low-incidence countries of 16% ([95% CI 10-22%], I2 = 93.3%, p < 0.001). The incidence of active TB in low-burden countries ranged from 0.61 to 450 every 10,000.

Reception centres for asylum seekers

Kunst and colleagues [14] conducted a systematic review of 46 studies to investigate the prevalence of LTBI and TB among migrants in the European context. The median yield of reported cases at the reception centres was 431 cases per 100,000 assessed. N = 20 studies that investigated screening for LTBI were included. Positivity (≥ 10 mm of intradermal hardening) to TST (interquartile range) ranged between 27.8% and 44.9%, while Interferon Gamma Release Assay test (IGRA) positivity was found to be between 17.4% and 29%.

Lönnroth and colleagues [15] conducted a systematic review to investigate the prevalence of TB among migrants in low-incidence countries. The authors reported that the absolute number of TB notifications in subjects born abroad increased in 14 of the 30 low-incidence countries in the period 2009-2015. They found that asylum seekers and refugees were at increased risk of TB, owing to the difficulties faced during migration, overcrowded conditions in both the countries of origin and destination, and factors such as malnutrition, exposure to infectious cases and a higher incidence of HIV in these individuals.

Bozorgmehr and colleagues [16] investigated the screening data on asylum seekers in Germany from 1995 to 2015 through a systematic meta-analysis. They reported that the prevalence of TB among asylum seekers was between 0.72 (95% CI: 0.45-1.10) and 6.41 (95% CI: 4.19-9.37) per 1,000 subjects. The aggregate estimated prevalence of active TB cases in the studies included was 3.47 (95% CI: 1.78-5.73; I2 = 94.9%; p < 0.0001) per 1,000 asylum seekers.

In 2017, Scotto and colleagues [17] conducted a study on the incidence of TB among migrants in Italy in the period between 2000 and 2016. They reported that, in 2014, 66% of new cases of TB were recorded in the migrant population.

Homeless shelters

Bamrah and colleagues [18] conducted a study to analyse cases of active TB reported in the United States between 1994 and 2010. Overall, 270,948 active TB cases were reported in this time window, 16,527 (16.4%) of which occurred among homeless people.

A systematic review by Nava-Aguilera and colleagues [19] revealed that recent transmission of TB was concentrated in some vulnerable population groups, including: ethnic minorities (OR 3.03, 95% CI: 2.21-4.16); urban residents (OR 1.52, 95% CI: 1.35-1.72); drug abusers (OR 3.01, 95% CI: 2.14-4.22); alcohol abusers (OR 2.27, 95% CI 1.69-3.06); homeless persons (OR 2.87, 95% CI: 2.04-4.02); former prison inmates (OR 2.21, 95% CI: 1.71-2.86); HIV-infected subjects (OR 1.66, 95% CI: 1.36-2.05); and the young (OR 2.09, 95% CI: 1.69-2.59).

Isler and colleagues [20] investigated the results of an LTBI screening program for employees of homeless shelters in the Montreal metropolitan area between 1998 and 2005. Both subjects who were cuti-positive (10 mm cut-off) at the start of the study and cuti-converted subjects (10 mm cut-off) were included. The prevalence of subjects with TST positivity at the start of the study was 12.9%. The incidence of cuti-conversion ranged from 2.3/100 person-years to 3.5/100 person-years. The incidence of cuti-conversion was not significantly associated with demographic or occupational items such as the type of employment.

Drug rehabilitation communities

A review by Grenfell and colleagues [21] investigated the prevalence of LTBI and TB in intravenous drug users. In studies conducted in Europe, the prevalence of LTBI ranged from 17% to 52%, with a higher incidence in prison settings. In North America, intravenous drug users had an LTBI prevalence between 12% and 39%. The prevalence of active TB ranged from 0.5% to 66%; this wide heterogeneity among studies was due to differences in the methods of detection and definition of active TB.

Deiss and colleagues [22] also conducted a review investigating the prevalence of LTBI and TB in intravenous drug users. They reported that this vulnerable population was at increased risk of both LTBI and TB, with

an observed prevalence of LTBI between 10% and 59%. Prolonged intravenous drug abuse and age proved to be associated with a higher prevalence of LTBI.

Brief final remarks

The studies mentioned, which were performed in countries with a low incidence of TB, provide a brief summary of the epidemiology of LTBI and TB in congregate settings and workplaces.

Adequate knowledge of up-to-date epidemiological data is a crucial first step in the risk assessment of occupational settings and a useful means of identifying reservoirs of TB infection at the community level, thereby contributing to the mapping of risk factors and the identification of groups at risk.

Furthermore, such environments (e.g. prisons), are frequently characterized by poor hygiene conditions, poor ventilation and a high prevalence of subjects at higher risk of active TB (e.g., drug abusers, alcoholics, immigrants from high-incidence areas for TB, individuals with HIV/AIDS, individuals with reduced access to care services). A lack of Infection and Prevention Controls (IPC) (e.g., administrative and environmental controls) in these settings may help to explain the increased risk of transmission of MT.

The heterogeneity of work environments makes it more difficult to assess the risk of LTBI, which is influenced by age and demographic structure, cultural factors, population density and migration patterns. Environmental factors, such as overcrowding and poor ventilation, have a direct impact on air exchange from person to person, which increases the likelihood of transmission.

Recognizing risk factors and settings at increased risk might contribute to eliminating tuberculosis in low-incidence countries, a challenge which requires tailored responses. As LTBI is most often a prerequisite for the development of TB in immunocompetent individuals, it appears essential to identify, as early as possible, those who have been infected after proven or suspected exposure to a case of contagious TB (pulmonary or laryngeal), in order to properly assess the risk of developing active TB and to implement preventive measures. Containment of the seedbeds of TB is essential in order to reach the goal of eliminating the disease: indeed, as long as a large reservoir of infected subjects remains, new active TB cases may arise at any time. Given the estimated prevalence of LTBI and the current shortage of tests and treatments, a further major effort is required. This effort should include: a surveillance system, scaling up targeted testing for LTBI in populations at risk, expanding short-term treatment regimens, involving both affected communities and medical service providers, and increasing the healthcare personnel involved in implementation and supervision. Such efforts would greatly benefit from the development of new tools, such as tests that more accurately assess the risk of reactivation as well as shorter LTBI treatment [23, 24].

In order to direct these efforts and make targeted choices, an essential first step is to identify subjects with the highest risk of exposure, who should be targeted for LTBI testing; occupational medicine could play a key role in this activity. Strategies for risk assessment are based on: (I) the workplace environment (e.g. local epidemiological and environmental features); (II) specific care activities performed; (III) risk factors for the increased likelihood of progression from LTBI to active disease. These key principles stress the role of occupational and preventive medicine in directing tailored policies for LTBI surveillance. Moreover, Occupational Health Surveillance programs, in close collaboration with the Departments of Prevention of the Local Health Authorities, could aim to reduce losses at steps of LTBI cascade of care, thereby enhancing the public health impact of proper diagnosis and treatment and contributing to achieving major results in terms of Public health.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

AM conceived and designed the study and the search strategy. PD reviewed and approved the search strategy. AM and GD searched the literature, selected the studies and analyzed the data. PD and LS controlled the review process. AM and GD drafted the initial manuscript. PD, LS, and AR reviewed and edited the manuscript. All authors read and approved the final manuscript.

References

[1] World Health Organization. Global tuberculosis report 2019. Geneva, World Health Organization 2019.

[2] European Centre for Disease Prevention and Control/WHO Regional Office for Europe. Tuberculosis surveillance and monitoring in Europe 2020-2018 data. Stockholm: ECDC; 2020.

[3] Lo Bue PA, Mermin JH: Latent tuberculosis infection: the final frontier of tuberculosis elimination in the USA. Lancet Infect Dis 2017;17(10):e327-e333. https://doi.org/10.1016/S1473-3099(17)30248-7

[4] Matteelli A, Rendon A, Tiberi S, Seif Al-Abri, Constantia Voniatis, Anna Cristina C. Carvalho, Rosella Centis, Lia D'Ambrosio, Dina Visca, Antonio Spanevello, Giovanni Battista Migliori. Tuberculosis elimination: where are we now? Eur Respir Rev 2018;27(148):180035. https://doi.org/10.1183/16000617.0035-2018

[5] Cohen A, Mathiasen VD, Schön T, Wejse C. The global prevalence of latent tuberculosis: a systematic review and meta-

analysis. Eur Respir J 2019;54(3):1900655. https://doi.org/10.1183/13993003.00655-2019

[6] Centers for Disease Control and Prevention (CD). Community Congregate Settings, Dec, 2018 Available at: https://www.cdc.gov/eis/field-epi-manual/chapters/community-settings.html (accessed on the 02-08-2020)

[7] Tricco AC, Antony J, Zarin W, Strifler L, Ghassemi M, Ivory J, Perrier L, Hutton B, Moher D, Straus SE. A scoping review of rapid review methods. BMC Med 2015;13:224. https://doi.org/10.1186/s12916-015-0465-6

[8] Baussano I, Williams BG, Nunn P, Beggiato M, Fedeli U, Scano F. Tuberculosis incidence in prisons: a systematic review. PLoS Med 2010;7(12):e1000381. https://doi.org/10.1371/journal.pmed.1000381

[9] Kawatsu L, Uchimura K, Izumi K, Ohkado A. [a systematic review on the prevalence and incidence of latent tuberculosis infection among prison population]. Kekkaku 2016;91:457-64.

[10] Moreira TR, Lemos AC, Colodette RM, Gomes AP, Batista RS. Prevalência de tuberculose na população privada de liberdade: revisão sistemática e metanálise [Prevalence of tuberculosis in incarcerated populations: systematic review and meta-analysis-Prevalencia de tuberculosis en la población privada de libertad: revisión sistemática y metanálisis]. Rev Panam Salud Publica 2019;43:e16. Portuguese. doi: 10.26633/RPSP.2019.16

[11] Lambert LA, Armstrong LR, Lobato MN, Ho C, France AM, Haddad MB. Tuberculosis in jails and prisons: United States, 2002-2013. Am J Public Health 2016;106:2231-7. https://doi.org/10.2105/AJPH.2016.303423

[12] Binswanger IA, O'Brien K, Benton K, Gardner EM, Hirsh JM, Felton S, Belknap R. Tuberculosis testing in correctional officers: a national random survey of jails in the United States. Int J Tuberc Lung Dis 2010;14:464-70.

[13] Grenzel ML, Grande AJ, Paniago AMM, Pompilio MA, de Oliveira SMVL, Trajman A. Tuberculosis among correctional facility workers: a systematic review and meta-analysis. PLoS One 2018;13:e0207400. https://doi.org/10.1371/journal.pone.0207400

[14] Kunst H, Burman M, Arnesen TM, Fiebig L, Hergens MP, Kalkouni O, Klinkenberg E, Orcau À, Soini H, Sotgiu G, Zenner D, de Vries G. Tuberculosis and latent tuberculous infection screening of migrants in Europe: comparative analysis of policies, surveillance systems and results. Int J Tuberc Lung Dis 2017;21(8):840-51.https://doi.org/10.5588/ijtld.17.0036.

[15] Lönnroth K, Mor Z, Erkens C, Bruchfeld J, Nathavitharana RR, van der Werf MJ, Lange C. Tuberculosis in migrants in low-incidence countries: epidemiology and intervention entry points. Int J Tuberc Lung Dis 2017;21(6):624-37. https://doi.org/10.5588/ijtld.16.0845.

[16] Bozorgmehr K, Razum O, Saure D, Joggerst B, Szecsenyi J, Stock C. Yield of active screening for tuberculosis among asylum seekers in Germany: a systematic review and meta-analysis. Euro Surveill 2017;22:30491. https://doi.org/10.2807/1560-7917.ES.2017.22.12.30491.

[17] Scotto G, Fazio V, Lo Muzio L. Tuberculosis in the immigrant population in Italy: state-of-the-art review. Infez Med. 2017 Sep 1.

[18] Bamrah S, Yelk Woodruff RS, Powell K, Ghosh S, Kammerer JS, Haddad MB. Tuberculosis among the homeless, United States, 1994-2010. Int J Tuberc Lung Dis 2013;17(11):1414-9. https://doi.org/10.5588/ijtld.13.0270

[19] Nava-Aguilera E, Andersson N, Harris E, Mitchell S, Hamel C, Shea B, López-Vidal Y, Villegas-Arrizón A, Morales-Pérez A. Risk factors associated with recent transmission of tuberculosis: systematic review and meta-analysis. Int J Tuberc Lung Dis 2009;13:17-26.

[20] Isler MA, Rivest P, Mason J, Brassard P. Screening employees of services for homeless individuals in Montréal for tuberculosis infection. J Infect Public Health 2013;6:209-15. https://doi.org/10.1016/j.jiph.2012.11.010.

[21] Grenfell P, Baptista Leite R, Garfein R, de Lussigny S, Platt L, Rhodes T. Tuberculosis, injecting drug use and integrated HIV-TB care: a review of the literature. Drug Alcohol Depend 2013;129(3):180-209. https://doi.org/10.1016/j.drugalcdep.2012.11.013

[22] Deiss RG, Rodwell TC, Garfein RS. Tuberculosis and illicit drug use: review and update. Clin Infect Dis 2009;48(1):72-82. https://doi.org/10.1086/594126

[23] Drain PK, Bajema KL, Dowdy D, Dheda K, Naidoo K, Schumacher SG, Ma S, Meermeier E, Lewinsohn DM, Sherman DR. Incipient and Subclinical Tuberculosis: a Clinical Review of Early Stages and Progression of Infection. Clin Microbiol Rev 2018;31(4):e00021-18. https://doi.org/10.1128/CMR.00021-18

[24] Zellweger JP, Sotgiu G, Corradi M, Durando P. The diagnosis of latent tuberculosis infection (LTBI): currently available tests, future developments, and perspectives to eliminate tuberculosis (TB). Med Lav 2020;111(3):170-83. https://doi.org/10.23749/mdl.v111i3.9983

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Correspondence: Alfredo Montecucco, Department of Health Sciences (DISSAL), University of Genoa and IRCCS Polyclinic of San Martino Hospital, Genoa, Italy - E-mail: alfredo.montecucco@edu.unige.it

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Review

Do statins play any role in reducing the incidence and mortality of ovarian cancer? A systematic review and meta-analysis

ABDOLLAH MOHAMMADIAN-HAFSHEJANI[1,2], CATHERINE M.T. SHERWIN[2], SAEID HEIDARI-SOURESHJANI[3]
[1] Department of Epidemiology and Biostatistics, School of Public Health, Shahrekord University of Medical Sciences, Shahrekord, Iran; [2] Pediatric Clinical Pharmacology, Department of Pediatrics, Wright State University Boonshoft School of Medicine, Dayton Children's Hospital, One Children's Plaza, Dayton, Ohio, USA; [3] Shahrekord University of Medical Sciences, Shahrekord, Iran

Keywords

Statin • Ovarian cancer • Incidence • Mortality

Summary

Introduction. *This systematic review and meta-analysis aimed to investigate the relationship between statin consumption and risk of incidence of ovarian cancer (OC) and associated mortality.*

Methods. *Computerized searches were conducted in three electronic databases (PubMed, Web of Science, and Scopus). Two calibrated authors performed the publications selection, data extraction, and quality assessment of the selected publications. The quality of the included articles was evaluated using the Newcastle-Ottawa Scale (NOS) for observational studies, and Jadad criteria for randomized clinical trials (RCTs). The electronic searches retrieved 2272 titles/abstracts. After the deletion of duplicate publications, 2030 titles/abstracts were assessed. Eighteen articles were included.*

Results. *Meta-analysis demonstrated that risk ratio (RR) of the association between statin consumption and OC incidence was 0.88 (95% CI = 0.75-1.03, P = 0.109). Patients receiving statin were less likely to die than those who did not receive statin, with a statistically significant association [RR = 0.76 (95% CI 0.67-0.86, P = 0.0001)]. There was no evidence of publication bias in examining the association between statin consumption and the risk of incidence and mortality from OC.*

Conclusions. *This study determined that statin use reduced the incidence risk of OC and significantly increased the survival in OC patients.*

Introduction

Ovarian cancer (OC) and its predominant pathological subtype, referred to as epithelial OC, are considered the seventh leading cancer among women worldwide [1]. The incidence of OC was reportedly 24469 cases, of whom 14,008 cases die due to the disease, in the USA (USA) in 2018 [2]. OC imposes a high direct and indirect economic burden on the healthcare system and society [3].

Several risk factors such as genetic predisposition, older age at menopause, breast cancer, hormone replacement therapy (HRT), and environmental and lifestyle factors such as pollutant exposure and smoking are associated with increased incidence of OC [4-6]. Current advances in prevention strategies and molecular mechanisms are being utilized to improve women's health outcomes and quality of life [7].

Several choices of treatment and prevention have so far been investigated to reduce OC in women. Hydroxymethylglutaryl-coenzyme A reductase inhibitor, which is commonly called statin and is a cholesterol-lowering drug, is one of the promising drugs for OC [8]. This drug is widely used to treat and prevent hypercholesterolemia and to reduce the risk of coronary heart disease [9, 10].

Recent studies have reported that statin could reduce the risk of OC incidence [11, 12] and also its post-diagnosis use can improve the survival of OC patients [11, 13-15]. In contrast, some studies have not reported any strong evidence on the protective effect of statins in reducing the risk of incidence [16-18] and mortality from OC [19-21].

Although the protective effects of statins against the onset of certain types of cancers have been proven, contradictory results have been reported by studies on the effects of statins on the incidence and mortality of OC, so that some investigations have considered statins as the protective agents [11, 13, 15], as risk factors [17, 22] and neutral [18, 21, 23]. There is not any definitive conclusion on the effects of Statins on the risk of incidence and mortality of OC. Undoubtedly, the results of meta-analysis studies can be helpful in this regard. Therefore, this meta-analysis aimed to investigate the relationship between statin consumption and risk of OC to correlate the current reports on this association.

Methods

Data sources and search strategy

This meta-analysis was performed in accordance with PRISMA guideline (http://www.prisma-statement.org). An extensive systematic review was done on 15 July 2019

in PubMed, Web of Science (ISI) and Scopus databases. The main and MeSH keywords below were used to conduct the search: ((statin*) or (("hydroxymethylglutaryl-CoA reductase") or ("HMGCoA reductase") and (inhibitor*)) or (anticholesteremic) or (simvastatin) or (rosuvastatin) or (pravastatin) or (atorvastatin) or (fluvastatin) or (cerivastatin) or (pitavastatin) or (lovastatin)) and (ovary) or (ovarian)) and (cancer) or (neoplasms) or (carcinoma) or (tumor) or (malignancy). In addition, manual searches and a search in Google Scholar, up to the first 100 hits, were also conducted. Reference lists of similar studies were explored to find more relevant publications that might not be retrieved by manual searching [24, 25].

Study selection

The studies were entered in the *EndNote X8* (released 8 November 2016, Thomson Reuters), and duplicate publications were identified and deleted by the software. Two researchers independently evaluated the titles and abstracts of the studies based on the predetermined inclusion and exclusion criteria. The full texts of all studies that passed this stage were independently reviewed. If any disagreements existed, consensus was achieved by discussion with the third team member. Studies of various types such as cross-sectional, cohort, case-control, and clinical trials were included in the meta-analysis. The included studies addressed the association between statin consumption and the incidence and mortality from OC. Risk Ratio (RR), Odds Ratio (OR) and Hazard Ratio (HR) of the relationship between statin consumption and the incidence or mortality from OC was given a 95% confidence interval (CI) if it had been presented in the article or was calculable based on the information presented in the publication.

Data extraction and quality assessment

Data were extracted by two individuals independently, and potential inconsistencies were resolved through discussion. From the included articles, the following information was drawn: First author's name, year of publication, country where the study was done, sample size, duration of follow-up, and odds ratio (OR) or risk ratio (RR) of incidence and Hazard Ratio (HR) of mortality from OC with 95% confidence interval (CI). For the quality assessment scale, Newcastle-Ottawa Scale (NOS) was used for observational studies, and Jadad criteria was used to assess the quality of the randomized clinical trials (RCTs). Using NOS, we assessed the studies as at extremely high risk of bias (0 to 3 NOS), high risk of bias (4 to 6), and low risk of bias (7 to 9) [26] and for RCTs the score between of 0 (very poor) and for RCTs, the scores of 0 (extremely poor) and 3 or higher (high quality) were considered to investigate their quality [27].

Statistical analysis

In this meta-analysis, we used the RR to estimate the risk of incidence and morbidity of OC. The effect size of the relationship between statin consumption and incidence and mortality from OC were reported by RR with 95% confidence interval (CI), and a two-tailed $P < 0.05$ was considered significance level. Overall summary estimates were calculated using the inverse variance-weighted random-effects meta-analysis. Individual HR and summary estimates were illustrated graphically as forest plots. Heterogeneity among studies was tested by Cochran's Q test (reported with a χ^2 value and P value, with $P < 0.1$ considered as significance level) and the I^2 statistics [28]. Twelve statistics with values of 25%, 50%, and 75% demonstrated low, moderate, and high levels of heterogeneity, respectively [29].

Based on a priori decisions, subgroup analyses were conducted according to the geographical location (Europe, America, and Asia), study quality (low *vs* high risk), sample size for incidence of OC (≥ 100,000 *vs* < 100,000) and mortality from OC (≥ 1,000 *vs* < 1,000), and type of study (case-control, Cohort and RCTs).

A series of sensitivity analyses were performed to more clearly reveal the sources of statistical heterogeneity between studies, as well as to evaluate the robustness of the findings. First, we aimed to examine the effect of individual studies on the summary estimates, and therefore influence analyses were conducted, in which the pooled estimates are recalculated by omitting one study at a time. Secondly, a meta-regression analysis was conducted to assess differences between subgroups. Publication bias was assessed using Begg's and Egger's tests [30, 31]. $P < 0.05$ was considered significance level. All statistical analyses were performed using Stata 12.0 software (Stata LLC, College Station, TX, USA).

Results

Search results, study characteristics of selected studies

A flowchart of the search strategy is illustrated in Figure 1. In the electronic searches, a total of 2,272 titles/abstracts were retrieved. After deletion of duplicate publications, 2,030 titles/abstracts remained. After studying the titles and abstracts of these articles, 1,758 articles that were not related to our subject were excluded. Moreover, 248 publications were excluded because of being published in non-English language, being review articles, meeting, letter to editor, and in vivo and in vitro studies. In this systematic review and meta-analysis, only studies published since 2000 were evaluated. After the study of the finally enrolled 24 articles, two other studies were excluded because the effect size of statin on the incidence or mortality of OC had not been calculated or not been reported in the study and four studies were found as being irrelevant to my research according to the information of the full texts of the articles. Eighteen articles were selected for final analysis, of which ten articles were included for the assessment of the relationship between statin use and the risk of developing OC [11, 12, 16-18, 22, 23, 32-34], and nine articles for the relationship between post-diagnosis statin consumption and OC mortality [11, 13-15, 19-21, 35, 36]. It should be noted that Lavie et al. study [11], addressed both the risk of incidence and mortality of from OC (Fig. 1).

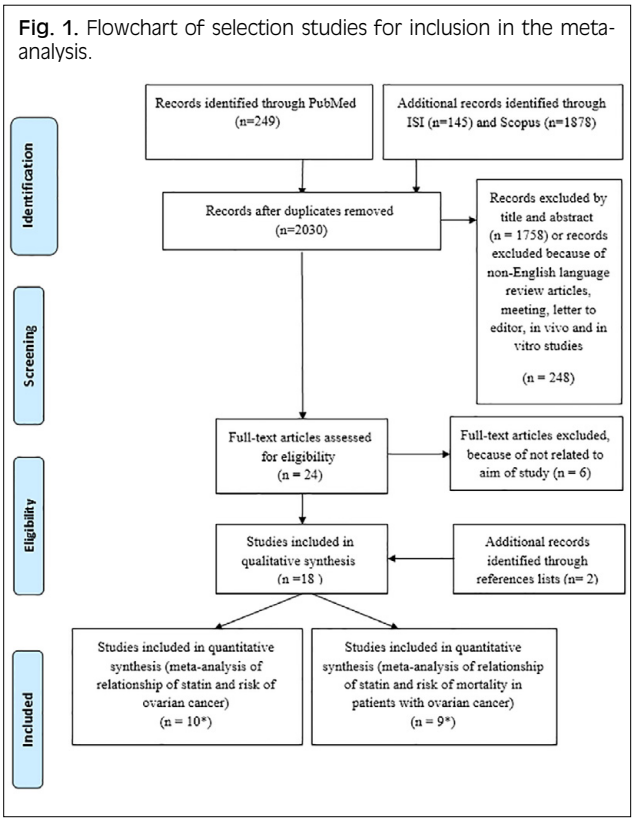


Fig. 1. Flowchart of selection studies for inclusion in the meta-analysis.

Characteristics of selected studies regarding the association between statins consumption and the risk of OC

Based on ten studies, a total of 1,254,501 participants were entered into the study based on the inclusion criteria. There were 7,943 cases of OC incidence reported in the included studies. Among the studies included [11, 12, 16-18, 22, 23, 32-34], four studies were cohort studies with total sample size of 428,613 individuals [17, 22, 33, 34], five studies were case-control with total sample size equal to 82,4891 individuals [11, 12, 16, 18, 32] and one was a clinical trial with total sample size equal to 997 individuals [23]. The reviewed articles had been published between 2001-2018 Six of the included studies were conducted in USA [12, 17, 22, 23, 33, 34], three in Europe [16, 18, 32] and one in Asia [11]. The sample size was 682-748, 282 participants and the mean follow-up of the participants period was 59-130 months (Tab. I).

Statin consumption and the incidence risk of OC

This meta-analysis included ten studies investigating the association between statin consumption and the risk of developing OC [11, 12, 16-18, 22, 23, 32-34]. Adjusted variables in the assessment of the relationship between statin use and risk of OC incidence are shown in Table II. The crude RR of the association between statin consumption and OC incidence is illustrated in Figure 2. People who received statin(s) were less likely to develop OC than those who did not, although the association was not statistically significant (RR = 0.88, 95% CI = 0.75-1.03, P = 0.109).

There was a significant heterogeneity among the results of the meta-analysis (χ^2 = 20.77, df = 9, P = 0.014, I^2 = 56.7%). Sensitivity analysis was performed by excluding studies from analysis one by one at each run. However, the number of pooled RRs did not change significantly, which indicates the robustness of the meta-analysis results (Fig. 2).

Subgroup analysis was performed to determine the association between statin consumption and risk of OC based on study design, sample size, and geographical location. The RR of OC in statin recipients was (RR = 0.82, 95% CI = 0.65-1.03, P = 0.09) in case-control studies; (RR = 0.98, 95% CI = 0.77-1.23, P = 0.848) in cohort studies; and (RR = 0.2, 95% CI = 0.01-4.15, P = 0.298) in clinical trials. Based on an analysis of geographical location, the RR of OC was (RR = 0.88, 95% CI = 0.69-1.12, P = 0.292) in North America; (RR = 0.98, 95% CI = 0.88-1.09, P = 0.732) in Europe; and (RR = 0.49, 95% CI = 0.28-0.81, P = 0.005) in Asia. Moreover, the RR of OC in studies with sample size < 10,000 was (RR = 0.65,

Tab. I. Characteristics of included studies for reviewing the incidence of OC.

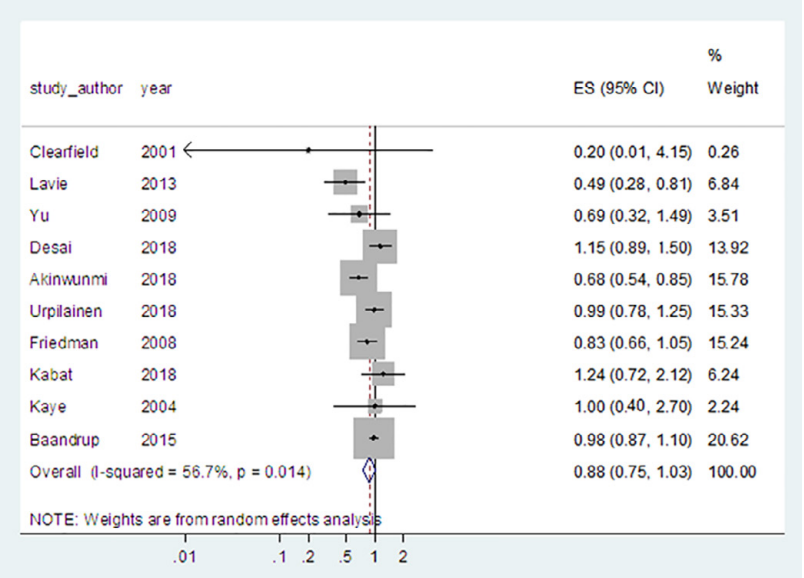
Publication first author	Year	Study setting	Study design	Sample size	Percentage of cancer cases	RR	95 % CI	Study period	Follow-up (median)	NOS
Urpilainen [16]	2018	Finland	Case-control study	748,282	0.04%	0.99	(0.78-1.25)	1996-2011	65	7
Friedman [33]	2008	USA	Cohort	169,261	0.05%	0.83	(0.66-1.05)	1994-2003	59	7
Kabat [17]	2018	USA	Cohort	24,208	0.48%	1.24	(0.72-2.12)	1993-1998	-	6
Kaye [18]	2004	UK	Case-control study	8,978	1.01%	1	(0.4-2.7)	1990-2002	77	8
Baandrup [32]	2015	Denmark	Case-control study	62,809	6.53%	0.98	(0.87-1.10)	2000-2011	-	8
Clearfield [23]	2001	USA	RCT	997	0.20%	0.2	(0.01-4.15)	-	62.4	6*
Lavie [11]	2013	Israel	Case-control study	682	18.48%	0.49	(0.28-0.81)	2003-2010	-	8
Yu [34]	2009	USA	Cohort	73,336	0.44%	0.69	(0.32-1.49)	1990-2004	67	8
Desai [22]	2018	USA	Cohort	161,808	0.47%	1.15	(0.89-1.50)	1993-1998	130	8
Akinwunmi [12]	2018	USA	Case-control study	4,140	49.28%	0.68	(0.54-0.85)	1992-2008	-	8

*: Jadad criteria was applied to assess the quality of the randomized clinical trials.

Tab. II. Adjusted variables in assessment relationship of statin use and risk of incidence of OC.

Publication first author	Year	Adjusted variables
Urpilainen [16]	2018	Age and duration of diabetes medication
Friedman [33]	2008	Calendar year
Kabat [17]	2018	Lipids or insulin
Kaye [18]	2004	Age, smoking, sex, smoking, body mass index (BMI) (kg/m^2)
Baandrup [32]	2015	Duration, intensity, term use
Clearfield [23]	2001	-
Lavie [11]	2013	Age
Yu [34]	2009	Age and BMI at the beginning of the study period, diabetes, high triglyceride and another lipid-lowering drug use, which were treated as time-varying covariates
Desai [22]	2018	Age, BMI, ethnicity, smoking status, education, current medical provider, baseline Hormone Therapy (HT) type and baseline HT duration
Akinwunmi [12]	2018	Age, study center, study phase, BMI, parity, educational status, use of oral contraceptive pills, history of tubal ligation, family history of OC, smoking status, and menopausal status

Fig. 2. Overall analysis of statin use and the incidence of OC.



95% CI = 0.53-0.80, P = 0.0001) and in studies with sample size >10,000 (RR = 0.98 95% CI = 0.90-1.07, P = 0.611) (Tab. III).

Evaluation of publication bias related to statin consumption and the incidence risk of OC

There was no evidence of publication bias in examining the association between statin consumption and the risk of incidence of OC. Therefore, tests of publication bias assessment were not statistically significant (Begg's test, p-value = 0.815; Eggers test, P value = 0.310).

Characteristics of studies in terms of the association between statins intake and risk of mortality in patients with OC

In nine meta-analysis studies, a total of 14,382 participants were enrolled based on our inclusion criteria. Among the included studies [11, 13-15, 19-21, 35, 36], seven studies were cohort with total sample size of 8,630 cases with

Tab. III. Subgroup analysis of the association between statin consumption and the incidence of OC.

Characteristics		Study n.	RR (95%CI)	P-value	Heterogeneity
Study type	RCT	1	0.20 (0.01-4.15)	0.298	0%
	Cohort	5	0.93 (0.78-1.10)	0.414	32.3%
	Case-control	5	0.82 (0.65-1.03)	0.09	72.3%
Study location	North America	7	0.86 (0.72-1.04)	0.122	49.8%
	Europe	3	0.98 (0.88-1.09)	0.73	0%
	Asia	1	0.49 (0.30-0.81)	0.005	0%
Sample size	Less than 10,000	4	0.65 (0.53-0.80)	0.0001	0%
	More than 10,000	7	0.95 (0.87-1.05)	0.330	7.5%

OC [13-15, 19, 20, 35, 36] and two studies were case-control studies with total sample size of 210 cases with OC [11, 21]. The studies had been published between 2008 and 2019. The sample size of recruited participants in the studies ranged was 60-5,416. The mean follow-up of participants was 6-48.8 months. Four of the included studies had been conducted in the USA [14, 15, 35, 36], two in Europe [13, 19] and three in Asia [11, 20, 21] (Tab. IV).

Statin intake and the risk of mortality from OC

This meta-analysis included nine studies that investigated the association between statin consumption and the risk of mortality from OC [11, 13-15, 19-21, 35, 36]. In the reviewed studies, adjusted variables had been included for the assessment of the relationship between statin consumption and the risk of mortality from OC (Tab. V). The crude RR of the association between statin consumption and the risk of mortality from OC is illustrated in Figure 3.

Patients receiving statins had reportedly lower mortality rate compared to those who did not. Notably, this association was statistically significant (RR = 0.76, 95% CI = 0.67-0.86, P = 0.0001) (Fig. 3).

There was a significant heterogeneity among the results of the meta-analysis (χ^2 = 4.19, df = 8, P = 0.077, I^2 = 43.6%). Sensitivity analysis was evaluated by excluding studies from analysis one by one at each run. However, the number of pooled RRs did not change significantly, indicating the robustness of the meta-analysis study results.

Subgroup analysis was done to investigate the association between statin consumption and the risk of mortality from OC based on study design, sample size, and geographical location. The RR of OC in statin recipients was (RR = 0.40, 95% CI = 0.19-0.85, P = 0.017) in case-control studies and (RR = 0.80, 95% CI = 0.74) in cohort studies. For geographical location, the results below were obtained: (RR = 0.70, 95% CI = 0.61-0.80, P = 0.0001) in North America; (RR = 0.84, 95% CI = 0.77-0.93,

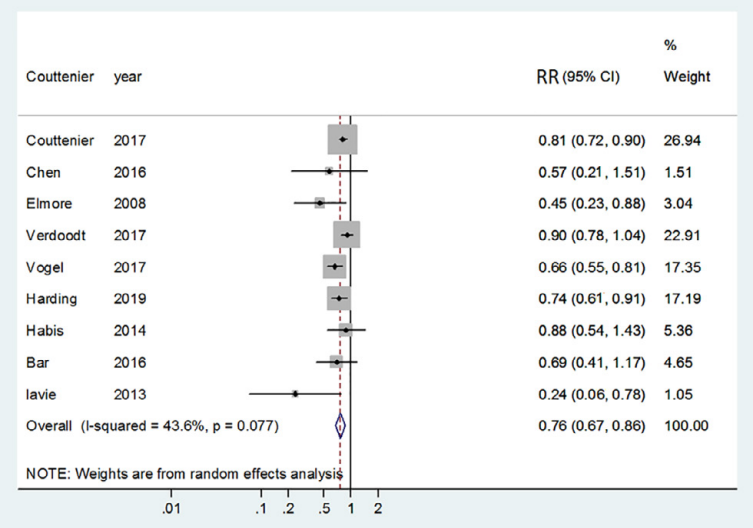
Tab. IV. Characteristics of included studies for survival

Publication first author	Year	Study setting	Study design	Sample size	Percentage of death	RR	95 % CI	Study period	Follow-up (median)	NOS
Couttenier [13]	2017	Belgium	Retrospective cohort	5416	37.64%	0.81	(0.72-90)	2004-2012	6 to 36 months	8
Chen [21]	2016	China	Retrospective	60	36.66%	0.57	(0.21-1.51)	2009-2013	30.3	6
Elmore [15]	2008	USA	Retrospective cohort	126	NA	0.45	(0.23-0.88)	1996-2001	54	7
Verdoodt [19]	2017	Denmark	Prospective cohort	4419	55.30%	0.9	(0.78-1.04)	2000-2013	29	9
Vogel [14]	2017	USA	Retrospective cohort	1431	NA	0.66	(0.55-0.81)	2007-2009	30.6	9
Harding [36]	2019	USA	Prospective cohort	2195	36.00%	0.74	(0.61-0.91)	2007-2012	26.5	8
Habis [35]	2014	USA	Retrospective cohort	442	NA	0.88	(0.54-1.43)	1992-2013	41.6	7
Bar [20]	2016	Israel	Retrospective cohort	143	54.54%	0.69	(0.41-1.17)	2000-2012	48.8	9
Lavie [11]	2013	Israel	Retrospective	150	40.66%	0.24	(0.06-0.78)	2003-2010	34	8

Tab. V. Adjusted variables in assessment relationship of statin use and mortality of OC.

Publication first author	Year	Adjusted variables
Couttenier [13]	2017	Age at diagnosis, year of diagnosis, comorbidities, cancer stage, and cancer treatments
Chen [21]	2016	Age, Federation International de Gynecologic at d'Obstétrique (FIGO) stage, tumor grade, histological subtype, cytoreductive surgery, cycles of chemotherapy, comorbidities (hypercholesterolemia and cardiovascular diseases)
Elmore [15]	2008	Age, diabetes mellitus, grade, stage, suboptimal cytoreduction
Verdoodt [19]	2017	Age at diagnosis, clinical stage, and year of diagnosis, tumour histology, chemotherapy, highest achieved education, disposable income, marital status, non-statin drug use and several comorbidities
Vogel [14]	2017	Age, race, median household income, stage, histology, platinum therapy, Charlson index, heart disease, diabetes, obesity, dyslipidemia
Harding [36]	2019	Age at diagnosis, year at diagnosis, race/ethnicity, marital status, surgical treatment received, grade of disease, stage at diagnosis, census tract poverty level, location of residence, Deyo-Charlson comorbidity score, comorbidities
Habis [35]	2014	Age, race, BMI, smoking status, comorbidities, physical status scores class, surgery characteristics, histologic subtype, FIGO stage, tumor site and grade of disease
Bar [20]	2016	Age, grade of disease, neoadjuvant chemotherapy, beta-blockers, aspirin, metformin, beta-blockers and comorbidity
Lavie [11]	2013	Age

Fig. 3. Overall analysis of statin use and the survival of OC.



Tab. VI. Subgroup analysis of the association between statin consumption and mortality of OC.

Characteristics		Study n.	RR (95%CI)	P-value	Heterogeneity
Study type	RCT	-	-	-	-
	Cohort	7	0.80 (0.74-0.85)	0.0001	38.7%
	Case-control	2	0.40 (0.19-0.85)	0.017	18.6%
Study location	North America	4	0.70 (0.61-0.80)	0.0001	3.7%
	Europe	2	0.84 (0.77-0.93)	0.0001	25%
	Asia	3	0.58 (0.37-0.88)	0.012	22.2%
Sample size	Less than 1,000	5	0.64 (0.48-0.86)	0.003	27.3%
	More than 1,000	4	0.80 (0.74-0.86)	0.0001	54.1%

P = 0.0001) in Europe; and (RR = 0.58, 95% CI = 0.37-0.88, P = 0.012) in Asia.

In addition, the RR related to mortality in OC patients associated with sample size < 1,000 was (RR = 0.64, 95% CI = 0.48-0.86, P = 0.003) and with sample size > 1,000 was (RR = 0.80, 95% CI = 0.74-0.86, P = 0.0001) (Tab. VI).

Evaluation of publication bias related to statin consumption and risk of mortality from OC

There was no evidence of publication bias based on our extensive analysis of the association between statin consumption and the risk of mortality from OC. The results of the analysis for the bias of assay tests were not statistically significant [Begg's test (P = 0.118); Egger's test (P = 0.118)].

Discussion

The purpose of this systematic review and meta-analysis was to investigate the relationship between statin consumption and the risk of OC incidence and survival in the patients. The study indicated that statin consumption reduced the risk of OC incidence by 12%. but given the 95% CI for the calculated RR, this relationship was not statistically significant.

However, statin consumption significantly reduces the risk of mortality in patients with OC. Overall; studies have also indicated that statins have beneficial effects on the prevention of death and incidence in gynecological cancers. Some studies reported that statin consumption could be inversely correlated with OC risk and mortality in gynecological cancers such as OC, endometrial and breast [25, 37-40]. However, some studies did not confirm that statins could have an impact on the risk of developing breast cancer, colorectal cancer, and lung cancer [41].

Statins help to treat hyperlipidemia through inhibiting hepatic cholesterol biosynthesis by blocking the rate-limiting phase in the mevalonate pathway via inhibiting hydroxymethylglutaryl-coenzyme A reductase (HMGCR) in hepatocytes [38]. Besides that, statins have shown anti-tumor activity and produce effect on metastasis formation so that they reduce the risk of cancer via various mechanisms, including increasing apoptosis cancer cell differentiation, activating anti-proliferative and pro-apoptotic signals, inhibiting cancer cell proliferation, modulating p53, p21, caspase 3 and caspase 6, sensitizing tumor cells to NK cell activity, blocking isoprenoids production (which play an important role in post-translational modifications of various proteins) or inhibiting activation of the proteasome pathway, inhibiting reactive oxygen species (ROS) production and inhibiting inflammation. These mechanisms have been discovered by means of cellular assays [38, 40, 41].

Moreover, obesity-related metabolic disorders including hypercholesterolemia can have an adverse effect on the prognosis of some cancers [38]. It should be noted that the effects of statins depend on their dosages (dose-response), duration of exposure of cells to the drug, the individual cell line, and statin type [42]. A meta-analysis showed that although statins were effective in preventing the risk of OC incidence, it did not have a significant effect on other gynecological cancers such as endometrial, cervical and vulvar [25].

However, a cohort study on women with type 2 diabetes showed that there was no relationship between statins or metformin consumption and OC incidence [16]. In another study, Desai et al. reported that pravastatin consumption could increase the risk of OC [22]. In addition, Baandrup et al. reported that statin consumption had no effective impact on reducing the risk of OC incidence [32]. The inconsistencies in the available research findings may be due to different sample size, genetic and demographic differences and regional diversities [43-45].

Similarly, subgroup analysis of the association between statin consumption and the incidence of OC indicated significant association for Asia (RR = 0.49, 95% CI 0.28-0.81, P = 0.005). The results of our meta-analysis demonstrates that patients receiving statins were significantly less likely to have increased risk of mortality compared to those who did not (RR = 0.76, 95% CI = 0.67-0.86, P = 0.0001). In agreement with this study, it has been suggested that long-term statin consumption is beneficial for primary prevention [46].

Other meta-analyses and review articles on gynecological cancers, such as breast and endometrial, have demonstrated that statin consumption generally has substantial survival-related benefits in terms of both disease-specific survival and overall survival, contributing to both pre- and post-diagnosis statin consumption [48-50]. Other meta-analyses have suggested that statin consumption is associated with an increase in survival for lung cancer [50], esophageal cancer [51], pancreatic cancer [52, 53] and endocrine-related gynecologic cancers [54].

A comparably comprehensive systematic review of cancer survival and incidence is one of the strengths of this study. In this study, subgroup analysis was also performed on several variables potentially affecting the incidence and mortality from OC, thereby increasing the power of the study and reliability of results. Most of the reviewed articles were of retrospective observational clinical type. Therefore, the analysis of the published studies may be affected by the available data or selection bias, comorbidity bias, and unmeasured or incomplete variables, as confounding variables, may also have affected the results of our systematic review. Other drugs are also likely to be taken during statin therapy, which might not have been included in the reviewed study, which may be a confounding variable. As mentioned above, simultaneous consumption of statins and other drugs can lead to interactions and even cytotoxic effects [10, 55, 56].

Although statins are mostly considered safe drug for a vast majority of patients, they may develop certain adverse effects including myalgia, rhabdomyolysis, and myopathy in some patients. Statins are known to interact with some cytochrome p450 enzyme groups [10].

There are few clinical trials on the effect of statins on the incidence and mortality of OC, and given the role of these studies in determining the therapeutic effects of drugs, it is recommended that multicenter randomized clinical trials studies with a large sample size be performed worldwide, so that we can use the results of these studies to make appropriate therapeutic decisions. Therefore, in order to obtain more reliable results, further trials should be carried out on cancer incidence and mortality. In addition, given the limited number of studies on this subject, we have only performed subgroup analysis based on geographical location, type of study, and sample size. Therefore, in this study, it was not possible to perform subgroup analysis based on other variables such as gender, age groups, duration of treatment with statins and drug type. Most studies have been conducted in the US and Europe, and no study has yet been conducted in Africa.

Conclusions

Given the lack of consensus on this subject and the relationship between statin consumption and ovarian cancer, this systematic review and meta-analysis robustly demonstrates that statin consumption can reduce the risk of OC incidence are by 12%, but the association is not statistically significant. Importantly, statin consumption was found to significantly increase the rate of survival in patients with OC. Therefore, statins may serve as a promising adjunctive anticancer drug for the prevention and reduction of mortality from OC. However, further clinical trials should be conducted to assess this relationship and its potential contribution to improving health outcomes for women at risk of OC.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

All authors contributed to the design of the review. SHS and AMH extracted and summarized the data. All authors contributed to drafting the manuscript. SHS,

CMS and AMH edited the first draft. Finally, all authors reviewed, commented, and approved the final draft.

References

[1] Momenimovahed Z, Tiznobaik A, Taheri S, Salehiniya H. Ovarian cancer in the world: epidemiology and risk factors. Int J Womens Health 2019;11:287-99. https://doi.org/10.2147/IJWH.S197604

[2] Torre LA, Trabert B, DeSantis CE. Ovarian cancer statistics, 2018. CA Cancer J Clin 2018;68:284-96. https://doi.org/10.3322/caac.21456

[3] Delgado-Ortega L, González-Domínguez A, Borrás JM, Oliva-Moreno J, González-Haba E, Menjón S, Pérez P, Vicente D, Cordero L, Jiménez M, Simón S, Hidalgo-Vega Á, Moya-Alarcón C. The economic burden of disease of epithelial ovarian cancer in Spain: the OvarCost study. Eur J Health Econ 2019;20:135-47. https://doi.org/10.1007/s10198-018-0986-y

[4] Reid BM, Permuth JB, Sellers TA. Epidemiology of ovarian cancer: a review. Cancer Biol Med 2017;14:9-32. https://doi.org/10.20892/j.issn.2095-3941.2016.0084

[5] Doubeni CA, Doubeni AR, Myers AE. Diagnosis and management of ovarian cancer. Am Fam Physician 2016;93:937-44.

[6] Roett MA, Evans P. Ovarian cancer: an overview. Am Fam Physician 2009;80:609-16.

[7] Modugno F, Edwards RP. Ovarian cancer: prevention, detection, and treatment of the disease and its recurrence. Molecular mechanisms and personalized medicine meeting report. Int J Gynecol Cancer 2012;22:S45-57. https://doi.org/10.1097/IGC.0b013e31826bd1f2

[8] Jawaid S, Gertz M, Corsino C, Cheung J, Seidle H, Couch RD. Human hydroxymethylglutaryl-coenzyme A reductase (HMGCR) and statin sensitivity. Indian Journal of Biochemistry & Biophysics 2010;47:331-9.

[9] Davies JT, Delfino SF, Feinberg CE, Johnson MF, Nappi VL, Olinger JT, Schwab AP, Swanson HI. Current and emerging uses of statins in clinical therapeutics: a review. Lipid Insights 2016;9:13-29. https://doi.org/10.4137/LPI.S37450

[10] Ramkumar S, Raghunath A, Raghunath S. Statin Therapy: review of safety and potential side effects. Acta Cardiol Sin 2016;32:631-9. https://doi.org/10.6515/ACS20160611A

[11] Lavie O, Pinchev M, Rennert HS, Segev Y, Rennert G. The effect of statins on risk and survival of gynecological malignancies. Gynecol Oncol 2013;130:615-9. https://doi.org/10.1016/j.ygyno.2013.05.025

[12] Akinwunmi B, Vitonis AF, Titus L, Terry KL, Cramer DW. Statin therapy and association with ovarian cancer risk in the New England Case Control (NEC) study. Int J Cancer 2019;144:991-1000. https://doi.org/10.1002/ijc.31758

[13] Couttenier A, Lacroix O, Vaes E, Cardwell CR, De Schutter H, Robert A. Statin use is associated with improved survival in ovarian cancer: a retrospective population-based study. PLoS One 2017;12:e0189233. https://doi.org/10.1371/journal.pone.0189233

[14] Vogel TJ, Goodman MT, Li AJ, Jeon CY. Statin treatment is associated with survival in a nationally representative population of elderly women with epithelial ovarian cancer. Gynecol Oncol 2017;146:340-5. https://doi.org/10.1016/j.ygyno.2017.05.009

[15] Elmore RG, Ioffe Y, Scoles DR, Karlan BY, Li AJ. Impact of statin therapy on survival in epithelial ovarian cancer. Gynecol Oncol 2008;111:102-5.

[16] Urpilainen E, Marttila M, Hautakoski A, Arffman M, Sund R, Ilanne-Parikka P, Arima R, Kangaskokko J, Puistola U, Laara E, Hinkula M. The role of metformin and statins in the incidence of epithelial ovarian cancer in type 2 diabetes: a cohort and nested case-control study. BJOG 2018;125:1001-8. https://doi.org/10.1111/1471-0528.15151

[17] Kabat GC, Kim MY, Chlebowski RT, Vitolins MZ, Wassertheil-Smoller S, Rohan TE. Serum lipids and risk of obesity-related cancers in postmenopausal women. Cancer Causes Control 2018;29:13-24. https://doi.org/10.1007/s10552-017-0991-y

[18] Kaye JA, Jick H. Statin use and cancer risk in the General Practice Research Database. Br J Cancer 2004;90:635-7. https://doi.org/10.1038/sj.bjc.6601566

[19] Verdoodt F, Kjaer Hansen M, Kjaer SK, Pottegard A, Friis S, Dehlendorff C. Statin use and mortality among ovarian cancer patients: a population-based cohort study. Int J Cancer 2017;141:279-86. https://doi.org/10.1002/ijc.30738

[20] Bar D, Lavie O, Stein N, Feferkorn I, Shai A. The effect of metabolic comorbidities and commonly used drugs on the prognosis of patients with ovarian cancer. Eur J Obstet Gynecol Reprod Biol 2016;207:227-31. https://doi.org/10.1016/j.ejogrb.2016.09.005

[21] Chen HY, Wang Q, Xu QH, Yan L, Gao XF, Lu YH, Wang L. Statin as a combined therapy for advanced-stage ovarian cancer: a propensity score matched analysis. Biomed Res Int 2016;2016:9125238. https://doi.org/10.1155/2016/9125238

[22] Desai P, Wallace R, Anderson ML, Howard BV, Ray RM, Wu C, Safford M, Martin LW, Rohan T, Manson JE, Simon MS. An analysis of the association between statin use and risk of endometrial and ovarian cancers in the Women's Health Initiative. Gynecol Oncol 2018;148:540-6. https://doi.org/10.1016/j.ygyno.2018.01.006

[23] Clearfield M, Downs JR, Weis S, Whitney EJ, Kruyer W, Shapiro DR, Stein EA, Langendorfer A, Beere PA, Gotto AM. Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS): efficacy and tolerability of long-term treatment with lovastatin in women. J Womens Health Gend Based Med 2001;10:971-81. https://doi.org/10.1089/152460901317193549

[24] Li X, Zhou J. Impact of postdiagnostic statin use on ovarian cancer mortality: a systematic review and meta-analysis of observational studies. Br J Clin Pharmacol 2018;84:1109-20. https://doi.org/10.1111/bcp.13559

[25] Liu Y, Qin A, Li T, Qin X, Li S. Effect of statin on risk of gynecologic cancers: a meta-analysis of observational studies and randomized controlled trials. Gynecol Oncol 2014;133:647-55. https://doi.org/10.1016/j.ygyno.2014.04.007

[26] Lo CK-L, Mertz D, Loeb M. Newcastle-Ottawa Scale: comparing reviewers' to authors' assessments. BMC Med Res Methodol 2014;14:45. https://doi.org/10.1186/1471-2288-14-45

[27] Kjaergard LL, Villumsen J, Gluud C. Reported methodologic quality and discrepancies between large and small randomized trials in meta-analyses. Ann Intern Med 2001;135:982-9. https://doi.org/10.7326/0003-4819-135-11-200112040-00010

[28] Laird NM, Mosteller F. Some statistical methods for combining experimental results. Int J Technol Assess Health Care 1990;6:5-30.

[29] Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557-60. https://doi.org/10.1136/bmj.327.7414.557

[30] Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629-34. https://doi.org/10.1136/bmj.315.7109.629

[31] Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics 1994:1088-101.

[32] Baandrup L, Dehlendorff C, Friis S, Olsen JH, Kjaer SK. Statin use and risk for ovarian cancer: a Danish nationwide case-control study. Br J Cancer 2015;112:157-61. https://doi.org/10.1038/bjc.2014.574

[33] Friedman GD, Flick ED, Udaltsova N, Chan J, Quesenberry CP, Jr., Habel LA. Screening statins for possible carcinogenic risk: up to 9 years of follow-up of 361,859 recipients. Pharmacoepidemiol Drug Saf 2008;17:27-36. https://doi.org/10.1002/pds.1507

[34] Yu O, Boudreau DM, Buist DS, Miglioretti DL. Statin use and

female reproductive organ cancer risk in a large population-based setting. Cancer Causes Control 2009;20:609-16. https://doi.org/10.1007/s10552-008-9271-1

[35] Habis M, Wroblewski K, Bradaric M, Ismail N, Yamada SD, Litchfield L, Lengyel E, Romero IL. Statin therapy is associated with improved survival in patients with non-serous-papillary epithelial ovarian cancer: a retrospective cohort analysis. PLoS One 2014;9:e104521. https://doi.org/10.1371/journal.pone.0104521

[36] Harding BN, Delaney JA, Urban RR, Weiss NS. Use of statin medications following diagnosis in relation to survival among women with ovarian cancer. Cancer Epidemiol Biomarkers Prev 2019. https://doi.org/10.1158/1055-9965.epi-18-1194

[37] Borgquist S, Broberg P, Tojjar J, Olsson H. Statin use and breast cancer survival - a Swedish nationwide study. BMC Cancer 2019;19:54. https://doi.org/10.1186/s12885-018-5263-z

[38] Borgquist S, Bjarnadottir O, Kimbung S, Ahern TP. Statins: a role in breast cancer therapy? J Intern Med 2018;284:346-57. https://doi.org/10.1111/joim.12806

[39] Beckwitt CH, Shiraha K, Wells A. Lipophilic statins limit cancer cell growth and survival, via involvement of Akt signaling. PloS one 2018;13:e0197422-e. https://doi.org/10.1371/journal.pone.0197422

[40] Borgquist S, Bjarnadottir O, Kimbung S, Ahern TP. Statins: a role in breast cancer therapy? J Intern Med 2018;284:346-57. https://doi.org/10.1111/joim.12806

[41] Stryjkowska-Góra A, Karczmarek-Borowska B, Góra T, Krawczak K. Statins and cancers. Contemp Oncol (Pozn) 2015;19:167-75. https://doi.org/10.5114/wo.2014.44294

[42] Matusewicz L, Meissner J, Toporkiewicz M, Sikorski AF. The effect of statins on cancer cells - review. Tumour Biol 2015;36:4889-904. https://doi.org/10.1007/s13277-015-3551-7

[43] Goldstein MR, Mascitelli L, Pezzetta F. Statins, Tregs and cancer. Atherosclerosis 2008;196:483-4. https://doi.org/10.1016/j.atherosclerosis.2007.09.028

[44] Wang Y, Ren F, Song Z, Chen P, Liu S, Ouyang L. Statin use and the risk of ovarian and endometrial cancers: a meta-analysis. 2019;19:730. https://doi.org/10.1186/s12885-019-5954-0

[45] Gbelcová H, Rimpelová S, Ruml T, Fenclová M, Kosek V, Hajšlová J, Strnad H, Kolář M, Vítek L. Variability in statin-induced changes in gene expression profiles of pancreatic cancer. Sci Rep 2017;7:44219.

[46] Gitsels LA, Kulinskaya E, Steel N. Survival benefits of statins for primary prevention: a cohort study. PloS one 2016;11:e0166847. https://doi.org/10.1371/journal.pone.0166847

[47] Wu QJ, Tu C, Li YY, Zhu J, Qian KQ, Li WJ, Wu L. Statin use and breast cancer survival and risk: a systematic review and meta-analysis. Oncotarget 2015;6:42988-3004. https://doi.org/10.18632/oncotarget.5557

[48] Zhong S, Zhang X, Chen L, Ma T, Tang J, Zhao J. Statin use and mortality in cancer patients: systematic review and meta-analysis of observational studies. Cancer Treat Rev 2015;41:554-67. https://doi.org/10.1016/j.ctrv.2015.04.005

[49] Li J, Liu R, Sun Z, Tang S, Wang L, Liu C, Zhao W, Yao Y, Sun C. The association between statin use and endometrial cancer survival outcome: a meta-analysis. Medicine 2018;97:e13264. https://doi.org/10.1097/md.0000000000013264

[50] Chen Y, Li X, Zhang R, Xia Y, Shao Z, Mei Z. Effects of statin exposure and lung cancer survival: a meta-analysis of observational studies. Pharmacol Res 2019;141:357-65. https://doi.org/10.1016/j.phrs.2019.01.016

[51] Deng HY, Lan X, Zheng X, Zha P, Zhou J, Wang RL, Jiang R, Qiu XM. The association between statin use and survival of esophageal cancer patients: a systematic review and meta-analysis. Medicine 2019;98:e16480. https://doi.org/10.1097/md.0000000000016480

[52] Wang D, Rodriguez EA, Barkin JS, Donath EM, Pakravan AS. Statin use shows increased overall survival in patients diagnosed with pancreatic cancer: a meta-analysis. Pancreas 2019;48:e22-e3. https://doi.org/10.1097/mpa.0000000000001276

[53] Jian-Yu E, Graber JM, Lu SE, Lin Y, Lu-Yao G, Tan XL. Effect of metformin and statin use on survival in pancreatic cancer patients: a systematic literature review and meta-analysis. Curr Med Chem 2018;25:2595-607. https://doi.org/10.2174/0929867324666170412145232

[54] Xie W, Ning L, Huang Y, Liu Y, Zhang W, Hu Y, Lang J, Yang J. Statin use and survival outcomes in endocrine-related gynecologic cancers: a systematic review and meta-analysis. Oncotarget 2017;8:41508-17. https://doi.org/10.18632/oncotarget.17242

[55] Taylor-Harding B, Orsulic S, Karlan BY, Li AJ. Fluvastatin and cisplatin demonstrate synergistic cytotoxicity in epithelial ovarian cancer cells. Gynecol Oncol 2010;119:549-56. https://doi.org/10.1016/j.ygyno.2010.08.017

[56] Izdebska M, Natorska-Chomicka D, Jagiello-Wojtowicz E. Preliminary studies evaluating cytotoxic effect of combined treatment with methotrexate and simvastatin on green monkey kidney cells. Acta Pol Pharm 2014;71:515-20.

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Correspondence: Saeid Heidari-Soureshjani, Circuit of Research and Technology, Shahrekord University of Medical Sciences, Shahrekord, Iran - Tel. +98 9131833509 - Fax: +98 383351031 - E-mail: heidari62@yahoo.com

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Review

Resources for assessing parents' vaccine hesitancy: a systematic review of the literature

PAOLA CELLA[1], GIANLUCA VOGLINO[2], ILARIA BARBERIS[3], ENRICO ALAGNA[4], CLAUDIA ALESSANDRONI[5], ALESSANDRO CUDA[6], FRANCESCO D'ALOISIO[7], GIULIA DALLAGIACOMA[6], SARA DE NITTO[8], FRANCESCA DI GASPARE[5], ORIANA GALLIPOLI[7], LEANDRO GENTILE[6], LUCIA KUNDISOV[9], MONICA NAVARO[10], SANDRO PROVENZANO[4], OMAR ENZO SANTANGELO[4], PASQUALE STEFANIZZI[8], VINCENZA GIANFREDI[11, 12]

[1] Post Graduate School of Hygiene and Preventive Medicine, Department of Medicine and Surgery, University of Parma, Italy; [2] Post Graduate School of Hygiene and Preventive Medicine, Department of Public Health, University of Turin, Italy; [3] Health Science Department, University of Genoa, Italy; [4] Post Graduate School of Hygiene and Preventive Medicine, Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties "G. D'Alessandro", University of Palermo, Italy; [5] Post Graduate School of Hygiene and Preventive Medicine, University of Rome Tor Vergata, Rome, Italy; [6] Post Graduate School of Hygiene and Preventive Medicine, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Italy; [7] Post Graduate School of Hygiene and Preventive Medicine, Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy; [8] Post Graduate School of Hygiene and Preventive Medicine, Department of Biomedical Science and Human Oncology, University of Bari Aldo Moro, Italy; [9] Post Graduate School of Public Health, University of Siena, Italy; [10] Post Graduate School of Hygiene and Preventive Medicine, Department of Experimental Medicine, University of Campania "L. Vanvitelli", Italy; [11] Post Graduate School of Hygiene and Preventive Medicine, Department of Experimental Medicine, University of Perugia, Italy; [12] School of Medicine, University Vita-Salute San Raffaele, Milan, Italy

Keywords

Vaccine • Hesitancy • Parents • Questionnaire • Review

Summary

The concept of Vaccine Hesitancy has begun to appear in the scientific landscape, referring to the reluctance of a growing proportion of people to accept the vaccination offer. A variety of factors were identified as being associated with vaccine hesitancy but there was no universal algorithm and currently there aren't any established metrics to assess either the presence or impact of vaccine hesitancy. The aim of this study was to systematically review the published questionnaires evaluating parental vaccine hesitancy, to highlight the differences among these surveys and offer a general overview on this matter. This study offers a deeper perspective on the available questionnaires, helping future researches to identify the most suitable one according to their own aim and study setting.

Introduction

Vaccines have long been considered as one of the most important public health achievements of the past century and they have largely contributed to the decline in morbidity and mortality related to various infectious diseases [1]. Due to the effectiveness of vaccination programs, many people nowadays have limited or no experience with vaccine-preventable diseases (VPDs), thus parents increasingly assume that the risks associated with VPDs are minimal compared to potential health and safety risks of vaccinations themselves [2, 3]. The concept of Vaccine Hesitancy has subsequently begun to appear in the scientific landscape, referring to the reluctance of a growing proportion of people to accept the vaccination offer [4]. In fact, urban centres with large clusters of vaccine-hesitant individuals are particularly vulnerable to VPD outbreaks among exposed, unimmunized children, as observed with the measles outbreaks in the USA, Canada, and Europe [5-7]. 2014-2015 the Disneyland measles outbreak was a stark reminder of the direct influence of vaccine hesitancy and refusal [8].

The World Health Organization (WHO) defines vaccine hesitancy as the ''delay in acceptance or refusal of vaccines despite availability of vaccination services. Vaccine hesitancy is complex and context specific, varying across time, place and for different vaccines. This phenomenon is influenced by factors such as complacency, convenience and confidence" [9]. The "3Cs" Model, that highlights these three categories, was first proposed in 2011 by the WHO EURO Vaccine Communications Working Group. In the "3 Cs" model, confidence is defined as trust in the effectiveness and safety of vaccines, and in the system that delivers them. This includes the reliability and competence of health services and health professionals and the motivations of policy-makers who decide on the needed vaccines. Vaccination complacency exists where the perceived risks of vaccine-preventable diseases are low and vaccination is not deemed a necessary preventive action. Vaccination convenience is a significant factor when physical availability, affordability, willingness-to-pay, geographical accessibility, ability to understand (language and health literacy) and appeal of immunization services affect the vaccination uptake [10]. There is a wide variety of determinants of vaccine hesitancy. In 2015, the WHO EURO Vaccine Communications Working Group developed the Vaccine Hesitancy

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Determinants Matrix which categorized determinants into the following groups: contextual, individual and group influences, and vaccine and vaccination-specific issues [11]. Contextual influences include historic, social, cultural, environmental, economic, political and institutional factors which might influence vaccine hesitant populations. The most common is conspiracy theories, which include a fear that vaccines are introduced to serve the economic and/or political interests of pharmaceutical companies [12, 13].

Individual and group influences include personal perceptions or beliefs about vaccines and influences from the social environment such as the belief that vaccines are unsafe. Parents are more afraid of the adverse events related to vaccines, which are thought to be more frequent and more serious than they really are, than of the complications that could arise from infectious diseases [14]. Moreover, some individuals do not perceive a medical need for certain vaccines. Vaccine Hesitancy is a global, complex and constantly changing phenomenon, currently representing one of the most significant problems of public health: in 2019 the World Health Organization (WHO) listed vaccine hesitancy in its top ten threats to global health [15]. To understand the impact that the various determinants have on vaccine hesitancy and what factors can influence vaccination decisions, numerous studies have been conducted over the years [16, 17].

Despite the growing number of articles on vaccine hesitancy published in recent years, there are some discrepancies among publications in terms of what exactly falls under the umbrella of "vaccine hesitancy", a term that was only introduced by the SAGE Working Group in 2015. Therefore, in order to obtain as much information as possible on this issue, all studies investigating the determinants of vaccine hesitancy, without specifically using the term "vaccine hesitancy", were also included in this review. A variety of factors were identified as being associated with vaccine hesitancy but there was no universal algorithm and currently there aren't any established metrics to assess either the presence or impact of vaccine hesitancy. Study methods used to measure "vaccine hesitancy" are too heterogeneous and this makes it difficult to make inferences about the influence of specific factors on vaccine-hesitant behaviour. The aim of this study was to systematically review the published questionnaires evaluating parental vaccine hesitancy, to highlight the differences among these surveys and offer a general overview on this matter. Administration channel, sample size, type of vaccine being investigated, and the type of questions used in the questionnaire are some of the variables that can be considered, when designing a study to investigate vaccine hesitancy. The characteristics of each study, as well as the variables investigated, have been analyzed in order to enable future researchers to choose the most suitable tool for evaluating and measuring vaccine hesitancy over time and in different settings, according to their own needs and goals.

Methods

Search strategy

This is a systematic review conducted in accordance with the PRISMA Guidelines [18]. Several databases were consulted, including PubMed/Medline, Web of Science and The Cochrane Library. The latter was used to identify existing systematic reviews with a similar objective, in order to further screen the lists of references of potentially related articles that might have not been retrieved in the other databases. The systematic search was performed with no time filter, from inception to December 14th, 2017; however, a language limit was adopted, indeed only English and Italian articles were included in our review. The predefined search strategy that was used to identify potential relevant articles included four main aspects: parents or caregivers, vaccine hesitancy/acceptance, immunization and survey. Mesh and text words were combined with Boolean operators AND and OR. The full search strategy is: ((((((questionnair*[Title/Abstract] OR survey[Title/Abstract] OR "Surveys and Questionnaires"[Mesh])) AND (vaccin*[Title/Abstract] OR immuniz*[Title/Abstract] OR immunis*[Title/Abstract] OR shot*[Title/Abstract] OR jab*[Title/Abstract] OR "Vaccines"[Mesh] OR "Immunization"[Mesh] OR "Vaccination"[Mesh])) AND (hesitanc*[Title/Abstract] OR doubt*[Title/Abstract] OR concern*[Title/Abstract] OR criticis*[Title/Abstract] OR rumo*r[Title/Abstract] OR sceptic*[Title/Abstract] OR fear*[Title/Abstract] OR refus*[Title/Abstract] OR reject*[Title/Abstract] OR delay[Title/Abstract] OR accept*[Title/Abstract] OR consen*[Title/Abstract] OR intent*[Title/Abstract] OR confidence[Title/Abstract] OR adherence[Title/Abstract] OR complian*[Title/Abstract] OR uptake[Title/Abstract] OR engagement[Title/Abstract] OR *trust[Title/Abstract] OR a*titude[Title/Abstract] OR perception*[Title/Abstract] OR opinion*[Title/Abstract] OR belief*[Title/Abstract] OR behavi*r[Title/Abstract] OR choice*[Title/Abstract] OR practic*[Title/Abstract] OR barrier*[Title/Abstract] OR facilitator*[Title/Abstract] OR "Health Knowledge, Attitudes, Practice"[Mesh] OR "Vaccination Refusal"[Mesh] OR "Trust"[Mesh] OR "Behavior"[Mesh] OR "Patient Acceptance of Health Care"[Mesh]))) AND (parent*[Title/Abstract] OR caregiver*[Title/Abstract] OR guardian*[Title/Abstract] OR tutor*[Title/Abstract] OR mother*[Title/Abstract] OR father*[Title/Abstract] OR "legally acceptable representative"[Title/Abstract] OR "Parents"[Mesh])). In order to include all publications related to the topic, the list of references was manually screened for all relevant papers. Endnote was used as a software to manage all the retrieved references.

Inclusion criteria

Studies that fulfilled the inclusion criteria were considered in this review. Papers aimed at investigating parents/caregivers vaccine hesitancy through a survey/

questionnaire were considered eligible, regardless of the attitudes and behaviours of the interviewed subjects. As a matter of fact, vaccine hesitancy is complex and driven by a wide variety of factors, as explained by the 3C model developed by the SAGE Working Group. Therefore, knowing the determinants of Vaccine Hesitancy in specific subgroups of parents (such as those who do not trust or have lost confidence in vaccinations) is extremely important in order to develop the right strategies to address it. In addition, including studies selecting the study population according to a negative/positive attitude/behaviour towards vaccination might be helpful for future researchers interested in studying Vaccine Hesitancy in a specific subgroup of parents. Because vaccine hesitancy is a complex phenomenon, strictly depending on several aspects that are country-specific, and because the introduction of vaccine hesitancy as a term in the scientific community is relatively new, we also included studies evaluating public trust/distrust, perceptions, concerns, confidence, attitudes, beliefs about vaccines and vaccination programs. Moreover, we included all types of available vaccines. Furthermore, we only assessed original articles, while other types of publications were not included in the analysis. Lastly, due to the aim of the research, only observational studies were considered: along with cross-sectional studies, we included cohort studies and case-control studies. The last two types of studies are particularly helpful in order to obtain as much information as possible and to have a broader overview of this phenomenon: as a matter of fact, they allowed us to include studies where questionnaires or surveys were used to investigate vaccine hesitancy among parents.

Exclusion criteria

Studies were excluded from this review when they investigated vaccine hesitancy in target populations different from parents/caregivers/guardians, for instance physicians, educators, or directly the adolescents. Papers written in languages other than English and Italian were excluded, as well as not original articles (reviews, letters to editor, conference papers, editorials). Additionally, surveys aimed at assessing aspects different than vaccine hesitancy were not included. Lastly, articles were excluded if the vaccines examined were not for humans or were not commercially available yet (such as the HIV vaccine), or if the publications were on vaccine development.

Data extraction

Eight couples of reviewers (VG and CA, MN and GV, SP and FD, OG and IB, EA and SDN, OES and LK, OG and AC, FDG and LG), independently performed the screening of titles and abstracts, followed by data extraction of the included articles. Disagreement was solved through a discussion between the authors, if disagreement persisted a third author was consulted (PC). Full-texts were downloaded and consulted only for the included articles. The extracted data were reported in a predefined, ad hoc spreadsheet elaborated in Excel. For each included article, the following items were evaluated: first author's name and year of publication, when and where the study was conducted, study design and study aim, population characteristics and sample size, types of survey and administration, if the questionnaire was previously validated and if it was attached to the manuscript, number and type of questions, type of vaccine analysed, immunization behaviour and beliefs about vaccines.

Data coding

The included articles were coded by study period, country, language, study type and study aim, population characteristics, way of administration, number of items and items categories in the questionnaire, types of vaccine, immunization behaviour, beliefs about vaccine safety/efficacy. Regarding the latter, beliefs were coded as follows: i) no assessment of perceived safety/efficacy vaccines; ii) the assessment was performed and most of the respondents believe vaccinations to be safe/effective; iii) the assessment was performed and most of the respondents do not believe vaccinations to be safe/effective; iv) the assessment was performed but data were not available); v) the assessment was performed, but only qualitative and descriptive data were available (numerical data not available). As for the immunization behaviour, it was classified as follows: i) "acceptance", if the whole population consisted of people receiving the vaccination; ii) "refusal", if the whole population consisted of parents refusing the vaccination; iii) "hesitancy/scepticism/doubt", if the population consisted of both parents accepting the vaccine and parents refusing it; iv) if no information was available, it was considered as missing data.

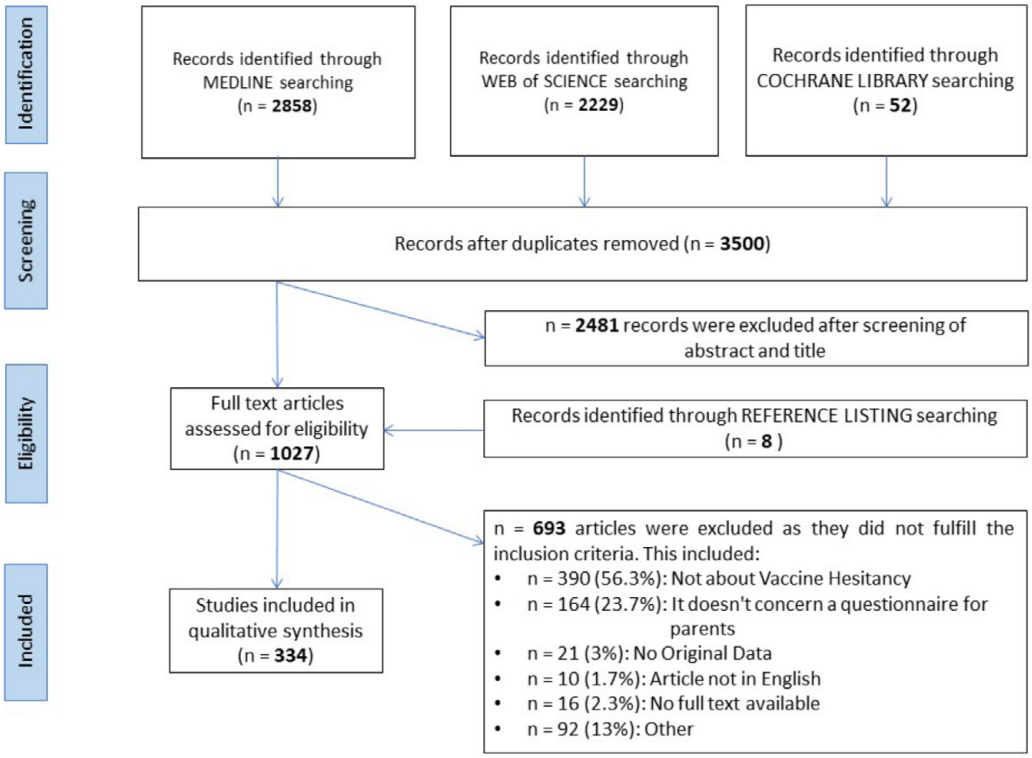
Results

We identified 5,139 records by running the pre-defined search strategies on the three selected databases (Medline, Web of Science, The Cochrane Library), and 8 additional records were retrieved from the manual searching of reference lists and citation chains of included papers. After removing duplicates, 3,500 papers were assessed for eligibility by title and abstract, and 2,481 papers were removed. After full text screening selection, 334 studies were included in the descriptive analysis and synthesis [3, 16, 19-350]. Figure 1 shows the selection flow. The main results of our systematic review are shown in Table I.

Study designs and study aims

Most of the included studies (60.4%, n = 202/334) were conducted between 2010 and 2019, while 30.5% (n = 102/334) articles reported a study period prior to 2008. In a total of 8.9% (n = 30/334) works the study period was not specified. Among the most recent studies, 48.8% (n = 100/334) were conducted in North or South America (Argentina, Brazil, Canada, Caribbean, El

Fig. 1. Flow chart of selection process.



Tab. I. Main characteristics of the included studies.

Author, year	Study period	Country	Study design	Administration channel	Sample size	Type of vaccine	Type of questions	Validation	Immunization behaviour
Adler A, 2007	2007	Israel	Cross-sectional	Paper-based	1,474	Varicella	Closed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Adorador A, 2011	2011	USA	Cross-sectional	Paper-based	108	Dtp	Closed	Not	Hesitancy/ scepticism/ doubt
Aharony N, 2017	2017	Israel	Cross-sectional	On-line	200	Child vaccines	Likert scale	With statistical methods	Refusal
Akis S, 2011	2011	Turkey	Cross-sectional	Paper-based	611	Flu	Closed	Not	Acceptance
Akmatov MK, 2009	2009	Kyrgyzstan	Cross-sectional	Paper-based	934	Child vaccines	Closed	Not	Acceptance
Alberts CJ, 2017	2017	Netherlands	Cross-sectional	Mail	1,309	Hpv	Closed	Statistical methods not reported	Acceptance
Alfredsson R, 2004	2004	Sweden	Cross-sectional	Paper-based	300	Mmr	Closed	With statistical methods	Acceptance
Allen JD, 2010	2010	USA	Cross-sectional	On-line	476	Hpv	Closed	Not	Acceptance
Allison MA, 2010	2010	USA	Cross-sectional	Paper-based	259	Flu	Likert scale	With statistical methods	Hesitancy/ scepticism/ doubt
Allred NJ, 2005	2005	USA	Cross-sectional	Mail	7,810	Dt	Closed	With statistical methods	Acceptance
Alshammari TM, 2018	2018	Saudi Arabia	Cross-sectional	Paper-based	467	Child vaccines	Closed	Statistical methods not reported	Acceptance

continues

follows

Tab. I. Main characteristics of the included studies.

Author, year	Study period	Country	Study design	Administration channel	Sample size	Type of vaccine	Type of questions	Validation	Immunization behaviour
Ambe JP, 2001	2001	Nigeria	Cross-sectional	Paper-based	500	Measles	Closed	Statistical methods not reported	Refusal
Aharon AA, 2017	2017	Israel	Cross-sectional	Paper-based	731	Hbv/dtp/mmr	Likert scale	Not	Refusal
Arrossi S, 2012	2012	Argentina	Cross-sectional	Paper-based	1,200	Hpv	Closed	With statistical methods	Acceptance
Azizi FSM, 2017	2017	Malesya	Cross-sectional	Paper-based	545	Child vaccines	Closed	With statistical methods	Hesitancy/ scepticism/ doubt
Baglioni A, 2014	2014	Italy	Cross-sectional	Paper-based	648	Hpv	Closed	With statistical methods	Acceptance
Bakhache P, 2013	2013	Multinational	Cross-sectional	On-line	2,460	Menb	Closed	Statistical methods not reported	Acceptance
Baldwin AS, 2013	2008-2010	USA	Cross-sectional	Paper-based	256	Hpv	Likert scale	With statistical methods	Acceptance
Bardenheier B, 2003	2000	USA	Cross-sectional	Paper-based	648	Hav	Closed	Not	Acceptance
Bardenheier B, 2004	2001	USA	Case-control	Paper-based	3,586	Mmr/ dtp/hbv	Likert scale	Not	Acceptance
Bardenheier BH, 2004	1997-1998	USA	Cross-sectional	Paper-based	3,552	Dtp/hib/ hbv/ polio	Closed	Not	Hesitancy/ scepticism/ doubt
Barnack JL, 2010	2006	USA	Cross-sectional	Mail	200	Hpv	Likert scale	Not	Acceptance
Barnack-Tavlaris JL, 2016	2009	USA	Cross-sectional	Telephone	4,666	Hpv	Closed	Not	Acceptance
Bazzano A, 2012	2007	USA	Cross-sectional	Telephone	197	Child vaccines	Closed	With statistical methods	Hesitancy/ scepticism/ doubt
Bedford H, 2007	2004	UK	Cross-sectional	Paper-based	859	New vaccines	Likert scale	Not	Acceptance
Beel ER, 2013	2010-2012	USA	Cross-sectional	Paper-based	511	Child vaccines	Closed	With statistical methods	Acceptance
Ben Natan M, 2011	2008	Israel	Cross-sectional	Paper-based	103	Hpv	Likert scale	With statistical methods	Hesitancy/ scepticism/ doubt
Ben Natan M, 2016	2015	Israel	Cross-sectional	Paper-based	200	Flu	Likert scale	With statistical methods	Acceptance
Ben Natan M, 2017	2016	Israel	Cross-sectional	Paper-based	200	Hpv	Likert scale	With statistical methods	Acceptance
Berenson AB, 2014	2011-2013	USA	Cross-sectional	Paper-based	1,256	Hpv	Closed	With statistical methods	Acceptance
Bettinger JA, 2016	2011	Canada	Cross-sectional	Mail	34	Flu	Closed	Not	Acceptance
Bham SQ, 2016	2015	Pakistan	Cross-sectional	Paper-based	210	Polio	Closed	Not	Acceptance
Bianco A, 2014	2014	Italy	Cross-sectional	Paper-based	566	Hpv	Likert scale	Not	Acceptance
Bigham M, 2006	2002-2003	Canada	Cross-sectional	Telephone	487	Hbv	Likert scale	With statistical methods	Hesitancy/ scepticism/ doubt

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Tab. I. Main characteristics of the included studies.

Author, year	Study period	Country	Study design	Administration channel	Sample size	Type of vaccine	Type of questions	Validation	Immunization behaviour
Alder S, 2015	2012	Argentina	Cross-sectional	Paper-based	180	Hpv	Closed	With statistical methods	Acceptance
Basu P, 2011	2008	India	Cross-sectional	Paper-based	522	Hpv	Closed	Statistical methods not reported	Acceptance
Blair A, 1997	1997	Australia	Cross-sectional	Paper-based	245	Child vaccines	Open field	Not	Acceptance
Blyth CC, 2014	2008–2012	Australia	Cross-sectional	Paper-based	2,576	Dtp/hib/hbv	Closed	Statistical methods not reported	Hesitancy/scepticism/doubt
Bodson J, 2016	2013	USA	Cross-sectional	Paper-based	119	Hpv	Closed	With statistical methods	Hesitancy/scepticism/doubt
Bonanni P, 2001	2001	Italy	Cross-sectional	Paper-based	300	Child vaccines	Closed	Not	Acceptance
Borena W, 2016	2015	Austria	Cross-sectional	Mail	439	Hpv	Closed	Not	Hesitancy/scepticism/doubt
Borras E, 2009	2003-2004	Spain	Cross-sectional	Telephone	630	Child vaccines	Closed	Not	Hesitancy/scepticism/doubt
Brabin L, 2006	2005	UK	Cross-sectional	Mail	317	Hpv	Mixed	With statistical methods	Hesitancy/scepticism/doubt
Brambleby P, 1989	1988	UK	Cross-sectional	Mail	977	Mmr	Mixed	Statistical methods not reported	Refusal
Breitkopf CR, 2009	2007	Vietnam	Cross-sectional	Paper-based	139	Hpv	Closed	Not	Hesitancy/scepticism/doubt
Brieger D, 2017	N.A.	Australia	Cross-sectional	Paper-based	201	Mmr	Mixed	Not	Hesitancy/scepticism/doubt
Brown B, 2017	2015-2016	USA	Cross-sectional	Paper-based	200	Hpv	Closed	Statistical methods not reported	Hesitancy/scepticism/doubt
Brown KF, 2011	2009	UK	Cross-sectional	Mail	535	Mmr	Mixed	Statistical methods not reported	Hesitancy/scepticism/doubt
Brunson EK, 2013	2010	USA	Cross-sectional	On-line	196	Child vaccines	Mixed	With statistical methods	Hesitancy/scepticism/doubt
Bults M, 2011	2009-2010	Netherlands	Cross-sectional	Face to face/mail	1900	Flu	Open field	With statistical methods	Refusal
Burdette AM, 2014	2014	USA	Cross-sectional	Telephone	20,000	Hpv	Closed	With statistical methods	Refusal
Busse JW, 2011	2010	Canada	Cross-sectional	Paper-based	95	Child vaccines	Closed	Statistical methods not reported	Hesitancy/scepticism/doubt
Buyuktiryaki B, 2014	2010	Turkey	Cross-sectional	Paper-based	625	Flu	Likert scale	Statistical methods not reported	Hesitancy/scepticism/doubt
Cacciatore MA, 2016	2014-2015	USA	Cross-sectional	On-line	2,000	Measles	Mixed	Not	Hesitancy/scepticism/doubt
Campbell H, 2017	2015	UK	Cross-sectional	Face to face	1,792	Child vaccines	Mixed	Not	Hesitancy/scepticism/doubt

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Tab. I. Main characteristics of the included studies.

Author, year	Study period	Country	Study design	Administration channel	Sample size	Type of vaccine	Type of questions	Validation	Immunization behaviour
Carlos RC, 2011	N.A.	USA	Cross-sectional	Mail	937	Hpv	Mixed	Not	Hesitancy/ scepticism/ doubt
Casiday R, 2006	2004	UK	Cross-sectional	Mail	996	Mmr	Likert scale	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Cassell JA, 2006	2004	Uk	Cross-sectional	Mail	452	Mmr	Mixed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Cataldi JR, 2016	2015	USA	Cross-sectional	On-line	343	Mmr	Mixed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Chan JY, 2014	2012	Hong Kong	Cross-sectional	Paper-based	1,285	Varicella	Mixed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Chaparro RM, 2016	2012	Argentina	Cross-sectional	Paper-based	77	Hpv	Likert scale	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Chau JPC, 2017	2013	Hong Kong	Cross-sectional	Paper-based	623	Flu	Likert scale	With statistical methods	Hesitancy/ scepticism/ doubt
Chen CH, 2015	2011	Taiwan	Cross-sectional	Mail	1,300	Flu	Likert scale	With statistical methods	Hesitancy/ scepticism/ doubt
Chen MF, 2011	2009	Taiwan	Cross-sectional	Paper-based	2,778	Flu	Mixed	With statistical methods	Hesitancy/ scepticism/ doubt
Cheruvu VK, 2017	2017	USA	Cross-sectional	Telephone	21,467	Hpv	Closed	Not	Refusal
Chung YM, 2017	2012-2014	USA	Cross-sectional	On-line	5,121	Child vaccines	Mixed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Cipriano JJ, 2018	2016	USA	Cross-sectional	On-line	75	Hpv	Mixed	Not	Acceptance
Clark SJ, 2016	2012	USA	Cross-sectional	On-line	1,799	Hpv	Mixed	With statistical methods	Acceptance
Clark SJ, 2016	2012	USA	Cross-sectional	On-line	1,799	Hpv	Mixed	Not	Hesitancy/ scepticism/ doubt
Clark SJ, 2016	2013	USA	Cross-sectional	On-line	1,799	Hpv	Mixed	Not	Hesitancy/ scepticism/ doubt
Cockcroft A, 2014	2011	Nigeria	Cross-sectional	Paper-based	5,257	Measles	Closed	Not	Acceptance
Colon-Lopez V, 2016	2013	Puerto Rico	Cross-sectional	Paper-based	200	Hpv	Mixed	Not	Acceptance
Colon-Lopez V, 2015	2013	Puerto Rico	Cross-sectional	Paper-based	200	Hpv	Mixed	Not	Acceptance
Coniglio MA,2011	2008	Italy	Cross-sectional	Paper-based	1,500	Child vaccines	Closed	Not	Hesitancy/ scepticism/ doubt
Constantine NA, 2007	2006	USA	Cross-sectional	Telephone	802	Hpv	Mixed	Not	Acceptance
Cooper Robbins SC, 2011	2007	Australia	Cross-sectional	Paper-based	169	Flu	Mixed	Not	Acceptance
Costa-Pinto JC, 2017	2014-2015	Australia	Cross-sectional	On-line	612	Child vaccines	Mixed	Not	Acceptance
Coyne-Beasley T, 2013	2008	USA	Cross-sectional	Telephone	1281	Mcv	Mixed	Not	Acceptance

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Tab. I. Main characteristics of the included studies.

Author, year	Study period	Country	Study design	Administration channel	Sample size	Type of vaccine	Type of questions	Validation	Immunization behaviour
Cuninghame CJ, 1994	1991-1992	UK	Cross-sectional	Face to face/ telephone/mail	93	Child vaccines	Mixed	Not	Acceptance
Cunningham-Erves J, 2016	2012-2013	USA	Cross-sectional	Paper-based	242	Hpv	Likert scale	Not	Acceptance
Dahlstrom LA, 2010	2007	Sweden	Cross-sectional	Web /paper based	13,946	Hpv	N.A.	With statistical methods	Acceptance
Daley MF, 2007	2003	USA	Cross-sectional	Telephone	472	Flu	Mixed	Not	Acceptance
Danchin MH, 2017	2015-2016	Australia	Cross-sectional	Questionnaire (ipad), follow-up phone surveys	975	Child vaccines	Mixed	Not	Acceptance
Danis K, 2010	2004–2005	Greece	Cross-sectional	Paper-based	3,434	Child vaccines	Mixed	Not	Acceptance
Dannetun E, 2007	2005	Sweden	Cross-sectional	Paper-based and online	1,229	Hbv	Mixed	Statistical methods not reported	Acceptance
Dannetun E, 2005	2003	Sweden	Cross-sectional	Paper-based	173	Mmr	Mixed	Not	Refusal
Danova J, 2015	2013-2014	Repubblica Ceca	Cross-sectional	Paper-based	480	Child vaccines	Closed	Not	Refusal
Darden PM, 2013	2008–2010	USA	Cross-sectional	Telephone		Dtp/ mcv/ hpv	N.A.	Not	Refusal
Davis K, 2004	2003	USA	Cross-sectional	Paper-based	575	Hpv	Mixed	Not	Acceptance
Dawar M, 2002	1999	Canada	Cross-sectional	Telephone	191	Hbv/ dtp/hib	Mixed	Not	Acceptance
de Courval FP, 2003	2000	Canada	Cross-sectional	Telephone	663	Varicella	Mixed	Not	Refusal
de Visser R, 2008	2008	UK	Cross-sectional	Paper-based	353	Hpv	Likert scale	Not	Acceptance
Dempsey AF, 2011	N.A.	USA	Cross-sectional	Mail	830	Hpv	Mixed	Not	Acceptance
Dempsey AF, 2015	2012-2013	USA	Cross-sectional	On-line	54	Hpv	Mixed	Not	Acceptance
Dempsey AF, 2006	2009	USA	Cross-sectional	On-line	1,178	Hpv	Likert scale	Not	Acceptance
DiAnna Kinder F, 2017	N.A.	USA	Cross-sectional	Paper-based	72	Hpv	Mixed	Not	Refusal
Dinh TA, 2007	2005	Vietnam	Cross-sectional	Paper-based	181	Hpv	Likert scale	Statistical methods not reported	Acceptance
Dorell C, 2014	2010	USA	Cross-sectional	Telephone	4103	Hpv	Closed	Not	Hesitancy/ scepticism/ doubt
Dorell C, 2013	2010-2011	USA	Cross-sectional	Telephone	8,652	Hpv/dtp/ mcv	Closed	Not	Hesitancy/ scepticism/ doubt
Dorell C, 2011	2009-2010	USA	Cross-sectional	Telephone	20,066	Hpv/ mcv/dtp	Closed	Not	Hesitancy/ scepticism/ doubt
Dube E, 2012	2008-2009	Canada	Cohort	Paper-based	413	Rotavirus	Mixed	Not	Acceptance
Dube E, 2015	2014	Canada	Cross-sectional	Telephone	703	Menb	Likert scale	Statistical methods not reported	Acceptance
Dube E, 2017	2015	Canada	Cross-sectional	On-line	20,13	Child vaccines	Mixed	Not	Acceptance
Dube E, 2016	2014	Canada	Cross-sectional	Telephone	589	Child vaccines	Likert scale	Statistical methods not reported	Hesitancy/ scepticism/ doubt

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Tab. I. Main characteristics of the included studies.

Author, year	Study period	Country	Study design	Administration channel	Sample size	Type of vaccine	Type of questions	Validation	Immunization behaviour
Ezat SW, 2013	2012	Malesya	Cross-sectional	Not reported	155	Hpv	N.A.	Not	Acceptance
Ezeanochie MC, 2014	2009	Nigeria	Cross-sectional	Paper-based	201	Hpv	Closed	Statistical methods not reported	Acceptance
Ezenwa BN, 2013	2012	Nigeria	Cross-sectional	Paper-based	290	Hpv	N.A.	Not	Acceptance
Farias CC, 2016	2015	Brazil	Cross-sectional	Paper-based	797	Hpv	Mixed	Not	Acceptance
Flood EM, 2010	2009	USA	Cross-sectional	On-line	500	Flu	Likert scale	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Flynn M, 2004	1999-2000	UK	Cohort	Paper-based	511	Mmr	Likert scale	Not	Hesitancy/ scepticism/ doubt
Freed GL, 2010	2009	USA	Cross-sectional	On-line	1,552	Mmr/ varicella/ mcv/ hpv	N.A.	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Freeman VA, 1999	1995	USA	Cross-sectional	Mail	247	Varicella	Closed	Not	Hesitancy/ scepticism/ doubt
Frew PM, 2016	2012-2014	USA	Cross-sectional	On-line	5,121	Child vaccines	Closed	Not	Acceptance
Frew PM, 2011	2009	USA	Cross-sectional	Not reported	223	Flu	Likert scale	Not	Refusal
Fry AM, 2001	1999-2000	USA	Case-control	Paper-based	66	Hib	Open field	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Fuchs EL, 2016	2011-2013	USA	Cross-sectional	Not reported	350	Hpv	Likert scale	Not	Hesitancy/ scepticism/ doubt
Garcia DA, 2014	2000	Colombia	Cross-sectional	Paper-based	4,802	Child vaccines	Mixed	Not	Hesitancy/ scepticism/ doubt
Gargano LM, 2013	2011	USA	Cross-sectional	Telephone	114	Flu/dtp/ mcv/ hpv	Closed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Gaudino JA, 2012	2004-2005	USA	Cross-sectional	On-line	1,588	Child vaccines	Likert scale	Not	Hesitancy/ scepticism/ doubt
Gefenaite G, 2012	2009	Netherlands	Case-control	Mail	469	Hpv	Closed	Not	Hesitancy/ scepticism/ doubt
Gellatly J, 2005	2003-2004	UK	Cross-sectional	Paper-based	110	Mmr	Likert scale	Not	Hesitancy/ scepticism/ doubt
Gellin BG, 2000	1999	USA	Cross-sectional	Telephone	1,600	Child vaccines	Likert scale	Not	Acceptance
Gentile A, 2015	2013	Argentina	Cross-sectional	Not reported	1,350	Flu	Likert scale	Not	Hesitancy/ scepticism/ doubt
Gerend MA, 2009	2008	USA	Cross-sectional	Paper-based	82	Hpv	Closed	Not	Acceptance
Gesser-Edelsburg A, 2016	2013	Israel	Cross-sectional	On-line	197	Polio	Open field	Not	Refusal
Giambi C, 2014	2012	Italy	Cross-sectional	Mail	1,738	Hpv	Mixed	Not	Refusal
Gilbert NL, 2016	2013	Canada	Cross-sectional	Telephone	5,720	Hpv	Likert scale	Not	Hesitancy/ scepticism/ doubt

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Tab. I. Main characteristics of the included studies.

Author, year	Study period	Country	Study design	Administration channel	Sample size	Type of vaccine	Type of questions	Validation	Immunization behaviour
Gilkey MB, 2017	2014-2015	USA	Cross-sectional	On-line	1,484	Hpv	Closed	Not	Hesitancy/ scepticism/ doubt
Glanz JM, 2013	2009-2011	USA	Cross-sectional	Mail	854	Child vaccines	Closed	Not	Hesitancy/ scepticism/ doubt
Glenn BA, 2015	2009	USA	Cross-sectional	Telephone	444	Hpv	Closed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Gomez Y, 2012	2010	USA	Cross-sectional	Paper-based	773	Flu	N.A.	Not	Hesitancy/ scepticism/ doubt
Gottlieb SL, 2009	2007	USA	Cross-sectional	Telephone	889	Hpv	Mixed	Not	Hesitancy/ scepticism/ doubt
Gowda C, 2013	2011	USA	Cross-sectional	On-line	79	Mmr	Likert scale	Not	Hesitancy/ scepticism/ doubt
Grabiel M, 2013	2012	USA	Cross-sectional	Paper-based	129	Hpv	Closed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Grandahl M, 2014	2012	Sweden	Cross-sectional	Face to face	25	Hpv	Open field	Statistical methods not reported	Refusal
Grandahl M, 2017	2012	Sweden	Cross-sectional	Paper-based	200	Hpv	Likert scale	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Greenberg J, 2017	2015	Canada	Cross-sectional	On-line	1,121	Mmr	Likert scale	Statistical methods not reported	Acceptance
Greenfield LS, 2015	2012	USA	Cross-sectional	Face to face	157	Dtp/ mcv/ hpv	Mixed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Griebeler M, 2012	2010	USA	Cross-sectional	Paper-based	102	Hpv	Likert scale	Statistical methods not reported	Acceptance
Guerry SL, 2011	2007-2008	USA	Cross-sectional	Telephone	509	Hpv	Likert scale	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Gundogdu Z, 2011	2009-2010	Turkey	Cross-sectional	Paper-based	300	Varicella	Likert scale	With statistical methods	Acceptance
Gunduz S, 2014	2011-2012	Turkey	Cross-sectional	Paper-based	285	Flu	Mixed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Gupta R, 2013	2009-2010	USA	Cross-sectional	Paper-based and online	381	Flu	Closed	Not	Hesitancy/ scepticism/ doubt
Gust D, 2005	2002	USA	Cross-sectional	Mail	697	Child vaccines	Likert scale	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Gust DA, 2006	2004-2005	USA	Cross-sectional	Telephone	2,286	Mmr/ dtp/hbv	Closed	Not	Hesitancy/ scepticism/ doubt
Gust DA, 2008	2003-2004	USA	Cross-sectional	Telephone	3,924	Child vaccines	Closed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Gust DA, 2005	2003	USA	Cross-sectional	Mail	642	Child vaccines	Likert scale	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Gust DA, 2004	2001	USA	Case-control	Mail	1,477	Mmr/ dtp/hbv	Likert scale	Statistical methods not reported	Hesitancy/ scepticism/ doubt

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Tab. I. Main characteristics of the included studies.

Author, year	Study period	Country	Study design	Administration channel	Sample size	Type of vaccine	Type of questions	Validation	Immunization behaviour
Gust DA, 2003	2002	USA	Cross-sectional	Mail	1,768	Child vaccines	Likert scale	Statistical methods not reported	Acceptance
Gustafson R, 2005	2003	Canada	Cross-sectional	Telephone	1,246	Varicella	Likert scale	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Haesebaert J, 2012	2008	France	Cross-sectional	Face to face	32	Hpv	Open field	Statistical methods not reported	Acceptance
Hagan D, 2016	2013	Ghana	Cross-sectional	Paper-based	303	Child vaccines	Closed	Statistical methods not reported	Acceptance
Hagemann C, 2017	2009-2011	Germany	Cross-sectional	Paper-based	1,998	Varicella/ measles	N.A.	With statistical methods	Hesitancy/ scepticism/ doubt
Hak E, 2005	N.A.	Netherland	Cross-sectional	Not reported	283	Influenza/ hbv/bcg	Likert scale	Not	Refusal
Hamama-Raz Y, 2016	2014	Israel	Cross-sectional	On-line	314	Child vaccines	Likert scale	Not	Acceptance
Han K, Zheng H, 2014	2010	China	Cross-sectional	Face to face	1,530	Bcg/dtp/ polio/ mcv/ hbv	Closed	With statistical methods	Hesitancy/ scepticism/ doubt
Hanley SJ, 2012	2010	Japan	Cross-sectional	Paper-based	862	Hpv	Likert scale	Not	Acceptance
Hanley SJ, 2014	2010	Japan	Cross-sectional	Paper-based	54	Hpv	Likert scale	Not	Acceptance
Harmsen IA, 2012	2011	Netherlands	Cross-sectional	Paper-based	906	Hbv	Likert scale	Not	Acceptance
He L, 2015	2013	China	Cross-sectional	Face to face	298	Flu	Open field	Not	Hesitancy/ scepticism/ doubt
Healy CM, 2014	N.A.	USA	Cross-sectional	Not reported	401	Hib/pcv/ mcv/ flu/hbv/ hav/hpv/ rotavirus	Closed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Henrikson NB, 2017	2013-2015	USA	Cohort	Telephone	237	Child vaccines	Closed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Hertweck SP, 2013	N.A.	USA	Cross-sectional	On-line	68	Hpv	Likert scale	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Hilyard KM, 2014	2010	USA	Cross-sectional	Not reported	684	Flu	Closed	Not	Hesitancy/ scepticism/ doubt
Hofman R, 2014	2009-2011	Netherlands	Cohort	Mail	793	Hpv	Likert scale	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Hofstetter AM, 2015	2011	USA	Cross-sectional	Face to face	128	Flu	Closed	With statistical methods	Hesitancy/ scepticism/ doubt
Hon KL, 2016	N.A.	Hong Kong	Cross-sectional	Paper-based	3,479	Flu	N.A.	Not	Hesitancy/ scepticism/ doubt
Hontelez JA, 2010	N.A.	Netherland	Cross-sectional	Paper-based	198	Hbv	Likert scale	Not	Hesitancy/ scepticism/ doubt
Horn L, 2010	2008	USA	Cross-sectional	Paper-based	325	Hpv	Likert scale	With statistical methods	Acceptance

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Tab. I. Main characteristics of the included studies.

Author, year	Study period	Country	Study design	Administration channel	Sample size	Type of vaccine	Type of questions	Validation	Immunization behaviour
How CH, 2016	2014	Singapore	Cross-sectional	Face to face	200	Pcv	Likert scale	Not	Hesitancy/ scepticism/ doubt
Hu Y, 2017	2014	China	Cross-sectional	Face to face	2,772	Child vaccines	N.A.	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Hwang JH, 2017	2014	South Korea	Cross-sectional	Face to face	638	Flu	Closed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Ilter E, 2010	2009	Turkey	Cross-sectional	Face to face	525	Hpv	Likert scale	Not	Hesitancy/ scepticism/ doubt
Imburgia TM, 2017	2014	USA	Cross-sectional	On-line	2,363	Flu	Likert scale	Not	Hesitancy/ scepticism/ doubt
Impicciatore P, 2000	1997	Italy	Cross-sectional	Face to face	1,035	Mmr	Closed	With statistical methods	Acceptance
Jani JV, 2008	2001	Mozambique	Cross-sectional	Face to face	668	Child vaccines	N.A.	Statistical methods not reported	Acceptance
Jaspers L, 2011	2009	Indonesia	Cross-sectional	Face to face	746	Hpv	Closed	Not	Acceptance
Jessop LJ, 2010	2001-2004	UK	Cohort	Paper-based	749	Mmr	Closed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Jolley D, 2014	2012	UK	Cross-sectional	On-line	89	Child vaccines	Likert scale	Statistical methods not reported	N.A.
Joseph NP, 2012	2008-2009	USA	Cross-sectional	Face to face	70	Hpv	Open field	With statistical methods	Hesitancy/ scepticism/ doubt
Joseph NP, 2015	N.A.		Cross-sectional	Paper-based	55	Hpv	Closed	With statistical methods	N.A.
Jung M, 2013	N.A.		Cross-sectional	On-line	639	Flu	N.A.	Not	Hesitancy/ scepticism/ doubt
Kadis JA, 2011	2009	USA	Cross-sectional	On-line	496	Hpv	Closed	Statistical methods not reported	Acceptance
Kahn JA, 2009	2006-2007	USA	Cross-sectional	Paper-based	7,207	Hpv	Likert scale	With statistical methods	Hesitancy/ scepticism/ doubt
Kalucka SK, 2016	N.A.	Poland	Cross-sectional	Paper-based	140	Child vaccines	Closed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Katz ML, 2012	2009	USA	Cross-sectional	Paper-based	111	Hpv	Likert scale	Not	Hesitancy/ scepticism/ doubt
Haesebaert J, 2014	2008	France	Cross-sectional	Paper-based	99	Hpv	Open field	With statistical methods	Hesitancy/ scepticism/ doubt
Kaya A, 2017	2016	Turkey	Cross-sectional	Paper-based	102	Flu	Mixed	Not	Acceptance
Kelley CA, 2015	N.A.		Case-control	Paper-based	229	Child vaccines	Mixed	Statistical methods not reported	Refusal
Kempe A, 2007	2003	USA	Cross-sectional	Telephone	472	Flu	Mixed	Statistical methods not reported	Hesitancy/ scepticism/ doubt

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Tab. I. Main characteristics of the included studies.

Author, year	Study period	Country	Study design	Administration channel	Sample size	Type of vaccine	Type of questions	Validation	Immunization behaviour
Kennedy A, 2011	2009	USA	Cross-sectional	Mail	475	Child vaccines	Likert scale	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Kennedy A, 2011	2010	USA	Cross-sectional	Mail	376	Child vaccines	Likert scale	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Kennedy AM, 2005	2002	USA	Cross-sectional	Mail	1527	Child vaccines	Likert scale	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Kepka D, 2015	2013	USA	Cross-sectional	Paper-based	118	Hpv	Closed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Kepka D, 2015	2013	USA	Cross-sectional	Paper-based	67	Hpv	Mixed	With statistical methods	Hesitancy/ scepticism/ doubt
Kepka DL, 2012	2009	USA	Cross-sectional	Paper-based	578	Hpv	Closed	With statistical methods	Hesitancy/ scepticism/ doubt
Kester LM, 2013	2010	USA	Cross-sectional	On-line	501	Hpv	Mixed	Not	Hesitancy/ scepticism/ doubt
Kettunen C, 2017	N.A.	USA	Cross-sectional	Mail	84	Child vaccines	Mixed	Statistical methods not reported	Acceptance
Kim KM, 2017	2014-2015	South Korea	Cross-sectional	Paper-based	200	Hpv	Mixed	Not	Acceptance
Kinder FD. 2016	N.A.	USA	Cross-sectional	Paper-based	72	Hpv	Mixed	Not	Refusal
Ko HS, 2015	N.A.	South Korea	Cross-sectional	Paper-based	308	Dtp	Closed	Not	Acceptance
Kong KA, 2014	2013	South Korea	Cross-sectional	Telephone	800	Hav	Mixed	Statistical methods not reported	Acceptance
Krawczyk A, 2015	2010	Canada	Cross-sectional	Mail	774	Hpv	Likert scale	Not	Hesitancy/ scepticism/ doubt
Krawczyk A, 2015	2010	Canada	Cross-sectional	Mail	708	Hpv	Open field	Not	Refusal
Krieger JL, 2011	N.A.	USA	Cross-sectional	Paper-based	182	Hpv	Closed	Not	Acceptance
Lavail KH, 2013	2010	USA	Case-control	Mail	376	Child vaccines	Likert scale	Not	Acceptance
Le Ngoc Tho S, 2015	2013	France	Cross-sectional	Paper-based	1,270	Menb	Mixed	With statistical methods	Acceptance
Lechuga J, 2012	N.A.	USA	Cross-sectional	Paper-based	150	Hpv	Open field	Not	Acceptance
Lee KN, 2017	2015-2016	South Korea	Cross-sectional	Paper-based	140	Hpv	Mixed	Not	Acceptance
Lee Mortensen G, 2015	2013	Multinational	Cross-sectional	Paper-based	1,837	Hpv	Closed	Not	Acceptance
Lehmann BA, 2017	2015	Netherlands	Cross-sectional	Paper-based	1,615	Child vaccines	Mixed	Not	Acceptance
Lewis T, 1988	1988	USA	Cohort	Mail	2,029	Dtp	N.A.	Not	N.A.
Liao Q, 2016	2012-2013	Hong Kong	Cross-sectional	Telephone	1,226	Flu	Mixed	Not	Acceptance
Lin CJ, 2006	2003-2004	USA	Cross-sectional	Paper-based	951	Flu	Mixed	Not	Acceptance
Linam WM, 2014	2010-2011	USA	Cross-sectional	Paper-based	372	Flu	Mixed	Not	Acceptance
Lindley MC, 2016	2013	USA	Cross-sectional	Mail	6,676	Hpv	Mixed	Not	Acceptance

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Tab. I. Main characteristics of the included studies.

Author, year	Study period	Country	Study design	Administration channel	Sample size	Type of vaccine	Type of questions	Validation	Immunization behaviour
Livni G, 2017	2012	Israel	Cross-sectional	Paper-based	186	Flu	Mixed	Statistical methods not reported	Acceptance
Loke AY, 2017	2010	Hong Kong	Cross-sectional	Paper-based	170	Hpv	Mixed	Not	Hesitancy/ scepticism/ doubt
Low MSF, 2017	2015-2016	Singapore	Cross-sectional	On-line	332	Flu	Mixed	Statistical methods not reported	Acceptance
Luthy KE, 2010	N.A.	USA	Cross-sectional	Paper-based	86	Child vaccines	Mixed	Not	Hesitancy/ scepticism/ doubt
Luthy KE, 2013	N.A.	USA	Cross-sectional	Paper-based	801	Child vaccines	Mixed	Not	Hesitancy/ scepticism/ doubt
Luthy KE, 2009	N.A.	USA	Cross-sectional	Paper-based	86	Child vaccines	Closed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Maayan-Metzger A, 2005	2003	Israel	Case-control	Paper-based	204	Hbv	Closed	Not	Hesitancy/ scepticism/ doubt
MacDonald SE, 2014	N.A.	Canada	Case-control	Mail	444	Child vaccines	Closed	Not	Hesitancy/ scepticism/ doubt
MacDougall DM, 2016	2010-2012	Canada	Case-control	Paper-based	722	Rotavirus	Mixed	Statistical methods not reported	Acceptance
Madhivanan P, 2014	2010	India	Cross-sectional	Mail	797	Hpv	Likert scale	Not	Acceptance
Mameli C, 2014	2013	Italy	Cross-sectional	Paper-based	1,842	Menb	Closed	Not	Hesitancy/ scepticism/ doubt
Marlow LA, 2007	2006	UK	Cross-sectional	Paper-based	684	Hpv	Mixed	Not	Acceptance
Marlow LA, 2007	2006	UK	Cross-sectional	Paper-based	684	Hpv	Likert scale	With statistical methods	Acceptance
Marshall H, 2014	2012	Australia	Cross-sectional	Paper-based	966	Menb	N.A.	With statistical methods	Acceptance
Marshall H, 2007	2006	Australia	Cross-sectional	Telephone	2,002	Hpv	Closed	With statistical methods	Acceptance
Mayet AY, 2017	2013	Saudi Arabia	Cross-sectional	Paper-based	998	Flu	Mixed	Not	Hesitancy/ scepticism/ doubt
McCauley MM, 2012	2010	USA	Cross-sectional	Telephone	690	Child vaccines	Likert scale	Statistical methods not reported	N.A.
McHale P, 2016	2012-2013	UK	Cross-sectional	Telephone	47	Mmr	Open field	Statistical methods not reported	N.A.
Melman ST, 1999	1995-1997	USA	Cross-sectional	Paper-based	1,059	Child vaccines	Open field	Not	N.A.
Meszaros JR, 1996	N.A.	USA	Cross-sectional	Paper-based	294	Pertussis	Mixed	Not	N.A.
Michael CA, 2014	2012	Nigeria	Cross-sectional	Paper-based	48	Polio	Open field	Not	N.A.
Michael CE, 2014	2009	Nigeria	Cross-sectional	Paper-based	201	Hpv	Closed	Not	Acceptance

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Tab. I. Main characteristics of the included studies.

Author, year	Study period	Country	Study design	Administration channel	Sample size	Type of vaccine	Type of questions	Validation	Immunization behaviour
Middleman AB, 2002	2000	USA	Cross-sectional	Paper-based	563	Hbv	Closed	Not	Hesitancy/ scepticism/ doubt
Milteer RM, 1996	1991-1994	USA	Cross-sectional	Paper-based	175	Child vaccines	Open field	Not	N.A.
Morales-Campos DY, 2017	2011-2013	Cameron	Cross-sectional	Paper-based	317	Hpv	Mixed	Not	Hesitancy/ scepticism/ doubt
Morhason-Bello IO, 2015	2012	Nigeria	Cross-sectional	Paper-based	1,002	Hpv	Likert scale	With statistical methods	Acceptance
Morrone T, 2017	2015	Italy	Cross-sectional	Paper-based	543	Menb	N.A.	With statistical methods	N.A.
Moulsdale P, 2017	2014	UK	Cross-sectional	Paper-based	86	Flu	Likert scale	Not	Acceptance
Muhwezi WW, 2014	2012	Uganda	Cross-sectional	Paper-based	870	Hpv	Closed	With statistical methods	Acceptance
Murakami H, 2014	2007	Pakistan	Cross-sectional	Paper-based	630	Polio	Open field	Not	Refusal
My C, 2017	2012	Australia	Cross-sectional	On-line	452	Flu	Closed	Not	Hesitancy/ scepticism/ doubt
Naeem M, 2011	2010	Pakistan	Cross-sectional	Paper-based	548	Polio	Closed	Not	Hesitancy/ scepticism/ doubt
Naeem M, 2011	2010	Pakistan	Cross-sectional	Paper-based	506	Hbv	Closed	Not	Hesitancy/ scepticism/ doubt
Namuigi P, 2005	2003	Papua New Guinea	Cross-sectional	Paper-based	120	Measles	Closed	Not	Hesitancy/ scepticism/ doubt
Niederhauser VP, 2007	2003-2004	USA	Cross-sectional	Paper-based	64	Child vaccines	Open field	Not	Hesitancy/ scepticism/ doubt
Oladokun RE, 2010	2009	Nigeria	Cross-sectional	Paper-based	248	Bcg/ polio/ dtp/ masles/ hbv	Closed	Statistical methods not reported	Acceptance
Onnela JP, 2016	2012	India	Cohort	Paper-based	2,462	Polio	Closed	Statistical methods not reported	Acceptance
Oria PA, 2013	2010	Kenya	Cross-sectional	Paper-based	7,177	Flu	Mixed	Not	N.A.
Ozawa S, 2017	2013	Nigeria	Cross-sectional	Paper-based	198	Dtp/ measles/ polio	Closed	Statistical methods not reported	N.A.
Paek HJ, 2015	2014	South Korea	Cross-sectional	Paper-based	1,017	Child vaccines	Mixed	Not	N.A.
Painter JE, 2011	2009	USA	Cross-sectional	Paper-based	102	Flu	Mixed	Not	N.A.
Parrella A, 2013	2011	Australia	Cross-sectional	Telephone	469	Child vaccines	Likert scale	Not	N.A.
Parrella A, 2012	2010	New Zeland	Cross-sectional	Telephone	179	Dtp/ polio/ hbv/hib/ rotavirus/ mmrv/ mcv/pcv/ flu	Closed	Statistical methods not reported	N.A.
Paulussen TG, 2006	1999	Netherland	Cross-sectional	On-line	491	Dtp/ polio/ hib/mmr	Closed	Not	N.A.

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Tab. I. Main characteristics of the included studies.

Author, year	Study period	Country	Study design	Administration channel	Sample size	Type of vaccine	Type of questions	Validation	Immunization behaviour
Peleg N, 2015	2011	Israel	Cross-sectional	Paper-based	273	Flu	Closed	Statistical methods not reported	N.A.
Pelucchi C, 2010	2008	Italy	Cross-sectional	Paper-based	3,026	Hpv	N.A.	Not	N.A.
Perez S, 2016	N.A.	Canada	Cross-sectional	On-line	2,272	Hpv	Closed	Statistical methods not reported	N.A.
Perez S, 2017	2014	Canada	Cross-sectional	On-line	2,272	Hpv	Closed	With statistical methods	N.A.
Perez S, 2016	2014	Canada	Cross-sectional	On-line	2,272	Hpv	Closed	Statistical methods not reported	N.A.
Perez S, 2016	2014	Canada	Cross-sectional	On-line	2,272	Hpv	Closed	Statistical methods not reported	N.A.
Podolsky R, 2009	N.A.	Usa	Cross-sectional	Paper-based	308	Hpv	Mixed	Statistical methods not reported	N.A.
Pot M, 2017	2015-2016	Netherlands	Cross-sectional	On-line	8,062	Hpv	Closed	Not	N.A.
Reiter PL, 2013	2008-2010	Usa	Cross-sectional	Mail	1,951	Hpv	Mixed	Statistical methods not reported	N.A.
Restivo V, 2015	2012-2013	Italy	Cross-sectional	Telephone	443	Mmr	Closed	Not	N.A.
Roberts JR, 2015	2011-2012	USA	Cross-sectional	Paper-based	363	Dtp/ mcv/ hpv	Closed	Statistical methods not reported	N.A.
Robitz R, 2011	2007-2008	USA	Cross-sectional	Telephone	484	Hpv	Closed	Statistical methods not reported	N.A.
Rogers C, 2014	N.A.	USA	Cross-sectional	On-line	51	Child vaccines	Closed	Not	N.A.
Ruffin MT, 2012	2006-2008	USA	Case-control	Telephone	1,131	Hpv	Closed	Not	N.A.
Salmon DA, 2005	2002-2003	USA	Case-control	Mail	1,367	Polio/ mmrv/ dtp/hib/ hbv	Closed	Not	N.A.
Salmon DA, 2009	N.A.	USA	Case-control	Mail	963	Child vaccines	Closed	Not	N.A.
Sam IC, 2009	2007	Malaysia	Cross-sectional	Paper-based	362	Hpv	Mixed	Not	N.A.
Sampson R, 2011	2008	UK	Cross-sectional	I part mail, ii part interview	7	Flu	Closed	Not	N.A.
Rickert VI, 2015	2012-2013	USA	Cross-sectional	On-line	501	Child vaccines/ flu/pcv/ mmr/ varicella/ dtp/hav/ hbv/hpv/ mcv	Closed	Not	N.A.
Rose SB, 2012	2008-2009	New Zeland	Cross-sectional	Paper-based	769	Hpv	Closed	Not	N.A.
Santibanez TA, 2016	2011-2012	USA	Cross-sectional	Telephone	19,178	Flu	Closed	Not	Hesitancy/ scepticism/ doubt

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Tab. I. Main characteristics of the included studies.

Author, year	Study period	Country	Study design	Administration channel	Sample size	Type of vaccine	Type of questions	Validation	Immunization behaviour
Saqer A, 2017	2017	Emirati Arabi	Cross-sectional	Paper-based	400	Hpv	Closed	With statistical methods	Hesitancy/ scepticism/ doubt
Schollin Ask L, 2017	2014	Sweden	Cross-sectional	On-line	1,063	Rotavirus	Closed	With statistical methods	Hesitancy/ scepticism/ doubt
Schwarz NG, 2009	2009	Gabon	Cross-sectional	Paper-based	40	Child vaccines	Closed	Statistical methods not reported	Acceptance
Selmouni F, 2015	2015	Marocco	Cross-sectional	Paper-based	1,312	Hpv	Open field	Not	Acceptance
Sengupta B, 1998	1998	India	Cross-sectional	Paper-based	656	Polio	Mixed	Not	Hesitancy/ scepticism/ doubt
Seven M, 2015	2015	Turkey	Cross-sectional	Paper-based	368	Hpv	Closed	Not	N.A.
Shao SJ, 2015	2014	Caraibi	Cross-sectional	Paper-based	35	Hpv	Closed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Shapiro GK, 2016	2014	Canada	Cross-sectional	On-line	1,427	Hpv	Mixed	Not	Hesitancy/ scepticism/ doubt
Shapiro GK, 2017	2016-2017	Canada	Cross-sectional	On-line	4,606	Hpv	Open field	Statistical methods not reported	Acceptance
Shawn DH, 1987	1986	Canada	Cross-sectional	Paper-based	133	Hib	Closed	Statistical methods not reported	Hesitancy/ scepticism/ doubt
Sheikh A, 2013	2012-2013	Pakistan	Cross-sectional	Paper-based	1,044	Polio/ tetanus/ measles	Closed	Not	Hesitancy/ scepticism/ doubt
Shuaib FM, 2010	2008	Jamaica	Case-control	Paper-based	285	Child vaccines	Closed	Not	Hesitancy/ scepticism/ doubt
Skinner J, 1995	1992	Australia	Cohort	Mail	1,004	Child vaccines	Mixed	Statistical methods not reported	Acceptance
Smailbegovic MS, 2003	1999	UK	Case-control	On-line	129	Child vaccines	Closed	Not	Hesitancy/ scepticism/ doubt
Smith MJ, 2009	2009	USA	Cross-sectional	On-line	121	Child vaccines	Mixed	Not	Hesitancy/ scepticism/ doubt
Smith PJ, 2011	2009	USA	Cross-sectional	Telephone	11,206	Child vaccines	Closed	Not	Hesitancy/ scepticism/ doubt
Smith PJ, 2010	2003	USA	Cross-sectional	Telephone	2,921	Child vaccines	Closed	Not	Hesitancy/ scepticism/ doubt
Smith PJ, 2006	2001-2002	USA	Cross-sectional	Telephone	7,695	Child vaccines	Closed	Not	Hesitancy/ scepticism/ doubt
Smith PJ, 2015	2010-2013	USA	Cross-sectional	Telephone	19,144	Measles	Closed	Not	Hesitancy/ scepticism/ doubt
Smith PJ, 2016	2010-2014	USA	Cross-sectional	Telephone	8,490	Hpv	Closed	Not	Hesitancy/ scepticism/ doubt
Sohail MM, 2015	N.A.	Pakistan	Cross-sectional	Paper-based	200	Child vaccines	Mixed	Not	Acceptance

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Tab. I. Main characteristics of the included studies.

Author, year	Study period	Country	Study design	Administration channel	Sample size	Type of vaccine	Type of questions	Validation	Immunization behaviour
Songthap A, 2012	2012	Thailandia	Cross-sectional	Paper-based	664	Hpv	Closed	Not	Hesitancy/ scepticism/ doubt
Soyer OU, 2011	2003	USA	Cross-sectional	Telephone	500	Flu	Mixed	Not	Acceptance
Staras SA, 2014	2009	Usa	Cross-sectional	Telephone	2,422	Hpv	Closed	Not	Acceptance
SteelFisher GK, 2015	2013-2014	Multinational	Cross-sectional	Paper-based	6,025	Polio	Closed	Not	Acceptance
Stefanoff P, 2010	2008-2009	Multinational	Cross-sectional	Telephone, paper-based, mail	6,611	Child vaccines	Mixed	Not	Acceptance
Stein Zamir C, 2017	2015	Israel	Cross-sectional	Paper-based	45	Child vaccines	Closed	Not	Acceptance
Stephenson JD, 1987	1986	Canada	Cross-sectional	Paper-based	133	Hib	Closed	Not	Hesitancy/ scepticism/ doubt
Stockwell MS, 2014	2007-2008	USA	Cross-sectional	Paper-based	705	Child vaccines	Mixed	Not	Acceptance
Strelitz B, 2015	2013-2014	USA	Cross-sectional	Paper-based	152	Flu	Likert scale	Not	Hesitancy/ scepticism/ doubt
Stretch R, 2008	2007-2008	UK	Cross-sectional	Paper-based	651	Hpv	Likert scale	Statistical methods not reported	Acceptance
Suarez-Castaneda E, 2014	2011	El Salvador	Cross-sectional	Paper-based	2,550	Child vaccines	Mixed	Not	Acceptance
Sundaram SS, 2010	N.A.	UK	Cross-sectional	Paper-based	50	Hpv	Likert scale	Not	Acceptance
Tadesse H, 2009	2008	Ethiopia	Case-control	Paper-based	266	Child vaccines	Mixed	Statistical methods not reported	Acceptance
Tagbo BN, 2014	2014	Nigeria	Cross-sectional	Paper-based	426	Polio	Likert scale	With statistical methods	Hesitancy/ scepticism/ doubt
Taiwo L, 2017	2015	Nigeria	Cross-sectional	Paper-based	379	Child vaccines	Mixed	Not	Hesitancy/ scepticism/ doubt
Takahashi K, 2014	1999-2003	Japan	Cross-sectional	Paper-based	120	Measles	Mixed	Not	Refusal
Tam WW, 2015	2003	HongKong	Cross-sectional	Paper-based	5,617	Varicella	Likert scale	Not	Acceptance
Tan TNQ, 2017	2011-2013	USA	Cross-sectional	Paper-based	516	Hpv	Mixed	Not	Acceptance
Tang CW, 2011	2006-2008	Taiwan	Cross-sectional	Paper-based	539	Child vaccines	Mixed	Statistical methods not reported	Acceptance
Taylor JA, 1996	1993	USA	Case-control	Paper-based	194	Child vaccines	Likert scale	Statistical methods not reported	Acceptance
Taylor JA, 2002	1998-2000	USA	Cross-sectional	Paper-based	13,520	Child vaccines	Mixed	Not	Acceptance
Thomas T, 2015	N.A.	Georgia	Cross-sectional	Paper-based	37	Hpv	Mixed	Not	Hesitancy/ scepticism/ doubt
Thomas TL, 2012	2010-2011	USA	Cross-sectional	Paper-based	400	Hpv	Mixed	Not	Hesitancy/ scepticism/ doubt
Thomas TL, 2013	2009	USA	Cross-sectional	Paper-based	200	Hpv	Likert scale	Statistical methods not reported	N.A.

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Tab. I. Main characteristics of the included studies.

Author, year	Study period	Country	Study design	Administration channel	Sample size	Type of vaccine	Type of questions	Validation	Immunization behaviour
Thomas TL, 2017	2010-2011	USA	Cross-sectional	Paper-based	341	Hpv	Likert scale	Not	Acceptance
Thompson EL, 2017	2012-2015	USA	Cross-sectional	Telephone	59,897	Hpv	Closed	With statistical methods	Hesitancy/ scepticism/ doubt
Tisi G, 2013	2011	Italy	Cross-sectional	Paper-based	161	Hpv	Mixed	Not	Acceptance
Taylor, JA, 2000	1997-1998	USA	Cross-sectional	Paper-based	598	Varicella	Likert scale	Not	Hesitancy/ scepticism/ doubt
Schwartz B, 2000	2000-2001	USA	Cross-sectional	Telephone	12,060	Hbv/dtp/ measles/ varicella	Mixed	Not	N.A.
Streng A, 2010	2006-2008	Germany	Cross-sectional	Paper-based	1,088	Varicella	Mixed	Statistical methods not reported	Acceptance
Opel DJ, 2011	2010	USA	Cross-sectional	Mail	228	Dtp/ polio/ mmr	Likert scale	Not	Hesitancy/ scepticism/ doubt
World Health Organization, 1997	1994	Italy	Cross-sectional	Face to face	1,800	Dtp/ polio	Closed	With statistical methods	Hesitancy/ scepticism/ doubt

N.A.: not available.

Salvator, Puerto Rico and USA). 14.4% of the studies (n = 48/334)) investigated the Asian population (Hong Kong, China, India, Indonesia, Israel, Japan, South Korea, Kyrgyzstan, Malaysia, Pakistan, Saudi Arabia, United Arab Emirates, Singapore, Taiwan, Thailand and Turkey), 12.8% (n = 26/334) the studies were about European people (Italy, Sweden, United Kingdom, Spain, Greece, France, Germany and the Netherlands), and 11.7% (n = 24/334) of the studies the population was from African and Oceanic countries (Cameroon, Ghana, Kenya, Gabon, Nigeria, Uganda, Morocco, Australia and New Zealand). Only 1.9% of the studies (n = 5/334) were carried out in multiple countries and were therefore classified as multinational surveys.

Almost all of the examined studies, 92.8% (n = 310/334), are cross-sectional; 4.8% (n = 16/334) are case-control studies and finally 2.4% (n = 8/334) are cohort studies.

Even though all the studies included in our review aimed at investigating the phenomenon of vaccine hesitancy, each of them focused on specific aspects of this behaviour. In this respect, the main purpose in 30.8% (n = 103/334) of the studies was to investigate parental knowledge, attitudes, practices, beliefs, awareness, concerns and sources of information about childhood vaccinations. In 21.6% (n = 72/334) of the studies the main objective was focused on investigating parents' attitudes towards childhood vaccinations and exploring possible influential or determining factors. 18.6% (n = 62/334) and 15.0% (n = 50/334) of the studies were aimed respectively at identifying the factors associated with the parental decision to vaccinate and at examining the potential reasons for refusing immunization of their children. In 4.5% (n = 15/334) of the cases, a broad assessment of the vaccine hesitancy phenomenon was specifically investigated.

According to the 3C model, vaccine convenience is determined by physical availability, affordability and willingness-to-pay, geographical accessibility, ability to understand (consisting of both language and health literacy) and appeal of immunization services [9]. Therefore, potential barriers to immunization were also considered in our research and were investigated in 3.6% (n = 12/334) of the studies. Particular attention to the aspect of non-compliance with the vaccination schedule, such as following the correct timing and the complete administration of all the required vaccine doses, was only investigated in 3.3% (n = 11/334) of the analysed studies. Finally, 9 studies (n = 2.7%) explored the various determinants that can condition parental decisions or attitudes towards the immunization of children with pre-existing pathologies or health problems.

Population characteristics

The population interviewed mainly consisted of parents – without any further details (73.1%, n = 244/334) – in approximately 20% of the studies (n = 66/334) the mother was the only parent surveyed, and only 1 study recruited selectively fathers. The sample size ranged from 7 to 59,897, the mean population included was about 1,647 people.

In primary studies, parents were recruited regardless of their attitudes and beliefs in 68.9% of the studies (n = 230/334), while in the remaining 103 articles, the primary

studies selected the population based on their attitude: about 13.5% of the studies (45/334) were conducted in people with a positive attitude of acceptance, 38 studies (11.4%) were conducted among a hesitant population and 20 (6%) selected a population with an attitude of refusal towards vaccines. The definition of "acceptant/hesitant/refusing" behaviour was described in every article considered, and even though the specific characteristics might be slightly different among different studies, we relied on the classification provided by each article to analyse our results. Recruiting parents on the basis of their attitude towards vaccinations was very important in order to analyse the determinants of Vaccine Hesitancy in each different subgroup.

Questionnaires characteristics

The Authors reported both the number and the type of items only in 38.0% (n = 127/334) of the included studies. Regarding the type, more than half (37.7%, n = 126/334) consisted of closed questions. Likert scales were the second most common type used in the questionnaires (23.6%, n = 79/334), while open-ended questions were used in 14.9% of the studies (n = 50/334).

Frequently the studies were conducted using a self-reported questionnaire (69.2%, n = 231/334), or interview (28.1%, n = 94/334), while in 2.7% (n = 9/334) of the studies data were collected in a multi-phase study. Considering the questionnaires, they were mainly administered either on paper (41.6%, n = 139/334) or as an online version (13.5%, n = 45/334). Other administration channels were mail, face to face interviews (9.9%, n = 33/334) or telephone interviews (13.5% 45/334).

However, in 80.2% of the studies, the questionnaire was not attached to the paper and for this reason it was not possible to obtain any further information. Lastly, in 42.8% of the studies (n = 143/334) the questionnaire had been previously validated; however, statistical methods were reported only in 14.8% of the sample (n = 51/334); while in 57.2% (n = 191/334) of the papers the questionnaire had not been validated.

Vaccines and immunization behaviours

22.4% (n = 75/334) of the included articles regarded childhood vaccinations in general, without addressing a specific vaccine. The HPV vaccine was the most frequently investigated (39.2%, n = 133/334), followed by influenza (13.5%, n = 47/334), measles (10.8%, n = 36/334) and varicella or varicella containing vaccine (MMRV) (4.5%, n = 15/334). 67.4% (n = 225/334) of the papers assessed the attitude towards one specific vaccine (monovalent or combined): 5,7% (n = 19/334) of the articles assessed attitudes towards polio vaccine, while 6,3% (n = 22/334) assessed HBV vaccine; a lower percentage reported the behaviour towards meningococcal vaccinations (1.7% - n = 6/334 MenB and 3.6% - n = 12/334 quadrivalent vaccine), Hib vaccine, HAV vaccine, rotavirus and BCG vaccination. 7.5% (n = 25/334) of the studies focused on more than one vaccine, such as diphtheria, tetanus and pertussis vaccination.

Data about the immunization behaviours were reported in 88% of the studies (n = 294/334). In particular, the subjects involved in the studies showed a behaviour defined as "acceptance" in 38.6% studies (38.6%, n = 129/334), as "hesitancy/scepticism/doubt" in 43.4% (n = 145/334) of the studies (and as "refusal" in 6.6% (n = 22/334) of the studies. In 10.5% (n = 35/334) of the studies assessed this information was not detected.

Parents' beliefs about vaccine safety/efficacy

Parents' beliefs about vaccine safety/efficacy were evaluated in most (58.7%; n = 196/334) of the papers included in the review. In particular, 52.4% (n = 175/334) gave a quantitative evaluation, among which 53.7% (n = 94/175) showed that the majority of the sample believed vaccines to be safe and effective, 4.6% of the studies (n = 8/175) showed that the minority of the subjects interviewed believed in vaccine safety/efficacy, while 41.7% (73/175) outlined how the beliefs about vaccines' safety/efficacy are one of the most important barriers in vaccination. Other studies (10.7%, n = 21/196) gave a qualitative and descriptive approach to the issue of "vaccine safety/efficacy". No information was given in 41.3% of the studies (n = 138/334).

Discussion

This manuscript shows the results of an extensive systematic review conducted using three scientific databases (PubMed/Medline, Web of Science and The Cochrane Library). Out of 3,508 retrieved studies, 334 papers were included in the qualitative evaluation. The inclusion of a great number of relevant studies, of which two thirds have been conducted in the last 10 years, reflects the relevance of this issue nowadays: investigating and therefore understanding the phenomenon of vaccine hesitancy is a necessary step in the process of overcoming it. As a matter of fact, it is extremely important to counteract this attitude, as it might lead to a decrease in vaccination coverage and therefore increase the risk of future epidemics of VPDs. The original papers included in the analysis were mainly studies conducted in western countries, while 1/4 were performed in Asia and 1/7 in Africa and Oceania.

Even if all the studies included in the review aimed at exploring VH among parents or guardians, they differ in their study design, overall number of items, context and response formats. Most of the times, three different types of questions were used in the articles examined: closed questions, likert scales questions and open-ended questions (however, a combination of these types of questions was often used as well). Most of studies had a cross-sectional design and were conducted in the last ten years, aimed to investigate parental knowledge, attitudes, practices and beliefs about childhood vaccinations, while only a small percentage (4.5%) investigated the specific

reasons for vaccine hesitancy. Closed questions were the most frequent, mainly through the administration of a self-reported questionnaire, but in most cases it was impossible to get more information about the tool used, because only in 20% of studies the questionnaire was attached to the article.

Closed questions, allowing a quantitative analysis, are a very useful tool although they don't permit to explain with more details the reasons behind VH for vaccine preventable diseases. In fact, it can be defined as "the means for testing objective theories by examining the relationship among variables which in turn can be measured so that numbered data can be analyzed using statistical procedures". On the other hand, a qualitative approach is more likely to use open questions as research tool. Open ended questions don't enable comparisons between different studies but they do provide more detailed information of the issues examined. In fact, a qualitative approach is useful when statistical procedures and numeric data may be insufficient to capture how patients and health care professionals feel about patients' care , enabling researchers to understand the world as another experiences it [351]. Qualitative tools are connected to the way human behavior can be explained, within the framework of the social structures in which that behavior takes place. However, closed questions represent the easiest way to explore a topic and simplify the analysis for the Authors. It should be considered that the way of administration varied among the studies and might have had an impact on the quality of the data generated [352]. Moreover, since questionnaires are a sort of "diagnostic" epidemiological tool, they should be previously validated in order to effectively measure their outcomes [353]. However, only a small percentage of questionnaire had been previously validated in the studies analysed. This aspect should be taken into account, since effectively monitoring VH and identifying beliefs about vaccines is extremely important in order to fully understand the nature of such hesitancy, to compare the phenomenon among countries and over the time, and lastly, to implement the appropriate types of intervention. In addition, only 14.8% of the included studies reported the statistical methods used to validate the questionnaire in detail.

This review shows that the most frequently analysed vaccines are HPV and flu, followed by measles and varicella containing vaccines. They were mainly investigated for the perception about risks and safety, as well for the low vaccine coverages (compared to the WHO target), which is partly due to the reduction in the perceived risk of these diseases [354]. In this perspective, the reinforcement of mandatory vaccination laws in some European countries (e.g. Italy and France) led to an increase in vaccination coverage, mainly because this intervention tackled the complacency component of VH [355, 356]. In Italy, the reinforcement of the mandatory vaccination law dramatically reduced the number of parents who missed the measles vaccination due to definitive informed dissent or unwillingness to attend the appointment [357].

Fathers were specifically investigated only in 1 study included [175] in this review: further studies should investigate this population, in order to determinate possible gender differences in VH definition. It can be speculated that fathers are little involved, by healthcare professionals, in the vaccination decisions of their children. On the contrary, the involvement of both parents could be important in order to recover the confidence of families, which has diminished over time. In this perspective, healthcare professionals should be adequately trained and properly equipped with communication skills to clearly, transparently and comprehensively deal with this problem [358, 359]. Healthcare professionals are the main source of information on the issue of vaccinations, but they are not the only one: parents frequently rely on the information they obtain on the internet, especially regarding vaccinations and the related diseases [360-362].

Before generalizing the results of this review, some limitation should be acknowledged. First, a small percentage of included studies was validated, and the questions identified didn't address all the determinants in the Vaccine Hesitancy Matrix.

Secondly, the findings from studies investigating specific vaccines should not be generalized to all vaccines. Moreover, VH evolves rapidly in time and some determinants could change quickly, not only according to the perception of danger of the diseases reported by media in different countries but also due to other socio-cultural influences. Monitoring the trend is important in order to measure parental VH in time and to better understand parents' concerns and behaviors. Generally speaking, the availability of a good and accurate tool, tested and validated in all settings, and subsequently refined, is necessary to compare the results, to assess the dynamic nature of VH and to develop tailored communication strategies [363-366]. Furthermore, most of the information, especially related to the vaccination status were self-reported and no vaccination cards or Immunization Information System (IIS) were used to verify the information. Healthcare professionals and scientists should be encouraged to use the new technologies, as for instance the IIS, to monitoring both the vaccination coverage and the VH trends [367, 368]. Moreover, according to a recent review, the IIS might greatly improve and counter VH [369].

Nevertheless, a point of strength of this review is the variety of vaccine preventable diseases included. Moreover, to the best of our knowledge, this is the first systematic review that extensively assessed the developed questionnaires aimed to evaluate the parents' VH.

Conclusions

To conclude, VH is a public health challenge as confirmed by the high number of studies and questionnaires retrieved. No questionnaire can be considered the absolute best a priori, but this study offers a deeper perspective on the available questionnaires, therefore

helping future researches to identify the most suitable one according to their own aim and study setting.
Further studies monitoring VH should take into account the questionnaires already available in literature, therefore allowing to improve intra- and inter-country comparability among countries and over time, reducing the time waste in developing a new questionnaire, and improving the financial sustainability of research. Moreover, using a validate questionnaire will improve the methodological quality of future studies.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

VG and PC conceived the study, PC, GV, IB, GD, MN, OG, FD, PS, SDN, SP, AC, LG, FDA, FDG, EA, OES, LK, CA, VG performed a search of the literature, drafted and revised the manuscript. GD revised the language. VG and IB revised critically the manuscript. All authors read and approved the last version of the manuscript.

References

[1] Centers for Disease Control and Prevention (CDC), Ten great public health achievements-United States, 1900-1999. MMWR Morb Mortal Wkly Rep 1999;48:241-3.

[2] Orenstein WA, Douglas RG, Rodewald LE, Hinman AR. Immunizations in the United States: success, structure, and stress. Health Aff (Millwood) 2005;24(3):599-6. https://doi.org/10.1377/hlthaff.24.3.599 .

[3] Freed GL, Clark SJ, Butchart AT, Singer DC, Davis MM. Parental vaccine safety concerns in 2009. Pediatrics 2010;125(4): 654-9. https://doi.org/10.1542/peds.2009-1962

[4] McClure CC, Cataldi JR, O'Leary ST. Vaccine Hesitancy: Where We Are and Where We Are Going. Clin Ther 2017;39(8): 1550-62. https://doi.org/10.1016/j.clinthera.2017.07.003

[5] Patel M, Lee AD, Redd SB, Clemmons NS, McNall RJ, Cohn AC, Gastanaduy PA. Increase in Measles Cases - United States, January 1-April 26, 2019. MMWR Morb Mortal Wkly Rep 2019;68(17): 402-4. https://doi.org/10.15585/mmwr.mm6817e1

[6] Public Health Agency of Canada. Measles & Rubella Weekly Monitoring Report – Week 20: May 12 to May 18, 2019. 2019 November 2019]; Available from: https://www.canada.ca/en/public-health/services/publications/diseases-conditions/measles-rubella-surveillance/2019/week-20.html

[7] European Center for Diseases Control and prevention, Monthly measles and rubella monitoring report, May 2019. 2019.

[8] Zipprich J, Winter K, Hacker J, Xia D, Watt J, Harriman K. Center for Disease Control and Prevention, Measles outbreak--California, December 2014-February 2015. MMWR Morb Mortal Wkly Rep 2015;64(6):153-4.

[9] MacDonald NE, Sage Working Group on Vaccine Hesitancy. Vaccine hesitancy: Definition, scope and determinants. Vaccine 2015;33(34):4161-4. https://doi.org/10.1016/j.vaccine.2015.04.036

[10] World Health Organization, Report of the SAGE working group on vaccine hesitancy, 2014.

[11] Larson HJ, Jarrett C, Eckersberger E, Smith DM, Paterson Understanding vaccine hesitancy around vaccines and vaccination from a global perspective: a systematic review of published literature, 2007-2012. Vaccine 2014;32(19):2150-9. https://doi.org/10.1016/j.vaccine.2014.01.081

[12] Gesser-Edelsburg A, Shir-Raz Y, Green M. Why do parents who usually vaccinate their children hesitate or refuse? General good vs. individual risk. Journal of Risk Research 2016;19(4):405-24. https://doi.org/10.1080/13669877.2014.983947

[13] Harmsen IA, Mollema L, Ruiter RA, Paulussen TG, de Melker HE, Kok G. Why parents refuse childhood vaccination: a qualitative study using online focus groups. BMC Public Health 2013;13:1183. https://doi.org/10.1186/1471-2458-13-1183

[14] European Centre for Disease Prevention and Control, Rapid literature review on motivating hesitant population groups in Europe to vaccinate. 2015: Stockholm.

[15] World Health Organization. Ten threats to global health in 2019, WHO/Rada Akbar. 2019 November 2019]; Available from: https://www.who.int/emergencies/ten-threats-to-global-health-in-2019.

[16] Dorell C, Yankey D. Kennedy A, Stokley S. Factors that influence parental vaccination decisions for adolescents, 13 to 17 years old: National Immunization Survey-Teen, 2010. Clin Pediatr (Phila) 2013;52(2):162-70. https://doi.org/10.1177/0009922812468208

[17] Smith LE, Amlot, R, Weinman J, Yiend J, Rubin GJ. A systematic review of factors affecting vaccine uptake in young children. Vaccine 2017;35(45):6059-69. https://doi.org/10.1016/j.vaccine.2017.09.046

[18] Moher D, Liberati A, Tetzlaff J, Altman DG, Group P Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med 2009;151(4):264-9, W64. https://doi.org/10.7326/0003-4819-151-4-200908180-00135

[19] Childhood vaccination coverage in Italy: results of a seven-region survey. The Italian Vaccine Coverage Survey Working Group. Bull World Health Organ 1994;72(6):885-95.

[20] Adler A, Herring E, Babilsky H, Gazala E, Cohen A, Levy I. Parent-dependent barriers to varicella immunization in Israel: the importance of adequate information. Acta Paediatr 2007;96 (3):428-31. https://doi.org/10.1111/j.1651-2227.2007.00118.x

[21] Adorador A, McNulty R, Hart D, Fitzpatrick, JP. Perceived barriers to immunizations as identified by Latino mothers. Journal of the American Academy of Nurse Practitioners 2011;23 (9):501-8.

[22] Aharony N, Goldman R. E-health literacy and the vaccination dilemma: an Israeli perspective. Information Research-an International Electronic Journal 2017;22 (2).

[23] Akis S, Velipasaoglu S, Camurda AD, Beyazova U, Sahn F. Factors associated with parental acceptance and refusal of pandemic influenza A/H1N1 vaccine in Turkey. Eur J Pediatr 2011; 170 (9):1165-72. https://doi.org/10.1007/s00431-011-1425-6

[24] Akmatov M, Mikolajczyk R, Kretzschmar M, Kramer A. Attitudes and beliefs of parents about childhood vaccinations in post-soviet countries: the example of Kyrgyzstan. The Pediatric infectious disease journal 2009;28(7):637-40.

[25] Alberts CJ, van der Loeff MF, Hazeveld Y, de Melker HE, van der Wal MF, Nielen A, El Fakiri, F, Prins M, Paulusse TG. A longitudinal study on determinants of HPV vaccination uptake

in parents/guardians from different ethnic backgrounds in Amsterdam, the Netherlands. BMC Public Health 2017;17(1): 220. https://doi.org/10.1186/s12889-017-4091-4

[26] Alfredsson R, Svensson E, Trollfors B, Borres M. Why do parents hesitate to vaccinate their children against measles, mumps and rubella? Acta paediatrica 2004;93(9):1232-7.

[27] Allison M, Reyes M, Young P, Calame L, Sheng X, Weng H. Parental attitudes about influenza immunization and school-based immunization for school-aged children. The Pediatric infectious disease journal 2010;29(8):751-5.

[28] Allen J, Othus M, Shelton R, Li Y, Norman N, Tom L. Parental decision making about the HPV vaccine. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 2010;19(9):2187-98.

[29] Allred NJ, Shaw KM, Santibanez TA, Rickert DL, Santoli JM. Parental vaccine safety concerns: results from the National Immunization Survey, 2001-2002. Am J Prev Med 2005;28(2): 221-4. https://doi.org/10.1016/j.amepre.2004.10.014

[30] Alshammari TM, Subaiea GM, Hussain T, Moin A, Yusuff KB. Parental perceptions, attitudes and acceptance of childhood immunization in Saudi Arabia: A cross sectional study. Vaccine 2018;36(1):23-28. https://doi.org/10.1016/j.vaccine.2017.11.050

[31] Ambe JP, Omotara BA, Mandu Baba M. Perceptions, beliefs and practices of mothers in sub-urban and rural areas towards measles and measles vaccination in Northern Nigeria. Trop Doct 2001; 31(2):89-90. https://doi.org/10.1177/004947550103100211

[32] Aharon A, Nehama H, Rishpon S, Baron-Epel O. Parents with high levels of communicative and critical health literacy are less likely to vaccinate their children. Patient Education and Counseling 2017;100(4):768-75.

[33] Arrossi S, Maceira V, Paolino M, Sankaranarayanan R. Acceptability and uptake of HPV vaccine in Argentina before its inclusion in the immunization program: a population-based survey. Vaccine 2012;30(14):2467-74. https://doi.org/10.1016/j.vaccine.2012.01.032

[34] Mohd Azizi FS, Kew Y, Moy FM. Vaccine hesitancy among parents in a multi-ethnic country, Malaysia. Vaccine 2017;35(22): 2955-61. https://doi.org/10.1016/j.vaccine.2017.04.010

[35] Baglioni A, Ceriale E, Bagnoli A, Mercone A, Nante N, Messina G. Parents' awareness and acceptance of HPV vaccination in Italy. Ig Sanita Pubbl 2014;70(5):489-98.

[36] Bakhache P, Rodrigo C, Davie S, Ahuja A, Sudovar B, Crudup T, Rose M. Health care providers' and parents' attitudes toward administration of new infant vaccines - a multinational survey. Eur J Pediatr 2013;172(4):485-92. https://doi.org/10.1007/s00431-012-1904-4

[37] Baldwin AS, Bruce CM, Tiro JA. Understanding how mothers of adolescent girls obtain information about the human papillomavirus vaccine: associations between mothers' health beliefs, information seeking, and vaccination intentions in an ethnically diverse sample. J Health Psychol 2013;18(7):926-38. https://doi.org/10.1177/1359105312445078

[38] Bardenheier B, Gonzalez IM, Washington ML, Bell BP, Averhoff F, Massoudi MS, Hyams I, Simard EP, Yusuf H. Parental knowledge, attitudes, and practices associated with not receiving hepatitis A vaccine in a demonstration project in Butte County, California. Pediatrics 2003;112(4):e269. https://doi.org/10.1542/peds.112.4.e269

[39] Bardenheier B, Yusuf H, Schwartz B, Gust D, Barker L, Rodewald L. Are parental vaccine safety concerns associated with receipt of measles-mumps-rubella, diphtheria and tetanus toxoids with acellular pertussis, or hepatitis B vaccines by children? Arch Pediatr Adolesc Med 2004;158(6):569-75. https://doi.org/10.1001/archpedi.158.6.569

[40] Bardenheier BH, Yusuf HR, Rosenthal J, Santoli JM, Shefer AM, Rickert DL, Chu SY. Factors associated with underimmunization at 3 months of age in four medically underserved areas. Public Health Rep 2004;119(5):479-85. https://doi.org/10.1016/j.phr.2004.07.005

[41] Barnack JL, Reddy DM, Swain C. Predictors of parents' willingness to vaccinate for human papillomavirus and physicians' intentions to recommend the vaccine. Womens Health Issues 20 https://doi.org/10. 20(1): 28-34. https://doi.org/10.1016/j.whi.2009.08.007

[42] Barnack-Tavlaris JL, Garcini LM, Macera CA, Brodine S, Klonoff EA. Human Papillomavirus Vaccination Awareness and Acceptability Among U.S.-Born and U.S. Foreign-Born Women Living in California. Health Care Women Int 2016;37(4): 444-62. https://doi.org/10.1080/07399332.2014.954702

[43] Bazzano A, Zeldin A, Schuster E, Barrett C, Lehrer D. Vaccine-related beliefs and practices of parents of children with autism spectrum disorders. Am J Intellect Dev Disabil 2012;117(3): 233-42. https://doi.org/10.1352/1944-7558-117.3.233

[44] Bedford H, Lansley M. More vaccines for children? Parents' views. Vaccine 2007;25(45):7818-23. https://doi.org/10.1016/j.vaccine.2007.08.057

[45] Beel ER, Rench MA, Montesinos DP, Mayes B, Healy CM. Knowledge and attitudes of postpartum women toward immunization during pregnancy and the peripartum period. Hum Vaccin Immunother 2013;9(9):1926-31. https://doi.org/10.4161/hv.25096

[46] Ben Natan M, Aharon O, Palickshvili S, Gurman V. Attitude of Israeli mothers with vaccination of their daughters against human papilloma virus. J Pediatr Nurs 2011;26(1):70-7. https://doi.org/10.1016/j.pedn.2009.07.006

[47] Ben Natan M, Kabha S, Yehia M, Hamza O. Factors That Influence Israeli Muslim Arab Parents' Intention to Vaccinate Their Children Against Influenza. J Pediatr Nurs 2016;31(3):293-8. https://doi.org/10.1016/j.pedn.2015.12.014

[48] Ben Natan M, Midlej K, Mitelman O, Vafiliev K. Intention of Mothers in Israel to Vaccinate their Sons against the Human Papilloma Virus. J Pediatr Nurs 2017;33:41-5. https://doi.org/10.1016/j.pedn.2017.01.001

[49] Berenson AB, Laz TH, Hirth JM, McGrath CJ, Rahman M. Effect of the decision-making process in the family on HPV vaccination rates among adolescents 9-17 years of age. Hum Vaccin Immunother 2014;10(7):1807-11. https://doi.org/10.4161/hv.28779

[50] Bettinger JA, Greyson D, Money D. Attitudes and Beliefs of Pregnant Women and New Mothers Regarding Influenza Vaccination in British Columbia. J Obstet Gynaecol Can 2016;38(11): 1045-52. https://doi.org/10.1016/j.jogc.2016.08.004

[51] Bham S, Saeed F, Shah M. Routine Immunization in Children and Unsatisfactory Polio Campaigns; A Cross Sectional Survey Conducted at Darul Sehat Hospital, Karachi. Annals Abbasi Shaheed Hospital & Karachi Medical & Dental College 2016; 21(1):29-36.

[52] Bianco A, Pileggi C, Iozzo F, Nobile CG, Pavia M. Vaccination against human papilloma virus infection in male adolescents: knowledge, attitudes, and acceptability among parents in Italy. Hum Vaccin Immunother 2014;10(9):2536-42. https://doi.org/10.4161/21645515.2014.969614

[53] Bigham M, Remple VP, Pielak K, McIntyre C, White R, Wu W. Uptake and behavioural and attitudinal determinants of immunization in an expanded routine infant hepatitis B vaccination program in British Columbia. Can J Public Health 2006; 97(2): 90-5.

[54] Alder S, Gustafsson S, Perinetti C, Mints M, Sundstrom K, Andersson S. Mothers' acceptance of human papillomavirus (HPV) vaccination for daughters in a country with a high prevalence of HPV. Oncol Rep 2015; 33(5):2521-8. https://doi.org/10.3892/or.2015.3817

[55] Basu P, Mittal S. Acceptability of human papillomavirus vaccine among the urban, affluent and educated parents of young girls residing in Kolkata, Eastern India. J Obstet Gynaecol Res 2011;37(5):393-401. https://doi.org/10.1111/j.1447-0756.2010.01371.x

[56] Blair A, Davies E, Nebauer M, Pirozzo S, Saba S, Turner C. Why immunise. Care giver understanding of childhood immunisation. Collegian 1997;4(3):10-7. https://doi.org/10.1016/s1322-7696(08)60235-8

[57] Blyth CC, Richmond PC, Jacoby P, Thornton P, Regan A, Robins C, Kelly H, Smith DW, Effler PV. The impact of pandemic A(H1N1)pdm09 influenza and vaccine-associated adverse events on parental attitudes and influenza vaccine uptake in young children. Vaccine 2014;32(32):4075-81. https://doi.org/10.1016/j.vaccine.2014.05.055

[58] Bodson J, Warner EL, Kepka D. Moderate Awareness and Limited Knowledge Relating to Cervical Cancer, HPV, and the HPV Vaccine Among Hispanics/Latinos in Utah. Health Promot Pract 2016;17(4):548-56. https://doi.org/10.1177/1524839916640271

[59] Bonanni P, Bergamini M. Factors influencing vaccine uptake in Italy. Vaccine 2001;20 Suppl 1:S8-12; discussion S1. https://doi.org/10.1016/s0264-410x(01)00284-5

[60] Borena W, Luckner-Hornischer A, Katzgraber F, Holm-von Laer D. Factors affecting HPV vaccine acceptance in west Austria: Do we need to revise the current immunization scheme? Papillomavirus Res 2016;2:173-7. https://doi.org/10.1016/j.pvr.2016.10.001

[61] Borras E, Dominguez A, Fuentes M, Batalla J, Cardenosa N, Plasencia A. Parental knowledge of paediatric vaccination. BMC Public Health 2009; 9:154. https://doi.org/10.1186/1471-2458-9-154

[62] Brabin L, Roberts SA, Farzaneh F, Kitchener HC. Future acceptance of adolescent human papillomavirus vaccination: a survey of parental attitudes. Vaccine 2006;24(16):3087-94. https://doi.org/10.1016/j.vaccine.2006.01.048

[63] Brambleby P, Hanrahan J. Measles immunisation non-acceptance: validation of computer-held records and raising the vaccine uptake at early school age; the Maidstone experience. Public Health 1989;103(4):289-94. https://doi.org/10.1016/s0033-3506(89)80042-3

[64] Breitkopf CR, Pearson HC, Dinh TA, Tran BC, Vu T, Phan GA, Ngo QV, Tran VD, Rosenthal SL. Human papillomavirus vaccine decision-making in Da Nang, Vietnam: perceived spousal and adolescent-parent concordance. Vaccine 2009;27(17):2367-71. https://doi.org/10.1016/j.vaccine.2009.02.021

[65] Brieger D, Edwards M, Mudgil P, Whitehall J. Knowledge, attitudes and opinions towards measles and the MMR vaccine across two NSW cohorts. Aust N Z J Public Health 2017;41(6):641-646. https://doi.org/10.1111/1753-6405.12720

[66] Brown B, Gabra MI, Pellman H. Reasons for acceptance or refusal of Human Papillomavirus Vaccine in a California pediatric practice. Papillomavirus Res 2017;3:42-5. https://doi.org/10.1016/j.pvr.2017.01.002

[67] Brown KF, Shanley R, Cowley NA, van Wijgerden J, Toff P, Falconer M, Ramsay M, Hudson MJ, Green J, Vincent CA, Kroll JS, Fraser G, Sevdalis N. Attitudinal and demographic predictors of measles, mumps and rubella (MMR) vaccine acceptance: development and validation of an evidence-based measurement instrument. Vaccine 2011;29(8):1700-9. https://doi.org/10.1016/j.vaccine.2010.12.030

[68] Brunson EK. The impact of social networks on parents' vaccination decisions. Pediatrics 2013;131(5):e1397-404. https://doi.org/10.1542/peds.2012-2452

[69] Bults M, Beaujean DJ, Richardus JH, van Steenbergen JE, Voeten HA. Pandemic influenza A (H1N1) vaccination in The Netherlands: parental reasoning underlying child vaccination choices. Vaccine 2011;29(37):6226-35. https://doi.org/10.1016/j.vaccine.2011.06.075

[70] Burdette AM, Gordon-Jokinen H, Hill TD. Social determinants of HPV vaccination delay rationales: Evidence from the 2011 National Immunization Survey-Teen. Prev Med Rep 2014;1:21-6. https://doi.org/10.1016/j.pmedr.2014.09.003

[71] Busse JW, Walji R, Wilson K. Parents' experiences discussing pediatric vaccination with healthcare providers: a survey of Canadian naturopathic patients. PLoS One 2011;6(8):e22737. https://doi.org/10.1371/journal.pone.0022737

[72] Buyuktiryaki B, Soyer OU, Erkocoglu M, Dogan A, Azkur D, Kocabas CN, Dallar Y, Tuncer A, Sekerel BE. What a pandemic teaches us about vaccination attitudes of parents of children with asthma. Vaccine 2014;32(20):2275-80. https://doi.org/10.1016/j.vaccine.2014.02.076

[73] Cacciatore MA, Nowak G, Evans NJ. Exploring The Impact Of The US Measles Outbreak On Parental Awareness Of And Support For Vaccination. Health Aff (Millwood) 2016;35(2):334-40. https://doi.org/10.1377/hlthaff.2015.1093

[74] Campbell H, Edwards A, Letley L, Bedford H, Ramsay M, Yarwood J. Changing attitudes to childhood immunisation in English parents. Vaccine 2017;35(22):2979-85. https://doi.org/10.1016/j.vaccine.2017.03.089

[75] Carlos RC, Dempsey AF, Resnicow K, Ruffin M, Patel DA, Straus CM, Vanessa KD. Maternal characteristics that predict a preference for mandatory adolescent HPV vaccination. Hum Vaccin 2011;7(2):225-9. https://doi.org/10.4161/hv.7.2.13691

[76] Casiday R, Cresswell T, Wilson D, Panter-Brick C. A survey of UK parental attitudes to the MMR vaccine and trust in medical authority. Vaccine 2006;24(2):177-84. https://doi.org/10.1016/j.vaccine.2005.07.063

[77] Cassell JA, Leach M, Poltorak MS, Mercer CH, Iversen A, Fairhead JR. Is the cultural context of MMR rejection a key to an effective public health discourse? Public Health 2006;120(9):783-94. https://doi.org/10.1016/j.puhe.2006.03.011

[78] Cataldi JR, Dempsey AF, O'Leary ST. Measles, the media, and MMR: Impact of the 2014-15 measles outbreak. Vaccine 2016;34(50):6375-80. https://doi.org/10.1016/j.vaccine.2016.10.048

[79] Chan JY, Leung KM, Tam WW, Lee A. Varicella vaccine uptake and associated factors in children in Hong Kong. Epidemiol Infect, 2014; 142(5): 994-1001. https://doi.org/10.1017/S0950268813001994

[80] Chaparro RM, Em Vargas V, Zorzo LR, Genero S, Cayre A. Acceptance of human papillomavirus vaccination and associated factors in the city of Resistencia, Argentina. Arch Argent Pediatr 2016;114(1):36-43. https://doi.org/10.5546/aap.2016.eng.36

[81] Chau JPC, Lo SHS, Choi KC, Chau MHK, Tong DWK, Kwong TKY, Thompson DR. Factors Determining the Uptake of Influenza Vaccination Among Children With Chronic Conditions. Pediatr Infect Dis J 2017;36(7):e197-e202. https://doi.org/10.1097/INF.0000000000001550

[82] Chen CH, Chiu PJ, Chih YC, Yeh GL. Determinants of influenza vaccination among young Taiwanese children. Vaccine 2015;3(16):1993-8. https://doi.org/10.1016/j.vaccine.2015.01.032

[83] Chen MF, Wang RH, Schneider JK, Tsai CT, Jiang DD, Hung MN, Lin LJ. Using the Health Belief Model to understand caregiver factors influencing childhood influenza vaccinations. J Community Health Nurs 2011;28(1):29-40. https://doi.org/10.1080/07370016.2011.539087

[84] Cheruvu VK, Bhatta MP, Drinkard LN. Factors associated with parental reasons for "no-intent" to vaccinate female adolescents with human papillomavirus vaccine: National Immunization Survey - Teen 2008-2012. BMC Pediatr 2017;17(1):52. https://doi.org/10.1186/s12887-017-0804-1

[85] Chung Y, Schamel J, Fisher A, Frew PM. Influences on Immunization Decision-Making among US Parents of Young Children. Matern Child Health J 2017;21(12):2178-87. https://doi.org/10.1007/s10995-017-2336-6

[86] Cipriano JJ, Scoloveno R, Kelly A. Increasing Parental Knowledge Related to the Human Papillomavirus (HPV) Vaccine. J Pediatr Health Care 2018;32(1):29-35. https://doi.org/10.1016/j.pedhc.2017.06.006

[87] Clark SJ, Cowan AE, Filipp SL, Fisher AM, Stokley S. Understanding Non-Completion of the Human Papillomavirus Vaccine Series: Parent-Reported Reasons for Why Adoles-

cents Might Not Receive Additional Doses, United States, 2012. Public Health Rep 2016;131(3):390-5. https://doi.org/10.1177/003335491613100304

[88] Clark SJ, Cowan AE, Filipp SL, Fisher AM, Stokley S. Parent Perception of Provider Interactions Influences HPV Vaccination Status of Adolescent Females. Clin Pediatr (Phila) 2016;55(8): 701-6. https://doi.org/10.1177/0009922815610629

[89] Clark SJ, Cowan AE, Filipp SL, Fisher AM, Stokley S. Parent HPV vaccine perspectives and the likelihood of HPV vaccination of adolescent males. Hum Vaccin Immunother 2016;12(1): 47-51. https://doi.org/10.1080/21645515.2015.1073426

[90] Cockcroft A, Usman MU, Nyamucherera OF, Emori H, Duke B, Umar NA, Andersson N. Why children are not vaccinated against measles: a cross-sectional study in two Nigerian States. Arch Public Health 2014;72(1):48. https://doi.org/10.1186/2049-3258-72-48

[91] Colon-Lopez V, Toro-Mejias LM, Conde-Toro A, Serra-Rivera MJ, Martinez TM, Rodriguez V, Rios AM, Berdiel L, Villanueva H. Views on HPV and HPV Vaccination: The Experience at a Federal Qualified Clinic in Puerto Rico. J Health Care Poor Underserved 2016;27(3):1411-26. https://doi.org/10.1353/hpu.2016.0126

[92] Colon-Lopez V, Quinones V, Del Toro-Mejias LM, Conde-Toro A, Serra-Rivera MJ, Martinez TM, Rodriguez V, Berdiel L, Villanueva H. HPV Awareness and Vaccine Willingness Among Dominican Immigrant Parents Attending a Federal Qualified Health Clinic in Puerto Rico. J Immigr Minor Health 2015; 17(4):1086-90. https://doi.org/10.1007/s10903-014-0067-y

[93] Coniglio MA, Platania M, Privitera D, Giammanco G, Pignato S. Parents' attitudes and behaviours towards recommended vaccinations in Sicily, Italy. BMC Public Health 2011;11:305. https://doi.org/10.1186/1471-2458-11-305

[94] Constantine NA, Jerman P. Acceptance of human papillomavirus vaccination among Californian parents of daughters: a representative statewide analysis. J Adolesc Health 2007;40(2): 108-15. https://doi.org/10.1016/j.jadohealth.2006.10.007

[95] Cooper Robbins SC, Leask J, Booy R. Parents' attitudes towards the influenza vaccine and influencing factors. J Paediatr Child Health 2011;47(7):419-22. https://doi.org/10.1111/j.1440-1754.2010.01993.x

[96] Costa-Pinto JC, Willaby HW, Leask J, Hoq M, Schuster T, Ghazarian A, O'Keefe J, Danchin MH. Parental Immunisation Needs and Attitudes Survey in paediatric hospital clinics and community maternal and child health centres in Melbourne, Australia. J Paediatr Child Health 2018;54(5):522-529. https://doi.org/10.1111/jpc.13790

[97] Coyne-Beasley T, Reiter PL, Liberty AC, Ford CA, Miles DR, Brewer NT. Awareness is not enough: the need to increase meningococcal vaccine uptake. Clin Pediatr (Phila) 2013;52(5): 441-50. https://doi.org/10.1177/0009922813481847

[98] Cuninghame CJ, Charlton CP, Jenkins SM. Immunization uptake and parental perceptions in a strictly orthodox Jewish community in north-east London. J Public Health Med 1994;16(3): 314-7.

[99] Cunningham-Erves J, Talbott LL, O'Neal MR, Ivankova NV, Wallston KA. Development of a Theory-based, Sociocultural Instrument to Assess Black Maternal Intentions to Vaccinate Their Daughters Aged 9 to 12 Against HPV. J Cancer Educ 2016;31(3): 514-21. https://doi.org/10.1007/s13187-015-0867-3

[100] Dahlstrom LA, Tran TN, Lundholm C, Young C, Sundstrom K, Sparen P. Attitudes to HPV vaccination among parents of children aged 12-15 years-a population-based survey in Sweden. Int J Cancer, 20 https://doi.org/10. 126(2): 500-7. https://doi.org/10.1002/ijc.24712

[101] Daley MF, Crane LA, Chandramouli V, Beaty BL, Barrow J, Allred N, Berman S, Kempe A. Misperceptions about influenza vaccination among parents of healthy young children. Clin Pediatr (Phila) 2007;46(5):408-17. https://doi.org/10.1177/0009922806298647

[102] Danchin MH, Costa-Pinto J, Attwell K, Willaby H, Wiley K, Hoq M, Leask J, Perrett KP, O'Keefe J, Giles ML, Marshall H. Vaccine decision-making begins in pregnancy: Correlation between vaccine concerns, intentions and maternal vaccination with subsequent childhood vaccine uptake. Vaccine 2018;36(44):6473-9. https://doi.org/10.1016/j.vaccine.2017.08.003

[103] Danis K, Georgakopoulou T, Stavrou T, Laggas D, Panagiotopoulos T. Socioeconomic factors play a more important role in childhood vaccination coverage than parental perceptions: a cross-sectional study in Greece. Vaccine 2010;28(7):1861-9. https://doi.org/10.1016/j.vaccine.2009.11.078

[104] Dannetun E, Tegnell A, Giesecke J. Parents' attitudes towards hepatitis B vaccination for their children. A survey comparing paper and web questionnaires, Sweden 2005; BMC Public Health 2007;7:86. https://doi.org/10.1186/1471-2458-7-86

[105] Dannetun E, Tegnell A, Hermansson G, Giesecke J. Parents' reported reasons for avoiding MMR vaccination. A telephone survey. Scand J Prim Health Care 2005;23(3):149-53. https://doi.org/10.1080/02813430510031306

[106] Danova J, Salek J, Kocourkova A, Celko AM. Factors Associated with Parental Refusal of Routine Vaccination in the Czech Republic. Cent Eur J Public Health 2015;23(4):321-3. https://doi.org/10.21101/cejph.a4395

[107] Darden PM, Thompson DM, Roberts JR, Hale JJ, Pope C, Naifeh M, Jacobson RM. Reasons for not vaccinating adolescents: National Immunization Survey of Teens, 2008-20 https://doi.org/10. Pediatrics 2013;131(4):645-51. https://doi.org/10.1542/peds.2012-2384

[108] Davis K, Dickman ED, Ferris D, Dias JK. Human papillomavirus vaccine acceptability among parents of 10- to 15-year-old adolescents. J Low Genit Tract Dis 2004;8(3):188-94. https://doi.org/10.1097/00128360-200407000-00005

[109] Dawar M, Dobson S, Kallos A, LaJeunesse C, Weatherill S, Daly P. Measuring hepatitis B uptake in a new universal infant program. Can J Public Health 2002;93(4):281-4.

[110] de Courval FP, De Serres G, Duval B. Varicella vaccine: factors influencing uptake. Can J Public Health 2003;94(4):268-71.

[111] de Visser R, McDonnell E. Correlates of parents' reports of acceptability of human papilloma virus vaccination for their school-aged children. Sex Health 2008;5(4):331-8. https://doi.org/10.1071/sh08042

[112] Dempsey AF, Butchart A, Singer D, Clark S, Davis M. Factors associated with parental intentions for male human papillomavirus vaccination: results of a national survey. Sex Transm Dis 2011;38(8):769-76. https://doi.org/10.1097/OLQ.0b013e318211c248

[113] Dempsey AF, Maertens J, Beaty B, O'Leary ST. Characteristics of users of a tailored, interactive website for parents and its impact on adolescent vaccination attitudes and uptake. BMC Res Notes 2015;8:739. https://doi.org/10.1186/s13104-015-1721-8

[114] Dempsey AF, Zimet GD, Davis RL, Koutsky L. Factors that are associated with parental acceptance of human papillomavirus vaccines: a randomized intervention study of written information about HPV. Pediatrics 2006;117(5): 1486-93. https://doi.org/10.1542/peds.2005-1381

[115] DiAnna Kinder F. Parental Refusal of Human Papillomavirus Vaccine: Multisite Study. J Pediatr Health Care 2018;32(2): 150-6. https://doi.org/10.1016/j.pedhc.2017.09.003

[116] Dinh TA, Rosenthal SL, Doan ED, Trang T, Pham VH, Tran BD, Tran VD, Phan GA, Chu HK, Breitkopf CR. Attitudes of mothers in Da Nang, Vietnam toward a human papillomavirus vaccine. J Adolesc Health 2007;40(6):559-63. https://doi.org/10.1016/j.jadohealth.2007.02.003

[117] Dorell C, Yankey D, Jeyarajah J, Stokley S, Fisher A, Markowitz L, Smith PJ. Delay and refusal of human papillomavirus vaccine for girls, national immunization survey-teen, 2010. Clin Pediatr (Phila) 2014;53(3):261-9. https://doi.org/10.1177/0009922813520070

[118] Dorell C, Yankey D, Strasser S. Parent-reported reasons for non-receipt of recommended adolescent vaccinations, national immunization survey: teen, 2009. Clin Pediatr (Phila) 2011;50(12): 1116-24. https://doi.org/10.1177/0009922811415104

[119] Dube E, Bettinger JA, Halperin B, Bradet R, Lavoie F, Sauvageau C, Gilca V, Boulianne N. Determinants of parents' decision to vaccinate their children against rotavirus: results of a longitudinal study. Health Educ Res 2012;27(6):1069-80. https://doi.org/10.1093/her/cys088

[120] Dube E, Gagnon D, Hamel D, Belley S, Gagne H, Boulianne N, Landry M, Bettinger JA. Parents' and adolescents' willingness to be vaccinated against serogroup B meningococcal disease during a mass vaccination in Saguenay-Lac-St-Jean (Quebec). Can J Infect Dis Med Microbiol 2015;26(3):163-7. https://doi.org/10.1155/2015/732464

[121] Dube E, Gagnon D, Ouakki M, Bettinger JA, Witteman HO, MacDonald S, Fisher W, Saini V, Greyson D, Canadian Immunization Research N. Measuring vaccine acceptance among Canadian parents: A survey of the Canadian Immunization Research Network. Vaccine 2018;36(4):545-52. https://doi.org/10.1016/j.vaccine.2017.12.005

[122] Dube E, Gagnon D, Zhou Z, Deceuninck G, Parental Vaccine Hesitancy in Quebec (Canada). PLoS Curr, 2016. 8. https://doi.org/10.1371/currents.outbreaks.9e239605f4d320c6ad27ce2aea5aaad2

[123] Ezat SW, Hod R, Mustafa J, Mohd Dali AZ, Sulaiman AS, Azman A. National HPV immunisation programme: knowledge and acceptance of mothers attending an obstetrics clinic at a teaching hospital, Kuala Lumpur. Asian Pac J Cancer Prev 2013;14(5): 2991-9. https://doi.org/10.7314/apjcp.2013.14.5.2991

[124] Ezeanochie MC, Olagbuji BN. Human papilloma virus vaccine: determinants of acceptability by mothers for adolescents in Nigeria. Afr J Reprod Health 2014;18(3):154-8.

[125] Ezenwa BN, Balogun MR Okafor IP. Mothers' human papilloma virus knowledge and willingness to vaccinate their adolescent daughters in Lagos, Nigeria. Int J Womens Health 2013;5: 371-7. https://doi.org/10.2147/IJWH.S44483

[126] Farias CC, Jesus DV, Moraes HS, Buttenbender IF, Martins IS, Souto MG. Goncalves Filho, P.H., Costa, R.M., Silva Sde, O., Ferreira, T.S., Coutinho, V.V., Minotto, H.R., and Fonseca, A.J., Factors related to non-compliance to HPV vaccination in Roraima-Brazil: a region with a high incidence of cervical cancer. BMC Health Serv Res 2016;16(1):417. https://doi.org/10.1186/s12913-016-1677-y

[127] Flood EM, Rousculp MD, Ryan KJ, Beusterien KM, Divino VM, Toback SL, Sasane M, Block SL, Hall MC, Mahadevia PJ. Parents' decision-making regarding vaccinating their children against influenza: A web-based survey. Clin Ther 2010;32(8):1448-67. https://doi.org/10.1016/j.clinthera.2010.06.020

[128] Flynn M, Ogden, J. Predicting uptake of MMR vaccination: a prospective questionnaire study. Br J Gen Pract 2004;54(504): 526-30.

[129] Freeman VA, Freed GL. Parental knowledge, attitudes, and demand regarding a vaccine to prevent varicella. Am J Prev Med 1999;17(2):153-5. https://doi.org/10.1016/s0749-3797(99)00063-x

[130] Frew PM, Fisher AK, Basket MM, Chung Y, Schamel J, Weiner JL, Mullen J, Omer SB, Orenstein WA. Changes in childhood immunization decisions in the United States: Results from 2012 & 2014 National Parental Surveys. Vaccine 2016;34(46):5689-96. https://doi.org/10.1016/j.vaccine.2016.08.001

[131] Frew PM, Hixson B, del Rio C, Esteves-Jaramillo A, Omer SB. Acceptance of pandemic 2009 influenza A (H1N1) vaccine in a minority population: determinants and potential points of intervention. Pediatrics 2011;127 Suppl 1:S113-9. https://doi.org/10.1542/peds.2010-1722Q

[132] Fry AM, Lurie P, Gidley M, Schmink S, Lingappa J, Fischer M, Rosenstein NE. Haemophilus influenzae Type b disease among Amish children in Pennsylvania: reasons for persistent disease. Pediatrics 2001;108(4): E60. https://doi.org/10.1542/peds.108.4.e60

[133] Fuchs EL, Rahman M, Berenson AB. Examining maternal beliefs and human papillomavirus vaccine uptake among male and female children in low-income families. Papillomavirus Res 2016;2: 8-40. https://doi.org/10.1016/j.pvr.2016.02.002

[134] Garcia LD, Velandia-Gonzalez M, Trumbo SP, Pedreira MC, Bravo-Alcantara P, Danovaro-Holliday MC. Understanding the main barriers to immunization in Colombia to better tailor communication strategies. BMC Public Health 2014;14:669. https://doi.org/10.1186/1471-2458-14-669

[135] Gargano LM, Herbert NL, Painter JE, Sales JM, Morfaw C, Rask K, Murray D., Di Clemente, RJ, Hughes JM. Impact of a physician recommendation and parental immunization attitudes on receipt or intention to receive adolescent vaccines. Hum Vaccin Immunother 2013;9(12):2627-33. https://doi.org/10.4161/hv.25823

[136] Gaudino JA, Robison S. Risk factors associated with parents claiming personal-belief exemptions to school immunization requirements: community and other influences on more skeptical parents in Oregon, 2006. Vaccine 2012;30(6):1132-42. https://doi.org/10.1016/j.vaccine.2011.12.006

[137] Gefenaite G, Smit M, Nijman HW, Tami A, Drijfhout IH, Pascal A, Postma MJ, Wolters BA, van Delden JJ, Wilschut JC, Hak E. Comparatively low attendance during Human Papillomavirus catch-up vaccination among teenage girls in the Netherlands: Insights from a behavioral survey among parents. BMC Public Health 2012;12:498. https://doi.org/10.1186/1471-2458-12-498

[138] Gellatly J, McVittie C, Tiliopoulos N. Predicting parents' decisions on MMR immunisation: a mixed method investigation. Fam Pract 2005;22(6):658-62. https://doi.org/10.1093/fampra/cmi066

[139] Gellin BG, Maibach EW, Marcuse EK. Do parents understand immunizations? A national telephone survey. Pediatrics 2000; 106(5):1097-102. https://doi.org/10.1542/peds.106.5.1097

[140] Gentile A, Juarez M, Hernandez S, Moya A, Bakir J, Lucion M. Influenza vaccine: Delayed vaccination schedules and missed opportunities in children under 2 years old. Vaccine 2015; 33(32):3913-7. https://doi.org/10.1016/j.vaccine.2015.06.065

[141] Gerend MA, Weibley E, Bland H. Parental response to human papillomavirus vaccine availability: uptake and intentions. J Adolesc Health 2009;45(5):528-31. https://doi.org/10.1016/j.jadohealth.2009.02.006

[142] Gesser-Edelsburg A, Shir-Raz Y, Green M. Why do parents who usually vaccinate their children hesitate or refuse? General good vs. individual risk. Journal of Risk Research 2016;19(4):405-24.

[143] Giambi C, D'Ancona F, Del Manso M, De Mei B, Giovannelli I, Cattaneo C, Possenti V, Declich S, Local Representatives for V. Exploring reasons for non-vaccination against human papillomavirus in Italy. BMC Infect Dis 2014;14:545. https://doi.org/10.1186/s12879-014-0545-9

[144] Gilbert NL, Gilmour H, Dube E, Wilson SE, Laroche J. Estimates and determinants of HPV non-vaccination and vaccine refusal in girls 12 to 14 y of age in Canada: Results from the Childhood National Immunization Coverage Survey, 2013. Hum Vaccin Immunother 2016;12(6):1484-90. https://doi.org/10.1080/21645515.2016.1153207

[145] Gilkey MB, Calo WA, Marciniak MW, Brewer NT. Parents who refuse or delay HPV vaccine: Differences in vaccination behavior, beliefs, and clinical communication preferences. Hum Vaccin Immunother 2017;13(3):680-6. https://doi.org/10.1080/21645515.2016.1247134

[146] Glanz JM, Wagner NM, Narwaney KJ, Shoup JA, McClure DL, McCormick EV, Daley MF. A mixed methods study of parental vaccine decision making and parent-provider trust. Acad Pediatr 2013;13(5) 481-8. https://doi.org/10.1016/j.acap.2013.05.030

[147] Glenn BA, Tsui J, Coronado GD, Fernandez ME, Savas LS,

Taylor VM, Bastani R. Understanding HPV vaccination among Latino adolescent girls in three U.S. regions. J Immigr Minor Health 2015;17(1):96-103. https://doi.org/10.1007/s10903-014-9996-8

[148] Gomez Y, Leguen F, Zhang G, O'Connell E. Correlates of 2009 H1N1 influenza vaccination among day care-aged children, Miami-Dade County. Vaccine 2012;30(27): 4002-6. https://doi.org/10.1016/j.vaccine.2012.04.037

[149] Gottlieb SL, Brewer NT, Sternberg MR, Smith JS, Ziarnowski K, Liddon N, Markowitz LE. Human papillomavirus vaccine initiation in an area with elevated rates of cervical cancer. J Adolesc Health 2009;45(5):430-7. https://doi.org/10.1016/j.jadohealth.2009.03.029

[150] Gowda C, Schaffer SE, Kopec K, Markel A, Dempsey AF. Does the relative importance of MMR vaccine concerns differ by degree of parental vaccine hesitancy?: An exploratory study. Hum Vaccin Immunother 2013;9(2):430-6. https://doi.org/10.4161/hv.22065

[151] Grabiel M, Reutzel TJ, Wang S, Rubin R, Leung V, Ordonez A, Wong M, Jordan E. HPV and HPV vaccines: the knowledge levels, opinions, and behavior of parents. J Community Health 2013;38(6):1015-21. https://doi.org/10.1007/s10900-013-9725-6

[152] Grandahl M, Oscarsson M, Stenhammar C, Neveus T, Westerling R, Tyden T. Not the right time: why parents refuse to let their daughters have the human papillomavirus vaccination. Acta Paediatr 2014;103(4):436-41. https://doi.org/10.1111/apa.12545

[153] Grandahl M, Tyden T, Westerling R, Neveus T, Rosenblad A, Hedin E, Oscarsson M. To Consent or Decline HPV Vaccination: A Pilot Study at the Start of the National School-Based Vaccination Program in Sweden. J Sch Health 2017;87(1):62-70. https://doi.org/10.1111/josh.12470

[154] Greenberg J, Dube E, Driedger M. Vaccine Hesitancy: In Search of the Risk Communication Comfort Zone. PLoS Curr,2017;9. https://doi.org/10.1371/currents.outbreaks.0561a011117a1d1f9596e24949e8690b

[155] Greenfield LS, Page LC, Kay M, Li-Vollmer M, Breuner CC, Duchin JS. Strategies for increasing adolescent immunizations in diverse ethnic communities. J Adolesc Health 2015;56(5 Suppl):S47-53. https://doi.org/10.1016/j.jadohealth.2014.10.274

[156] Griebeler M, Feferman H, Gupta V, Patel D. Parental beliefs and knowledge about male human papillomavirus vaccination in the US: a survey of a pediatric clinic population. Int J Adolesc Med Health 2012;24(4):315-20. https://doi.org/10.1515/ijamh.2012.045

[157] Guerry SL, De Rosa CJ, Markowitz LE, Walker S, Liddon N, Kerndt PR, Gottlieb SL. Human papillomavirus vaccine initiation among adolescent girls in high-risk communities. Vaccine 2011;29(12):2235-41. https://doi.org/10.1016/j.vaccine.2011.01.052

[158] Gundogdu Z, Gundogdu O. Parental attitudes and varicella vaccine in Kocaeli, Turkey. Prev Med 2011;52(3-4):278-80. https://doi.org/10.1016/j.ypmed.2011.01.011

[159] Gunduz S, Yuksel NC, Aktoprak HB, Canbal M, Kaya M. Attitudes towards influenza vaccination in high socioeconomic status Turkish parents. Turk J Med Sci 2014;44(4):649-55. https://doi.org/10.3906/sag-1305-43

[160] Gupta R, Alkhateeb FM, Latif DA, Farley KN. Parental attitudes affecting compliance with the recommendation for two doses of 2009 pandemic influenza A (H1N1) vaccine in children less than 10 years of age in West Virginia. W V Med J 2013;109(2):10-4.

[161] Gust D, Brown C, Sheedy K, Hibbs B, Weaver D, Nowak G. Immunization attitudes and beliefs among parents: beyond a dichotomous perspective. Am J Health Behav 2005;29(1):81-92. https://doi.org/10.5993/ajhb.29.1.7

[162] Gust DA, Campbell S, Kennedy A, Shui I, Barker L, Schwartz B. Parental concerns and medical-seeking behavior after immunization. Am J Prev Med 2006;31(1):32-5. https://doi.org/10.1016/j.amepre.2006.03.017

[163] Gust DA, Darling N, Kennedy A, Schwartz B. Parents with doubts about vaccines: which vaccines and reasons why. Pediatrics 2008;122(4):718-25. https://doi.org/10.1542/peds.2007-0538

[164] Gust DA, Kennedy A, Shui I, Smith PJ, Nowak G, Pickering LK. Parent attitudes toward immunizations and healthcare providers the role of information. Am J Prev Med 2005;29(2):105-12. https://doi.org/10.1016/j.amepre.2005.04.010

[165] Gust DA, Strine TW, Maurice E, Smith P, Yusuf H, Wilkinson M, Battaglia M, Wright R, Schwartz B. Underimmunization among children: effects of vaccine safety concerns on immunization status. Pediatrics 2004;114(1):e16-22. https://doi.org/10.1542/peds.114.1.e16

[166] Gust, D.A., Woodruff, R., Kennedy, A., Brown, C., Sheedy, K., and Hibbs, B., Parental perceptions surrounding risks and benefits of immunization. Semin Pediatr Infect Dis,2003;14(3):207-12. https://doi.org/10.1016/s1045-1870(03)00035-9

[167] Gustafson R, Skowronski DM. Disparities in varicella vaccine coverage in the absence of public funding. Vaccine 2005;23(27):3519-25. https://doi.org/10.1016/j.vaccine.2005.02.001

[168] Haesebaert J, Lutringer-Magnin D, Kalecinski J, Barone G, Jacquard AC, Regnier V, Leocmach Y, Vanhems P, Chauvin F, Lasset C. French women's knowledge of and attitudes towards cervical cancer prevention and the acceptability of HPV vaccination among those with 14 - 18 year old daughters: a quantitative-qualitative study. BMC Public Health 2012;12:1034. https://doi.org/10.1186/1471-2458-12-1034

[169] Hagan D, Phethlu DR. Determinants of parents' decisions on childhood immunisations at Kumasi Metropolis in Ghana. Curationis 2016;39(1): e1-e https://doi.org/10. https://doi.org/10.4102/curationis.v39i1.1554

[170] Hagemann C, Streng A, Kraemer A, Liese JG. Heterogeneity in coverage for measles and varicella vaccination in toddlers - analysis of factors influencing parental acceptance. BMC Public Health 2017;17(1):724. https://doi.org/10.1186/s12889-017-4725-6

[171] Hak E, Schonbeck Y, De Melker H, Van Essen GA, Sanders EA. Negative attitude of highly educated parents and health care workers towards future vaccinations in the Dutch childhood vaccination program. Vaccine 2005;23(24):3103-7. https://doi.org/10.1016/j.vaccine.2005.01.074

[172] Hamama-Raz Y, Ginossar-David E, Ben-Ezra M. Parental regret regarding children's vaccines-The correlation between anticipated regret, altruism, coping strategies and attitudes toward vaccines. Isr J Health Policy Res 2016;5:55. https://doi.org/10.1186/s13584-016-0116-1

[173] Han K, Zheng H, Huang Z, Qiu Q, Zeng H, Chen B, Xu J. Vaccination coverage and its determinants among migrant children in Guangdong, China. BMC Public Health 2014;14:203. https://doi.org/10.1186/1471-2458-14-203

[174] Hanley SJ, Yoshioka E, Ito Y, Konno R, Hayashi Y, Kishi R, Sakuragi N. Acceptance of and attitudes towards human papillomavirus vaccination in Japanese mothers of adolescent girls. Vaccine 2012;30(39):5740-7. https://doi.org/10.1016/j.vaccine.2012.07.003

[175] Hanley SJ, Yoshioka E, Ito Y, Konno R, Sasaki Y, Kishi R, Sakuragi N. An exploratory study of Japanese fathers' knowledge of and attitudes towards HPV and HPV vaccination: does marital status matter? Asian Pac J Cancer Prev 2014;15(4):1837-43. https://doi.org/10.7314/apjcp.2014.15.4.1837

[176] Harmsen IA, Lambooij MS, Ruiter RA, Mollema L, Veldwijk J, van Weert YJ, Kok G, Paulussen TG, de Wit GA, de Melker HE. Psychosocial determinants of parents' intention to vaccinate their newborn child against hepatitis B. Vaccine 2012;30(32):4771-7. https://doi.org/10.1016/j.vaccine.2012.05.034

[177] He L, Liao QY, Huang YQ, Feng S, Zhuang XM. Parents' per-

ception and their decision on their children's vaccination against seasonal influenza in Guangzhou. Chin Med J (Engl) 2015; 128(3):327-41. https://doi.org/10.4103/0366-6999.150099

[178] Healy CM, Montesinos DP, Middleman AB. Parent and provider perspectives on immunization: are providers overestimating parental concerns? Vaccine 2014;32(5): 579-84. https://doi.org/10.1016/j.vaccine.2013.11.076

[179] Henrikson NB, Anderson ML, Opel DJ, Dunn J, Marcuse EK, Grossman DC. Longitudinal Trends in Vaccine Hesitancy in a Cohort of Mothers Surveyed in Washington State, 2013-2015. Public Health Rep 2017;132(4):451-4. https://doi.org/10.1177/0033354917711175

[180] Hertweck SP, LaJoie AS, Pinto MD, Flamini L, Lynch T, Logsdon MC. Health care decision making by mothers for their adolescent daughters regarding the quadrivalent HPV vaccine. J Pediatr Adolesc Gynecol 2013;26(2):96-101. https://doi.org/10.1016/j.jpag.2012.10.009

[181] Hilyard KM, Quinn SC, Kim KH, Musa D, Freimuth VS. Determinants of Parental Acceptance of the H1N1 Vaccine. Health Educ Behav 2014;41(3):307-14. https://doi.org/10.1177/1090198113515244

[182] Hofman R, van Empelen P, Richardus JH, de Kok IM, de Koning HJ, van Ballegooijen M, Korfage IJ. Predictors of HPV vaccination uptake: a longitudinal study among parents. Health Educ Res 2014;29(1):83-96. https://doi.org/10.1093/her/cyt092

[183] Hofstetter AM, Barrett A, Stockwell MS. Factors impacting influenza vaccination of urban low-income Latino children under nine years requiring two doses in the 2010-2011 season. J Community Health 2015;40(2):227-34. https://doi.org/10.1007/s10900-014-9921-z

[184] Hon KL, Tsang YC, Chan LC, Ng DK, Miu TY, Chan JY, Lee A, Leung TF, Hong Kong Society of Paediatric Research and Allergy. A community-based cross-sectional immunisation survey in parents of primary school students. NPJ Prim Care Respir Med 2016;26:16011. https://doi.org/10.1038/npjpcrm.2016.11

[185] Hontelez JA, Hahne SJ, Oomen P, de Melker H. Parental attitude towards childhood HBV vaccination in The Netherlands. Vaccine, 20 https://doi.org/10. 28(4): 1015-20. https://doi.org/10.1016/j.vaccine.2009.10.128

[186] Horn L, Howard C, Waller J, Ferris DG. Opinions of parents about school-entry mandates for the human papillomavirus vaccine. J Low Genit Tract Dis 20 https://doi.org/10. 14(1): 43-8. https://doi.org/10.1097/LGT.0b013e3181b0fad4

[187] How CH, Phua See Chun P, Shafi F, Jakes RW. Parental knowledge, attitudes and perception of pneumococcal disease and pneumococcal conjugate vaccines in Singapore: a questionnaire-based assessment. BMC Public Health 2016;16:923. https://doi.org/10.1186/s12889-016-3597-5

[188] Hu Y, Li Q, Chen Y. Timeliness of Childhood Primary Immunization and Risk Factors Related with Delays: Evidence from the 2014 Zhejiang Provincial Vaccination Coverage Survey. Int J Environ Res Public Health 2017;14(9). https://doi.org/10.3390/ijerph14091086

[189] Hwang JH, Lim CH, Kim DH, Eun BW, Jo DS, Song YH, Kim YK. A Survey of Parental Perception and Pattern of Action in Response to Influenza-like Illness in Their Children: Including Healthcare Use and Vaccination in Korea. J Korean Med Sci 2017;32(2):204-11. https://doi.org/10.3346/jkms.2017.32.2.204

[190] Ilter E, Celik A, Haliloglu B, Unlugedik E, Midi A, Gunduz T, Ozekici U. Women's knowledge of Pap smear test and human papillomavirus: acceptance of HPV vaccination to themselves and their daughters in an Islamic society. Int J Gynecol Cancer 20 https://doi.org/10. 20(6): 1058-62. https://doi.org/10.1111/IGC.0b013e3181dda2b9

[191] Imburgia TM, Hendrix KS, Donahue KL, Sturm LA, Zimet GD. Predictors of influenza vaccination in the U.S. among children 9-13years of age. Vaccine 2017;35(18):2338-42. https://doi.org/10.1016/j.vaccine.2017.03.060

[192] Impicciatore P, Bosetti C, Schiavio S, Pandolfini C, Bonati M. Mothers as active partners in the prevention of childhood diseases: maternal factors related to immunization status of preschool children in Italy. Prev Med 2000;31(1):49-55. https://doi.org/10.1006/pmed.2000.0677

[193] Jani JV, De Schacht C, Jani IV, Bjune G. Risk factors for incomplete vaccination and missed opportunity for immunization in rural Mozambique. BMC Public Health 2008;8:161. https://doi.org/10.1186/1471-2458-8-161

[194] Jaspers L, Budiningsih S, Wolterbeek R, Henderson FC, Peters AA. Parental acceptance of human papillomavirus (HPV) vaccination in Indonesia: a cross-sectional study. Vaccine 2011;29 (44):7785-93. https://doi.org/10.1016/j.vaccine.2011.07.107

[195] Jessop LJ, Murrin C, Lotya J, Clarke AT, O'Mahony D, Fallon UB, Johnson H, Bury G, Kelleher, CC, Murphy AW, Lifeways Cohort Study Steering G. Socio-demographic and health-related predictors of uptake of first MMR immunisation in the Lifeways Cohort Study. Vaccine 20 https://doi.org/10. 28(38): 6338-43. https://doi.org/10.1016/j.vaccine.2010.06.092

[196] Jolley D, Douglas KM. The effects of anti-vaccine conspiracy theories on vaccination intentions. PLoS One 2014;9(2): e89177. https://doi.org/10.1371/journal.pone.0089177

[197] Joseph NP, Clark JA, Bauchner H, Walsh JP, Mercilus G, Figaro J, Bibbo C, Perkins RB. Knowledge, attitudes, and beliefs regarding HPV vaccination: ethnic and cultural differences between African-American and Haitian immigrant women. Womens Health Issues 2012;22(6): e571-9. https://doi.org/10.1016/j.whi.2012.09.003

[198] Joseph NP, Shea K, Porter CL, Walsh JP, Belizaire M, Estervine G, Perkins R. Factors Associated with Human Papillomavirus Vaccine Acceptance Among Haitian and African-American parents of Adolescent Sons. J Natl Med Assoc 2015;107(2):80-8. https://doi.org/10.1016/S0027-9684(15)30028-6

[199] Jung M, Lin L, Viswanath K. Associations between health communication behaviors, neighborhood social capital, vaccine knowledge, and parents' H1N1 vaccination of their children. Vaccine 2013;31(42):4860-6. https://doi.org/10.1016/j.vaccine.2013.07.068

[200] Kadis JA, McRee AL, Gottlieb SL, Lee MR, Reiter PL, Dittus PJ, Brewer NT. Mothers' support for voluntary provision of HPV vaccine in schools. Vaccine 2011;29(14):2542-7. https://doi.org/10.1016/j.vaccine.2011.01.067

[201] Kahn JA, Ding L, Huang B, Zimet GD, Rosenthal SL, Frazier AL. Mothers' intention for their daughters and themselves to receive the human papillomavirus vaccine: a national study of nurses. Pediatrics 2009;123(6):1439-45. https://doi.org/10.1542/peds.2008-1536

[202] Kalucka S, Lopata E. Age-conditioned differences in parents' attitudes towards compulsory vaccination. Family Medicine and Primary Care Review 2016;18(4):425-8.

[203] Katz ML, Kam JA, Krieger JL, Roberto AJ. Predicting human papillomavirus vaccine intentions of college-aged males: an examination of parents' and son's perceptions. J Am Coll Health 2012;60(6):449-59. https://doi.org/10.1080/07448481.2012.673523

[204] Haesebaert J, Lutringer-Magnin D, Kalecinski J, Barone G, Jacquard AC, Leocmach Y, Regnier V, Vanhems P, Chauvin F, Lasset C. Disparities of perceptions and practices related to cervical cancer prevention and the acceptability of HPV vaccination according to educational level in a French cross-sectional survey of 18-65 years old women. PLoS One 2014;9(10):e109320. https://doi.org/10.1371/journal.pone.0109320

[205] Kaya A, Altinel N, Karakaya G, Cetinkaya F. Knowledge and attitudes among patients with asthma and parents and physicians towards influenza vaccination. Allergol Immunopathol (Madr) 2017;45(3):240-3. https://doi.org/10.1016/j.aller.2016.08.003

[206] Kelley CA, Velazco CS, Delaney TV, Bensimhon A, Huang KN, Jarvis PR, Jolin JS, Schaberg KB, Burke M, Finley C, Carney JK. Factors contributing to suboptimal rates of childhood vac-

cinations in Vermont. J Child Health Care 2015;19(4):558-68. https://doi.org/10.1177/1367493514530955

[207] Kempe A, Daley MF, Crane LA, Barrow J, Chandramouli V, Beaty BL, Allred NJ, Berman S. Misperceptions regarding influenza vaccine safety for individuals with chronic medical illness. Prev Med 2007;45(1):80-2. https://doi.org/10.1016/j.ypmed.2006.12.001

[208] Kennedy A, Basket M, Sheedy K. Vaccine attitudes, concerns, and information sources reported by parents of young children: results from the 2009 HealthStyles survey. Pediatrics 2011;127 Suppl 1: S92-9. https://doi.org/10.1542/peds.2010-1722N

[209] Kennedy A, Lavail K, Nowak G, Basket M, Landry S. Confidence about vaccines in the United States: understanding parents' perceptions. Health Aff (Millwood) 2011;30(6):1151-9. https://doi.org/10.1377/hlthaff.2011.0396

[210] Kennedy AM, Brown CJ, Gust DA. Vaccine beliefs of parents who oppose compulsory vaccination. Public Health Rep 2005;120(3):252-8. https://doi.org/10.1177/003335490512000306

[211] Kepka D, Ding Q, Bodson J, Warner EL, Mooney K. Latino Parents' Awareness and Receipt of the HPV Vaccine for Sons and Daughters in a State with Low Three-Dose Completion. J Cancer Educ 2015;30(4):808-12. https://doi.org/10.1007/s13187-014-0781-0

[212] Kepka D, Warner EL, Kinney AY, Spigarelli MG, Mooney K. Low human papillomavirus (HPV) vaccine knowledge among Latino parents in Utah. J Immigr Minor Health 2015;17(1):125-31. https://doi.org/10.1007/s10903-014-0003-1

[213] Kepka DL, Ulrich AK, Coronado GD. Low knowledge of the three-dose HPV vaccine series among mothers of rural Hispanic adolescents. J Health Care Poor Underserved 2012;23(2):626-35. https://doi.org/10.1353/hpu.2012.0040

[214] Kester LM, Zimet GD, Fortenberry JD, Kahn JA, Shew ML. A national study of HPV vaccination of adolescent girls: rates, predictors, and reasons for non-vaccination. Matern Child Health J 2013;17(5):879-85. https://doi.org/10.1007/s10995-012-1066-z

[215] Kettunen C, Nemecek J, Wenger O. Evaluation of low immunization coverage among the Amish population in rural Ohio. Am J Infect Control 2017;45(6):630-4. https://doi.org/10.1016/j.ajic.2017.01.032

[216] Kim KM, Choi JS. Mothers' intentions to vaccinate their teenaged children against human papillomavirus, as predicted by sex in South Korea: An application of the theory of planned behavior. Jpn J Nurs Sci 2017;14(4):288-96. https://doi.org/10.1111/jjns.12155

[217] Kinder F. Parental Refusal of the Human Papillomavirus Vaccine. Journal of Pediatric Health Care 2016;30(6):551-7.

[218] Ko HS, Jo YS, Kim YH, Park YG, Wie JH, Cheon J, Moon HB, Lee Y, Shin JC. Knowledge and Acceptability about Adult Pertussis Immunization in Korean Women of Childbearing Age. Yonsei Med J 2015;56(4):1071-8. https://doi.org/10.3349/ymj.2015.56.4.1071

[219] Kong KA, Yoon SH, Cho SJ, Kim HW, Kim KH. Public acceptance and willingness to hepatitis a vaccination in children aged 7-18 years in Republic of Korea. J Korean Med Sci 2014;29(11):1528-35. https://doi.org/10.3346/jkms.2014.29.11.1528

[220] Krawczyk A, Knauper B, Gilca V, Dube E, Perez S, Joyal-Desmarais K, Rosberger Z. Parents' decision-making about the human papillomavirus vaccine for their daughters: I. Quantitative results. Hum Vaccin Immunother 2015;11(2):322-9. https://doi.org/10.1080/21645515.2014.1004030

[221] Krawczyk A, Perez S, King L, Vivion M, Dube E, Rosberger Z. Parents' decision-making about the human papillomavirus vaccine for their daughters: II. Qualitative results. Hum Vaccin Immunother 2015;11(2):330-6. https://doi.org/10.4161/21645515.2014.980708

[222] Krieger J, Kam J, Katz M, Roberto A. Does mother know best? An actor-partner model of college-age women's human papillomavirus vaccination behavior. Human Communication Research, 2011;31(1):107-24.

[223] Lavail KH, Kennedy AM. The role of attitudes about vaccine safety, efficacy, and value in explaining parents' reported vaccination behavior. Health Educ Behav 2013;40(5):544-51. https://doi.org/10.1177/1090198112463022

[224] Le Ngoc Tho S, Ader F, Ferry T, Floret D, Arnal M, Fargeas S, Chidiac C, Valour F. Vaccination against serogroup B Neisseria meningitidis: Perceptions and attitudes of parents. Vaccine 2015;33(30):3463-70. https://doi.org/10.1016/j.vaccine.2015.05.073

[225] Lechuga J, Swain G, Weinhardt LS. Perceived need of a parental decision aid for the HPV vaccine: content and format preferences. Health Promot Pract 2012;13(2):214-21. https://doi.org/10.1177/1524839910388622

[226] Lee KN, Chang KH, Cho SS, Park SH, Park ST. Attitudes Regarding HPV Vaccinations of Children among Mothers with Adolescent Daughters in Korea. J Korean Med Sci 2017;32(1):130-4. https://doi.org/10.3346/jkms.2017.32.1.130

[227] Lee Mortensen G, Adam M, Idtaleb L. Parental attitudes towards male human papillomavirus vaccination: a pan-European cross-sectional survey. BMC Public Health 2015;15:624. https://doi.org/10.1186/s12889-015-1863-6

[228] Lehmann BA, de Melker HE, Timmermans DRM, Mollema L. Informed decision making in the context of childhood immunization. Patient Educ Couns 2017;100(12):2339-45. https://doi.org/10.1016/j.pec.2017.06.015

[229] Lewis T, Osborn LM, Lewis K, Brockert J, Jacobsen J, Cherry JD. Influence of parental knowledge and opinions on 12-month diphtheria, tetanus, and pertussis vaccination rates. Am J Dis Child 1988;142(3):283-6. https://doi.org/10.1001/archpedi.1988.02150030053018

[230] Liao Q, Lam WW, Cowling BJ, Fielding R. Psychosocial Influences on Parental Decision-Making Regarding Vaccination Against Seasonal Influenza for Young Children in Hong Kong: a Longitudinal Study, 2012-2013. Int J Behav Med 2016;23(5):621-34. https://doi.org/10.1007/s12529-016-9551-1

[231] Lin CJ, Nowalk MP, Zimmerman RK, Ko FS, Zoffel L, Hoberman A, Kearney DH. Beliefs and attitudes about influenza immunization among parents of children with chronic medical conditions over a two-year period. J Urban Health 2006;83(5):874-83. https://doi.org/10.1007/s11524-006-9084-z

[232] Linam WM, Gilliam CH, Honeycutt M, Wisdom C, Swearingen CJ, Romero JR. Parental perceptions about required influenza immunization of pediatric healthcare personnel. Infect Control Hosp Epidemiol 2014;35(10):1301-3. https://doi.org/10.1086/678061

[233] Lindley MC, Jeyarajah J, Yankey D, Curtis CR, Markowitz LE, Stokley S. Comparing human papillomavirus vaccine knowledge and intentions among parents of boys and girls. Hum Vaccin Immunother 2016;12(6):1519-27. https://doi.org/10.1080/21645515.2016.1157673

[234] Livni G, Wainstein A, Birk E, Chodick G, Levy I. Influenza Vaccination Rate and Reasons for Nonvaccination in Children With Cardiac Disease. Pediatr Infect Dis J 2017;36(11):e268-e271. https://doi.org/10.1097/INF.0000000000001579

[235] Loke AY, Chan ACO, Wong YT. Facilitators and barriers to the acceptance of human papillomavirus (HPV) vaccination among adolescent girls: a comparison between mothers and their adolescent daughters in Hong Kong. BMC Res Notes 2017;10(1):390. https://doi.org/10.1186/s13104-017-2734-2

[236] Low MSF, Tan H, Hartman M, Tam CC, Hoo C, Lim J, Chiow S, Lee S, Thng R, Cai M, Tan Y, Lock J. Parental perceptions of childhood seasonal influenza vaccination in Singapore: A cross-sectional survey. Vaccine 2017;35(45):6096-102. https://doi.org/10.1016/j.vaccine.2017.09.060

[237] Luthy KE, Beckstrand RL, Callister LC. Parental hesitation in immunizing children in Utah. Public Health Nurs, 20 https://doi.org/10. 27(1):25-31. https://doi.org/10.1111/j.1525-1446.2009.00823.x

[238] Luthy KE, Beckstrand RL, Meyers CJ. Common perceptions of parents requesting personal exemption from vaccination. J Sch Nurs 2013;29(2):95-103. https://doi.org/10.1177/1059840512455365

[239] Luthy KE, Beckstrand RL, Peterson NE. Parental hesitation as a factor in delayed childhood immunization. J Pediatr Health Care 2009;23(6):388-93. https://doi.org/10.1016/j.pedhc.2008.09.006

[240] Maayan-Metzger A, Kedem-Friedrich P, Kuint J. To vaccinate or not to vaccinate--that is the question: why are some mothers opposed to giving their infants hepatitis B vaccine? Vaccine,2005; 23(16):1941-8. https://doi.org/10.1016/j.vaccine.2004.10.015

[241] MacDonald SE, Schopflocher DP, Vaudry W. Parental concern about vaccine safety in Canadian children partially immunized at age 2: a multivariable model including system level factors. Hum Vaccin Immunother 2014;10(9):2603-11. https://doi.org/10.4161/21645515.2014.970075

[242] MacDougall DM, Halperin BA, Langley JM, MacKinnon-Cameron D, Li L, Halperin SA, Maritime Universal Rotavirus Vaccination Program. Knowledge, attitudes, beliefs, and behaviors of parents and healthcare providers before and after implementation of a universal rotavirus vaccination program. Vaccine 2016; 34(5):687-95. https://doi.org/10.1016/j.vaccine.2015.09.089

[243] Madhivanan P, Li T, Srinivas V, Marlow L, Mukherjee S, Krupp K. Human papillomavirus vaccine acceptability among parents of adolescent girls: obstacles and challenges in Mysore, India. Prev Med 2014;64:69-74. https://doi.org/10.1016/j.ypmed.2014.04.002

[244] Mameli C, Faccini M, Mazzali C, Picca M, Colella G, Duca PG, Zuccotti GV. Acceptability of meningococcal serogroup B vaccine among parents and health care workers in Italy: a survey. Hum Vaccin Immunother 2014;10(10):3004-10. https://doi.org/10.4161/21645515.2014.971602

[245] Marlow LA, Waller J, Wardle J. Trust and experience as predictors of HPV vaccine acceptance. Hum Vaccin 2007;3(5):171-5. https://doi.org/10.4161/hv.3.5.4310

[246] Marlow LA, Waller J, Wardle J. Parental attitudes to pre-pubertal HPV vaccination. Vaccine 2007;25(11):1945-52. https://doi.org/10.1016/j.vaccine.2007.01.059

[247] Marshall H, Clarke M, Sullivan T. Parental and community acceptance of the benefits and risks associated with meningococcal B vaccines. Vaccine 2014;32(3):338-44. https://doi.org/10.1016/j.vaccine.2013.11.042

[248] Marshall H, Ryan P, Roberton D, Baghurst P. A cross-sectional survey to assess community attitudes to introduction of Human papillomavirus vaccine. Aust N Z J Public Health 2007;31(3): 235-42. https://doi.org/10.1111/j.1467-842x.2007.00054.x

[249] Mayet AY, Al-Shaikh GK, Al-Mandeel HM, Alsaleh NA, Hamad AF. Knowledge, attitudes, beliefs, and barriers associated with the uptake of influenza vaccine among pregnant women. Saudi Pharm J 2017;25(1):76-82. https://doi.org/10.1016/j.jsps.2015.12.001

[250] McCauley MM, Kennedy A, Basket M, Sheedy K. Exploring the choice to refuse or delay vaccines: a national survey of parents of 6- through 23-month-olds. Acad Pediatr 2012;12(5): 375-83. https://doi.org/10.1016/j.acap.2012.06.007

[251] McHale P, Keenan A, Ghebrehewet S. Reasons for measles cases not being vaccinated with MMR: investigation into parents' and carers' views following a large measles outbreak. Epidemiol Infect 2016;144(4):870-5. https://doi.org/10.1017/S0950268815001909

[252] Melman ST, Nguyen TT, Ehrlich E, Schorr M, Anbar RD. Parental compliance with multiple immunization injections. Arch Pediatr Adolesc Med 1999;153(12):1289-91. https://doi.org/10.1001/archpedi.153.12.1289

[253] Meszaros JR, Asch DA, Baron J, Hershey JC, Kunreuther H, Schwartz-Buzaglo J. Cognitive processes and the decisions of some parents to forego pertussis vaccination for their children. J Clin Epidemiol 1996;49(6):697-703. https://doi.org/10.1016/0895-4356(96)00007-8

[254] Michael CA, Ogbuanu IU, Storms AD, Ohuabunwo CJ, Corkum M, Ashenafi S, Achari P, Biya O, Nguku P, Mahoney F, Team NORS. An assessment of the reasons for oral poliovirus vaccine refusals in northern Nigeria. J Infect Dis 2014;210 Suppl 1:S125-30. https://doi.org/10.1093/infdis/jiu436

[255] Michael EC, Olagbuji BN. Human papilloma virus vaccine: determinants of acceptability by mothers for adolescents in Nigeria. Afr J Reprod Health 2014;18(3):154-8.

[256] Middleman AB, Guajardo AD, Sunwoo E, Sansaricq KM. Parent knowledge and attitudes about school-based hepatitis B immunization programs. J Sch Health 2002;72(8):348-51. https://doi.org/10.1111/j.1746-1561.2002.tb07923.x

[257] Milteer RM, Jonna S. Parental reasons for delayed immunizations in children hospitalized in a Washington, DC, public hospital. J Natl Med Assoc 1996;88(7):433-6.

[258] Morales-Campos DY, Parra-Medina D. Predictors of Human Papillomavirus Vaccine Initiation and Completion Among Latino Mothers of 11- to 17-Year-Old Daughters Living Along the Texas-Mexico Border. Fam Community Health 2017;40(2): 139-49. https://doi.org/10.1097/FCH.0000000000000144

[259] Morhason-Bello IO, Wallis S, Adedokun BO, Adewole IF. Willingness of reproductive-aged women in a Nigerian community to accept human papillomavirus vaccination for their children. J Obstet Gynaecol Res 2015;41(10):1621-9. https://doi.org/10.1111/jog.12775

[260] Morrone T, Napolitano F, Albano L, Di Giuseppe G. Meningococcal serogroup B vaccine: Knowledge and acceptability among parents in Italy. Hum Vaccin Immunother 2017;13(8): 1921-7. https://doi.org/10.1080/21645515.2017.1313940

[261] Moulsdale P, Grant A, Fletcher M, Finn A. Parents' perceptions of influenza and why they accept or decline the nasal vaccine for their child. Nurs Child Young People 2017;29(3):28-33. https://doi.org/10.7748/ncyp.2017.e854

[262] Muhwezi WW, Banura C, Turiho AK, Mirembe F. Parents' knowledge, risk perception and willingness to allow young males to receive human papillomavirus (HPV) vaccines in Uganda. PLoS One 2014;9(9):e106686. https://doi.org/10.1371/journal.pone.0106686

[263] Murakami H, Kobayashi M, Hachiya M, Khan ZS, Hassan SQ, Sakurada S. Refusal of oral polio vaccine in northwestern Pakistan: a qualitative and quantitative study. Vaccine 2014;32(12): 1382-7. https://doi.org/10.1016/j.vaccine.2014.01.018

[264] My C, Danchin M, Willaby HW, Pemberton S, Leask J. Parental attitudes, beliefs, behaviours and concerns towards childhood vaccinations in Australia: A national online survey. Aust Fam Physician 2017;46(3):145-51.

[265] Naeem M, Adil M, Abbas SH, Khan MZ, Naz SM, Khan A, Khan, MU. Coverage and causes of missed oral polio vaccine in urban and rural areas of Peshawar. J Ayub Med Coll Abbottabad 2011;23(4):98-102.

[266] Naeem M, Khan MZ, Abbas SH, Adil M, Khan MU, Naz SM. Factors associated with low hepatitis B vaccination; a user and provider perspective study in Peshawar. J Pak Med Assoc 2011;61(11):1125-7.

[267] Namuigi P, Phuanukoonnon S. Barriers to measles immunization: the beliefs and attitudes of caregivers in Goroka, Eastern Highlands Province, Papua New Guinea. P N G Med J,2005; 48(3-4):183-7.

[268] Niederhauser VP, Markowitz M. Barriers to immunizations: Multiethnic parents of under- and unimmunized children speak. J Am Acad Nurse Pract 2007;19(1):15-23. https://doi.org/10.1111/j.1745-7599.2006.00185.x

[269] Oladokun RE, Adedokun BO, Lawoyin TO. Children not receiving adequate immunization in Ibadan, Nigeria: what reasons and beliefs do their mothers have? Niger J Clin Pract 20 https://doi.org/10. 13(2):173-8.

[270] Onnela JP, Landon BE, Kahn AL, Ahmed D, Verma H, O'Malley AJ, Bahl S, Sutter RW, Christakis NA. Polio vaccine hesitancy in the networks and neighborhoods of Malegaon, India. Soc Sci Med 2016;153:99-106. https://doi.org/10.1016/j.socscimed.2016.01.024

[271] Oria PA, Arunga G, Lebo E, Wong JM, Emukule G, Muthoka P, Otieno N, Mutonga D, Breiman, RF, Katz MA. Assessing parents' knowledge and attitudes towards seasonal influenza vaccination of children before and after a seasonal influenza vaccination effectiveness study in low-income urban and rural Kenya, 2010-2011. BMC Public Health 2013;13:391. https://doi.org/10.1186/1471-2458-13-391

[272] Ozawa S, Wonodi C, Babalola O, Ismail T, Bridges J. Using best-worst scaling to rank factors affecting vaccination demand in northern Nigeria. Vaccine 2017;35(47):6429-37. https://doi.org/10.1016/j.vaccine.2017.09.079

[273] Paek HJ, Shin KA, Park K. Determinants of caregivers' vaccination intention with respect to child age group: a cross-sectional survey in South Korea. BMJ Open 2015;5(9):e008342. https://doi.org/10.1136/bmjopen-2015-008342

[274] Painter JE, Gargano LM, Sales JM, Morfaw C, Jones LM, Murray D, Di Clemente RJ, Hughes, JM. Correlates of 2009 H1N1 influenza vaccine acceptability among parents and their adolescent children. Health Educ Res 2011;26(5):751-60. https://doi.org/10.1093/her/cyr025

[275] Parrella A, Gold M, Marshall H, Braunack-Mayer A, Baghurst P. Parental perspectives of vaccine safety and experience of adverse events following immunisation. Vaccine 2013 31(16): 2067-74. https://doi.org/10.1016/j.vaccine.2013.02.011

[276] Parrella A, Gold M, Marshall H, Braunack-Mayer A, Watson M, Baghurst P. Parental views on vaccine safety and future vaccinations of children who experienced an adverse event following routine or seasonal influenza vaccination in 20 https://doi.org/10. Hum Vaccin Immunother 2012;8(5):662-7. https://doi.org/10.4161/hv.19478

[277] Paulussen TG, Hoekstra F, Lanting CI, Buijs GB, Hirasing RA. Determinants of Dutch parents' decisions to vaccinate their child. Vaccine 2006;24(5):644-51. https://doi.org/10.1016/j.vaccine.2005.08.053

[278] Peleg N, Zevit N, Shamir R, Chodick G, Levy I. Seasonal influenza vaccination rates and reasons for non-vaccination in children with gastrointestinal disorders. Vaccine 2015;33(1):182-6. https://doi.org/10.1016/j.vaccine.2014.10.086

[279] Pelucchi C, Esposito S, Galeone C, Semino M, Sabatini C, Picciolli I, Consolo S, Milani G, Principi N. Knowledge of human papillomavirus infection and its prevention among adolescents and parents in the greater Milan area, Northern Italy. BMC Public Health 2010;10:378. https://doi.org/10.1186/1471-2458-10-378

[280] Perez S, Shapiro GK, Tatar O, Joyal-Desmarais K, Rosberger Z. Development and Validation of the Human Papillomavirus Attitudes and Beliefs Scale in a National Canadian Sample. Sex Transm Dis 2016;43(10):626-32. https://doi.org/10.1097/OLQ.0000000000000506

[281] Perez S, Tatar O, Gilca V, Shapiro GK, Ogilvie G, Guichon J, Naz A, Rosberger Z. Untangling the psychosocial predictors of HPV vaccination decision-making among parents of boys. Vaccine 2017;35(36):4713-21. https://doi.org/10.1016/j.vaccine.2017.07.043

[282] Perez S, Tatar O, Ostini R, Shapiro GK, Waller J, Zimet G, Rosberger Z. Extending and validating a human papillomavirus (HPV) knowledge measure in a national sample of Canadian parents of boys. Prev Med 2016;91:43-49. https://doi.org/10.1016/j.ypmed.2016.07.017

[283] Perez S, Tatar O, Shapiro GK, Dube E, Ogilvie G, Guichon J, Gilca V, Rosberger Z. Psychosocial determinants of parental human papillomavirus (HPV) vaccine decision-making for sons: Methodological challenges and initial results of a pan-Canadian longitudinal study. BMC Public Health 2016;16(1):1223. https://doi.org/10.1186/s12889-016-3828-9

[284] Podolsky R, Cremer M, Atrio J, Hochman T, Arslan AA. HPV vaccine acceptability by Latino parents: a comparison of U.S. and Salvadoran populations. J Pediatr Adolesc Gynecol 2009; 22(4):205-15. https://doi.org/10.1016/j.jpag.2008.05.010

[285] Pot M, van Keulen HM, Ruiter RAC, Eekhout I, Mollema L, Paulussen T. Motivational and contextual determinants of HPV-vaccination uptake: A longitudinal study among mothers of girls invited for the HPV-vaccination. Prev Med 2017;100: 41-49. https://doi.org/10.1016/j.ypmed.2017.04.005

[286] Reiter PL, Katz ML, Paskett ED. Correlates of HPV vaccination among adolescent females from Appalachia and reasons why their parents do not intend to vaccinate. Vaccine 2013;31(31): 3121-5. https://doi.org/10.1016/j.vaccine.2013.04.068

[287] Restivo V, Napoli G, Marsala MG, Bonanno V, Sciuto V, Amodio E, Calamusa G, Vitale F, and Firenze A. Factors associated with poor adherence to MMR vaccination in parents who follow vaccination schedule. Hum Vaccin Immunother 2015;11(1): 140-5. https://doi.org/10.4161/hv.34416

[288] Roberts JR, Thompson D, Rogacki B, Hale JJ, Jacobson RM, Opel DJ, Darden PM. Vaccine hesitancy among parents of adolescents and its association with vaccine uptake. Vaccine 2015; 33(14):1748-55. https://doi.org/10.1016/j.vaccine.2015.01.068

[289] Robitz R, Gottlieb SL, De Rosa CJ, Guerry SL, Liddon N, Zaidi A, Walker S, Smith JS. Brewer, N.T., and Markowitz, L.E., Parent attitudes about school requirements for human papillomavirus vaccine in high-risk communities of Los Angeles, California. Cancer Epidemiol Biomarkers Prev 2011;20(7):1421-9. https://doi.org/10.1158/1055-9965.EPI-10-1236

[290] Rogers C. Parents' vaccine beliefs: a study of experiences and attitudes among parents of children in private pre-schools. R I Med J (2013), 2014;97(4):27-30.

[291] Ruffin MTT, Hade EM, Gorsline MR, DeGraffinreid CR, Katz ML, Kobrin SC, Paskett ED. Human papillomavirus vaccine knowledge and hypothetical acceptance among women in Appalachia Ohio. Vaccine 2012;30(36):5349-57. https://doi.org/10.1016/j.vaccine.2012.06.034

[292] Salmon DA, Moulton LH, Omer SB, DeHart MP, Stokley S, Halsey NA. Factors associated with refusal of childhood vaccines among parents of school-aged children: a case-control study. Arch Pediatr Adolesc Med 2005;159(5) 470-6. https://doi.org/10.1001/archpedi.159.5.470

[293] Salmon DA, Sotir MJ, Pan WK, Berg JL, Omer SB, Stokley S, Hopfensperger DJ, Davis JP, Halsey NA. Parental vaccine refusal in Wisconsin: a case-control study. WMJ, 2009;108(1):17-23.

[294] Sam IC, Wong LP, Rampal S, Leong YH, Pang CF, Tai YT, Tee HC, Kahar-Bador M. Maternal acceptance of human papillomavirus vaccine in Malaysia. J Adolesc Health 2009;44(6) 610-2. https://doi.org/10.1016/j.jadohealth.2008.11.014

[295] Sampson R, Wong L. Macvicar R. Parental reasons for non-uptake of influenza vaccination in young at-risk groups: a qualitative study. Br J Gen Pract 2011;61(588):e386-91. https://doi.org/10.3399/bjgp11X583155

[296] Rickert VI, Rehm SJ, Aalsma MC, Zimet GD. The role of parental attitudes and provider discussions in uptake of adolescent vaccines. Vaccine 2015;33(5):642-7. https://doi.org/10.1016/j.vaccine.2014.12.016

[297] Rose SB, Lawton BA, Lanumata TS, Hibma M, Baker MG. Predictors of intent to vaccinate against HPV/cervical cancer: a multi-ethnic survey of 769 parents in New Zealand. N Z Med J 2012;125(1350):51-62.

[298] Santibanez TA, Kennedy ED. Reasons given for not receiving an influenza vaccination, 2011-12 influenza season, United States. Vaccine 2016;34(24):2671-8. https://doi.org/10.1016/j.vaccine.2016.04.039

[299] Saqer A, Ghazal S, Barqawi H, Babi JA, Al Khafaji R, Elmekresh MM. Knowledge and Awareness about Cervical Cancer Vaccine (HPV) Among Parents in Sharjah. Asian Pac J Cancer Prev 2017;18(5):1237-41. https://doi.org/10.22034/APJCP.2017.18.5.1237

[300] Schollin Ask L, Hjern A, Lindstrand A, Olen O, Sjogren E, Blennow M, Ortqvist A. Receiving early information and trusting Swedish child health centre nurses increased parents' willingness to vaccinate against rotavirus infections. Acta Paediatr 2017;106(8):1309-16. https://doi.org/10.1111/apa.13872

[301] Schwarz NG, Gysels M, Pell C, Gabor J, Schlie M, Issifou S, Lell B, Kremsner PG, Grobusch MP, Pool R. Reasons for non-adherence to vaccination at mother and child care clinics (MCCs) in Lambarene, Gabon. Vaccine 2009;27(39):5371-5. https://doi.org/10.1016/j.vaccine.2009.06.100

[302] Selmouni F, Zidouh A, Nejjari C, Bekkali R. Acceptability of the human papilloma virus vaccine among Moroccan parents: a population-based crosssectional study. East Mediterr Health J 2015;21(8):555-63. https://doi.org/10.26719/2015.21.8.555

[303] Sengupta B, Sinha RN, Sarkar GN, Biswas AB, Mukherjee KL. Perception and practice regarding pulse polio immunisation in an urban community of Calcutta. J Indian Med Assoc 1998;96(8):247-8.

[304] Seven M, Guvenc G, Sahin E, Akyuz A. Attitudes to HPV Vaccination among Parents of Children Aged 10 to 13 Years. J Pediatr Adolesc Gynecol 2015;28(5):382-6. https://doi.org/10.1016/j.jpag.2014.11.005

[305] Shao SJ, Nurse C, Michel L, Joseph MA, Suss AL. Attitudes and Perceptions of the Human Papillomavirus Vaccine in Caribbean and African American Adolescent boys and Their Parents. J Pediatr Adolesc Gynecol 2015;28(5): 373-7. https://doi.org/10.1016/j.jpag.2014.11.003

[306] Shapiro GK, Holding A, Perez S, Amsel R, Rosberger Z. Validation of the vaccine conspiracy beliefs scale. Papillomavirus Res 2016;2:167-72. https://doi.org/10.1016/j.pvr.2016.09.001

[307] Shapiro GK, Perez S, Naz A, Tatar O, Guichon JR, Amsel R, Zimet GD, Rosberger Z. Investigating Canadian parents' HPV vaccine knowledge, attitudes and behaviour: a study protocol for a longitudinal national online survey. BMJ Open 2017;7(10):e017814. https://doi.org/10.1136/bmjopen-2017-017814

[308] Shawn DH, Gold R. Survey of parents' attitudes to the recommended Haemophilus influenzae type b vaccine program. CMAJ 1987;136(10):1038-40.

[309] Sheikh A, Iqbal B, Ehtamam A, Rahim M, Shaikh HA, Usmani HA, Nasir J, Ali S, Zaki M, Wahab TA, Wasim W, Aftab AA. Reasons for non-vaccination in pediatric patients visiting tertiary care centers in a polio-prone country. Arch Public Health 2013;71(1):19. https://doi.org/10.1186/0778-7367-71-19

[310] Shuaib FM, Kimbrough D, Roofe M, McGwin GJr, Jolly P. Factors associated with incomplete childhood immunization among residents in St. Mary, Jamaica. West Indian Med J 2010;59(5):549-54.

[311] Skinner J, March L, Simpson JM. A retrospective cohort study of childhood immunisation status in northern Sydney. Aust J Public Health 1995;19(1):58-63. https://doi.org/10.1111/j.1753-6405.1995.tb00298.x

[312] Smailbegovic MS, Laing GJ, Bedford H. Why do parents decide against immunization? The effect of health beliefs and health professionals. Child Care Health Dev 2003;29(4):303-11. https://doi.org/10.1046/j.1365-2214.2003.00347.x

[313] Smith MJ, Woods CR, Marshall GS. Parental vaccine concerns in Kentucky. J Ky Med Assoc 2009;107(9):342-9.

[314] Smith PJ, Humiston SG, Marcuse EK, Zhao Z, Dorell CG, Howes C, Hibbs B. Parental delay or refusal of vaccine doses, childhood vaccination coverage at 24 months of age, and the Health Belief Model. Public Health Rep 2011;126 Suppl 2:135-46. https://doi.org/10.1177/00333549111260S215

[315] Smith PJ, Humiston SG, Parnell T, Vannice KS, Salmon DA. The association between intentional delay of vaccine administration and timely childhood vaccination coverage. Public Health Rep 2010;125(4):534-41. https://doi.org/10.1177/003335491012500408

[316] Smith PJ, Kennedy AM, Wooten K, Gust DA, Pickering LK. Association between health care providers' influence on parents who have concerns about vaccine safety and vaccination coverage. Pediatrics 2006;118(5):e1287-92. https://doi.org/10.1542/peds.2006-0923

[317] Smith PJ, Marcuse EK, Seward JF, Zhao Z, Orenstein WA. Children and Adolescents Unvaccinated Against Measles: Geographic Clustering, Parents' Beliefs, and Missed Opportunities. Public Health Rep 2015;130(5):485-504. https://doi.org/10.1177/003335491513000512

[318] Smith PJ, Stokley S, Bednarczyk RA, Orenstein WA, Omer SB. HPV vaccination coverage of teen girls: the influence of health care providers. Vaccine 2016;34(13):1604-10. https://doi.org/10.1016/j.vaccine.2016.01.061

[319] Sohail M, Mahmood B, Asim M. Mother's Knowledge, Attitude and Practices about child immunization: A study in district Faisalabad, Pakistan. Rawal Medical Journal 2015;40(4): 441-4.

[320] Songthap A, Pitisuttithum P, Kaewkungwal J, Fungladda W, Bussaratid V. Knowledge, attitudes, and acceptability of a human papilloma virus vaccine among students, parents and teachers in Thailand. Southeast Asian J Trop Med Public Health 2012;43(2):340-53.

[321] Soyer OU, Hudaverdiyev S, Civelek E, Isik E, Karabulut E, Kocabas C, Sekerel BE. Parental perspectives on influenza vaccination in children with asthma. Pediatr Pulmonol 2011;46(2):139-44. https://doi.org/10.1002/ppul.21332

[322] Staras SA, Vadaparampil ST, Patel RP, Shenkman EA. Parent perceptions important for HPV vaccine initiation among low income adolescent girls. Vaccine 2014;32(46):6163-9. https://doi.org/10.1016/j.vaccine.2014.08.054

[323] Steel Fisher GK, Blendon RJ, Guirguis S, Brule A, Lasala-Blanco N, Coleman M, Petit V, Ahmed M, Mataruse N, Corkum M, Nisar M, Ben-Porath EN, Gigli S, Sahm, C. Threats to polio eradication in high-conflict areas in Pakistan and Nigeria: a polling study of caregivers of children younger than 5 years. Lancet Infect Dis 2015;15(10):1183-92. https://doi.org/10.1016/S1473-3099(15)00178-4

[324] Stefanoff P, Mamelund SE, Robinson M, Netterlid E, Tuells J, Bergsaker MA, Heijbel H, Yarwood J, Europe V. Tracking parental attitudes on vaccination across European countries: The Vaccine Safety, Attitudes, Training and Communication Project (VACSATC). Vaccine 2010;28(35):5731-7. https://doi.org/10.1016/j.vaccine.2010.06.009

[325] Stein Zamir C, Israeli A. Knowledge, Attitudes and Perceptions About Routine Childhood Vaccinations Among Jewish Ultra-Orthodox Mothers Residing in Communities with Low Vaccination Coverage in the Jerusalem District. Matern Child Health J 2017;21(5):1010-17. https://doi.org/10.1007/s10995-017-2272-5

[326] Stephenson JD. Survey of parents' attitudes to the recommended Haemophilus influenzae type b vaccine program. CMAJ, 1987;137(5):371-2.

[327] Stockwell MS, Irigoyen M, Andres Martinez R, Findley SE. Failure to return: parental, practice, and social factors affecting missed immunization visits for urban children. Clin Pediatr (Phila), 2014; 53(5): 420-7. https://doi.org/10.1177/0009922814527497

[328] Strelitz B. Gritton J, Klein EJ, Bradford MC, Follmer K, Zerr DM, Englund JA, Opel DJ. Parental vaccine hesitancy and acceptance of seasonal influenza vaccine in the pediatric emergency department. Vaccine 2015;33(15):1802-7. https://doi.org/10.1016/j.vaccine.2015.02.034

[329] Stretch R, Roberts SA, McCann R, Baxter D, Chambers G, Kitchener H, Brabin L. Parental attitudes and information needs in an adolescent HPV vaccination programme. Br J Cancer 2008;99(11):1908-11. https://doi.org/10.1038/sj.bjc.6604766

[330] Suarez-Castaneda E, Pezzoli L, Elas M, Baltrons R, Crespin-Elias EO, Pleitez OA, de Campos MI, Danovaro-Holliday MC. Routine childhood vaccination programme coverage, El Salvador, 2011-In search of timeliness. Vaccine 2014;32(4):437-44. https://doi.org/10.1016/j.vaccine.2013.11.072

[331] Sundaram SS, Roberts C, Rowen D, Patel R. Parental attitudes towards the human papillomavirus vaccine in ethnic minorities: a community survey. Int J STD AIDS 2010;21(6):449. https://doi.org/10.1258/ijsa.2010.010105

[332] Tadesse H, Deribew A, Woldie M. Predictors of defaulting from completion of child immunization in south Ethiopia, May 2008: a case control study. BMC Public Health,2009;9:150. https://doi.org/10.1186/1471-2458-9-150

[333] Tagbo BN, Ughasoro MD, Esangbedo DO. Parental acceptance of inactivated polio vaccine in Southeast Nigeria: a qualitative cross-sectional interventional study. Vaccine 2014;32(46): 6157-62. https://doi.org/10.1016/j.vaccine.2014.08.053

[334] Taiwo L, Idris S, Abubakar A, Nguku P, Nsubuga P, Gidado S, Okeke L, Emiasegen S, Waziri E. Factors affecting access to information on routine immunization among mothers of under 5 children in Kaduna State Nigeria, 2015. Pan Afr Med J 2017; 27:186. https://doi.org/10.11604/pamj.2017.27.186.11191

[335] Takahashi K, Kanda H, Kim JY. Reasons for Non-vaccination among Patients Who Acquired Measles: Lessons from the Local Measles Epidemics in Japan. West Indian Med J 2014;63(6): 647-9. https://doi.org/10.7727/wimj.2013.310

[336] Tam WW, Chan J, Lo KK, Lee A, Chan PK, Chan D, Nelson EA. Parental Attitudes and Factors Associated With Varicella Vaccination in Preschool and Schoolchildren in Hong Kong: A Cross-Sectional Study. Medicine (Baltimore) 2015;94(36): e1519. https://doi.org/10.1097/MD.0000000000001519

[337] Tan TQ, Gerbie MV. Perception, Awareness, and Acceptance of Human Papillomavirus Disease and Vaccine Among Parents of Boys Aged 9 to 18 Years. Clin Pediatr (Phila) 2017;56(8):737-43. https://doi.org/10.1177/0009922816682788

[338] Tang C, Huang S, Weng K, Ger L, Hsieh K. Parents' views about the vaccination program in Taiwan. Pediatrics and neonatology 2011;52(2):98-102

[339] Taylor JA, Cufley D. The association between parental health beliefs and immunization status among children followed by private pediatricians. Clin Pediatr (Phila) 1996;35(1):18-22. https://doi.org/10.1177/000992289603500104

[340] Taylor JA, Darden PM, Brooks DA, Hendricks JW, Wasserman RC, Bocian AB. Pediatric Research in Office, S., and National Medical, A., Association between parents' preferences and perceptions of barriers to vaccination and the immunization status of their children: a study from Pediatric Research in Office Settings and the National Medical Association. Pediatrics, 2002;110(6):1110-6. https://doi.org/10.1542/peds.110.6.1110

[341] Thomas T, Blumling A, Delaney A. The Influence of Religiosity and Spirituality on Rural Parents' Health Decision Making and Human Papillomavirus Vaccine Choices. ANS Adv Nurs Sci 2015;38(4):E1-E12. https://doi.org/10.1097/ANS.0000000000000094

[342] Thomas TL, Strickland OL, Di Clemente R, Higgins M, Haber M. Rural African American parents' knowledge and decisions about human papillomavirus vaccination. J Nurs Scholarsh 2012;44(4):358-67. https://doi.org/10.1111/j.1547-5069.2012.01479.x

[343] Thomas TL, Strickland OL, Di Clemente R, Higgins M, Williams B, Hickey K. Parental Human Papillomavirus Vaccine Survey (PHPVS): nurse-led instrument development and psychometric testing for use in research and primary care screening. J Nurs Meas 2013;21(1):96-109. https://doi.org/10.1891/1061-3749.21.1.96

[344] Thomas TL, Strickland OL, Higgins M. Mothers, Fathers, Sons, and Human Papillomavirus Immunization Practices. Fam Community Health,2017;40(3):278-87. https://doi.org/10.1097/FCH.0000000000000104

[345] Thompson EL, Rosen BL, Vamos CA, Kadono M, Daley EM. Human Papillomavirus Vaccination: What Are the Reasons for Nonvaccination Among U.S. Adolescents? J Adolesc Health 2017;61(3):288-93. https://doi.org/10.1016/j.jadohealth.2017.05.015

[346] Tisi G, Salinaro F, Apostoli P, Bassani R, Bellicini A, Groppi L, Donarini P, Pecorelli S. HPV vaccination acceptability in young boys. Ann Ist Super Sanita 2013;49(3):286-91. https://doi.org/10.4415/ANN_13_03_09

[347] Taylor JA, Newman RD. Parental attitudes toward varicella vaccination. The Puget Sound Pediatric Research Network. Arch Pediatr Adolesc Med 2000;154(3):302-6. https://doi.org/10.1001/archpedi.154.3.302

[348] Wright RA, Battaglia M, Wilkinson M, Huggins VJ, Abt Associates. The National Immunization Survey: design of a study on knowledge, attitudes, and practices (NIS-KAP). Proceedings of the section on Survey Research Methods, Indianapolis. American Statistical Association, 2000:697–702.

[349] Streng A, Seeger K, Grote V, Liese JG. Varicella vaccination coverage in Bavaria (Germany) after general vaccine recommendation in 2004. Vaccine 20 https://doi.org/10. 28(35): 5738-45. https://doi.org/10.1016/j.vaccine.2010.06.007

[350] Opel DJ, Taylor JA, Mangione-Smith R, Solomon C, Zhao C, Catz S, Martin D. Validity and reliability of a survey to identify vaccine-hesitant parents. Vaccine 2011;29(38):6598-605. https://doi.org/10.1016/j.vaccine.2011.06.115

[351] Austin Z, Sutton J. Qualitative research: getting started. Can J Hosp Pharm 2014;67(6):436-40. https://doi.org/10.4212/cjhp.v67i6.1406

[352] Gianfredi V, Nucci D, Salvatori T, Orlacchio F, Villarini M, Moretti M, PErCEIVE IN UMBRIA STUDY GROUP. "PErCEIVE in Umbria": evaluation of anti-influenza vaccination's perception among Umbrian pharmacists. J Prev Med Hyg 2018;59(1):E14-E19. https://doi.org/10.15167/2421-4248/jpmh2018.59.1.806

[353] Meadows KA. So you want to do research? 5: Questionnaire design. Br J Community Nurs 2003;8(12):562-70. https://doi.org/10.12968/bjcn.2003.8.12.11854

[354] Keszei, A.P., Novak, M., and Streiner, D.L., Introduction to health measurement scales. J Psychosom Res 20 https://doi.org/10. 68(4): 319-23. https://doi.org/10.1016/j.jpsychores.2010.01.006

[355] Tabacchi G, Costantino C, Napoli G, Marchese V, Cracchiolo M, Casuccio A, Vitale F, The Esculapio Working Group. Determinants of European parents' decision on the vaccination of their children against measles, mumps and rubella: A systematic review and meta-analysis. Hum Vaccin Immunother 2016;12(7): 1909-23. https://doi.org/10.1080/21645515.2016.1151990

[356] Gualano MR, Bert F, Voglino G, Buttinelli E, D'Errico MM, De Waure C, Di Giovanni P, Fantini MP, Giuliani, AR, Marranzano M, Masanotti G, Massimi A, Nante N, Pennino F, Squeri R, Stefanati A, Signorelli C, Siliquini R. Attitudes towards compulsory vaccination in Italy: Results from the NAVIDAD multicentre study. Vaccine 2018;36(23):3368-3374. https://doi.org/10.1016/j.vaccine.2018.04.029

[357] Gualano MR, Olivero E, Voglino G, Corezzi M, Rossello P, Vicentini C, Bert F, Siliquini R. Knowledge, attitudes and beliefs towards compulsory vaccination: a systematic review. Hum Vaccin Immunother 2019;15(4):918-31. https://doi.org/10.1080/21645515.2018.1564437

[358] Gianfredi V, D'Ancona F, Maraglino F, Cenci C, Iannazzo S. Polio and measles: reasons of missed vaccination in Italy, 2015-2017. Ann Ig 2019;31(3):191-201. https://doi.org/10.7416/ai.2019.2282

[359] Gianfredi V, Grisci C, Nucci D, Parisi V, Moretti M. [Communication in health.]. Recenti Prog Med 2018;109(7):374-83. https://doi.org/10.1701/2955.29706

[360] Gianfredi V, Odone A, Fiacchini D, Rosselli R, Battista T, Signorelli C. Trust and reputation management, branding, social media management nelle organizzazioni sanitarie: sfide e opportunità per la comunità igienistica italiana. J Prev Med Hyg 2019;60(3):E108-E109.

[361] Bragazzi NL, Barberis I, Rosselli R, Gianfredi V, Nucci D, Moretti M, Salvatori T, Martucci G, Martini M. How often

people google for vaccination: Qualitative and quantitative insights from a systematic search of the web-based activities using Google Trends. Hum Vaccin Immunother 2017;13(2):464-9. https://doi.org/10.1080/21645515.2017.1264742

[362] Bragazzi NL, Gianfredi V, Villarini M, Rosselli R, Nasr A, Hussein A, Martini M, Behzadifar M. Vaccines Meet Big Data: State-of-the-Art and Future Prospects. From the Classical 3Is ("Isolate-Inactivate-Inject") Vaccinology 1.0 to Vaccinology 3.0, Vaccinomics, and Beyond: A Historical Overview. Front Public Health 2018;6:62. https://doi.org/10.3389/fpubh.2018.00062

[363] Gianfredi V, Bragazzi NL, Mahamid M, Bisharat B, Mahroum N, Amital H, Adawi M. Monitoring public interest toward pertussis outbreaks: an extensive Google Trends-based analysis. Public Health 2018;165:9-15. https://doi.org/10.1016/j.puhe.2018.09.001

[364] Kazi AM, Khalid W. Questionnaire designing and validation. J Pak Med Assoc 2012;62(5):514-6.

[365] Gianfredi V, Nucci D, Ceccarelli F, Villarini M, Moretti M. PILATES (Physical Activity and Diet Survey): An Italian Self-Administered Questionnaire Evaluating Diet Habits of Gym-Goers. Validation Process. J Diet Suppl 2019;16(3):307-17. https://doi.org/10.1080/19390211.2018.1456501

[366] Nucci D, Licitra L, Sciara S, Moretti M, Gianfredi V. PRuNUS: design and validation of a questionnaire among prisoners - data of pilot study in the Penitentiary Institute of Perugia, Italy. Int J Prison Health 2019;16(2):165-83. https://doi.org/10.1108/IJPH-01-2019-0001

[367] Cuda A, Gentile L, Voglino G, Gianfredi V. and Gruppo di lavoro Vaccini e Vaccine Hesitancy Consulta degli Specializzandi, SII, [Design and validation of a questionnaire for assessing Public Health residents' opinions and knowledge regarding the new mandatory vaccination law in Italy (DL 73/2017)]. Ig Sanita Pubbl,2018;74(2):137-52.

[368] Derrough T, Olsson K, Gianfredi V, Simondon F, Heijbel H, Danielsson N, Kramarz P, Pastore-Celentano L. Immunisation Information Systems - useful tools for monitoring vaccination programmes in EU/EEA countries, 2016. Euro Surveill 2017;22(17). https://doi.org/10.2807/1560-7917.ES.2017.22.17.30519

[369] Gianfredi V, Balzarini F, Gola M, Mangano S, Carpagnano LF, Colucci ME, Gentile L, Piscitelli A, Quattrone F, Scuri S, Mantovani LG, Auxilia F, Castaldi S, Capolongo S, Pelissero G, Odone A, Signorelli C. Leadership in Public Health: Opportunities for Young Generations Within Scientific Associations and the Experience of the "Academy of Young Leaders". Front Public Health 2019;7:378. https://doi.org/10.3389/fpubh.2019.00378

[370] Gianfredi V, Moretti M, Lopalco PL. Countering vaccine hesitancy through immunization information systems, a narrative review. Hum Vaccin Immunother 2019;1-19. https://doi.org/10.1080/21645515.2019.1599675

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Correspondence: Ilaria Barberis, Health Science Department, University of Genoa, largo Rosanna Benzi 10, Pad. 3 San Martino Hospital, Italy - Tel./Fax +39 010 3538502 - E-mail: ilaria.barberis@edu.unige.it

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REVIEW

A review of quality of life of patients suffering from ichthyosis

GIANMARCO TROIANO, GIACOMO LAZZERI
Department of Molecular & Developmental Medicine, University of Siena, Italy

Keywords

Quality of life • Ichtyosis • Pain • Mental health

Summary

Introduction. *Ichthyoses include a heterogeneous group of skin diseases often characterized by persistent scaling and hyperkeratosis with variable erythema, pruritus, and sweating impairment. The aim of our review was to assess the quality of life in patients with ichthyosis.*
Methods. *In July 2018 we performed a systematic search in the electronic database PubMed (MEDLINE). The MESH term "quality of life" was combined, through the Boolean operator AND with the key word "ichthyosis". We considered eligible for the systematic review studies written in English.*
Results. *The literature search yielded 63 publications, but 7 studies were included in the review. Studies were published in 2003-2014 and involved a minimum of 10 and a maximum of 235 patients. Authors used 5 types of tools: Dermatology Life Quality Index (DLQI), Dermatitis Family Impact Questionnaire (DFI), Nottingham Health Profile (NHP) questionnaire, Short Form Questionnaire 36 and 12 (SF-36, SF-12). Many patients reported worse scores than general population. Patients referred physical problems related to pain (which negatively influenced the mobility).*
Conclusions. *Ichthyosis considerably impaired the QoL, especially for paediatric patients. Further studies and efforts should be done to manage and treat the pain.*

Introduction

The ichthyoses include a heterogeneous group of skin diseases linked by the common finding of abnormal barrier function, which leads to increased trans-epidermal water loss and compensatory hyperproliferation [1]. Ichthyosis vulgaris is caused by loss-of-function mutations in the filaggrin gene (FLG) [2]. Filaggrin is very important in the terminal differentiation of the skin and the formation of cornified envelope in the stratum corneum [3]. FLG mutations are observed in approximately 7.7% of Europeans and 3.0% of Asians, but appear to be infrequent in darker-skinned populations [2]. The inherited ichthyoses are classified as syndromic or non-syndromic, depending on the presence or absence of extracutaneous findings [4]. They are characterized by persistent scaling and hyperkeratosis with variable erythema, pruritus, and sweating impairment [5].
The new classification identifies 36 types of ichthyosis, which are subdivided according to their frequency, pattern of inheritance and extracutaneous involvement [6].
Among diseases that cause ichthyosis as one of the symptoms, there are some diseases that induce abnormalities in organs other than the skin. Of these, diseases with characteristic signs are regarded as syndromes. Although these syndromes are very rare, Netherton syndrome, Sjögren-Larsson syndrome, Conradi-Hünermann-Happle syndrome, Dorfman-Chanarin syndrome, ichthyosis follicularis, atrichia and photophobia (IFAP) syndrome, and Refsum syndrome have been described in texts as representative ones [7].
Quality of life may be adversely affected by the social and psychologic consequences of this disease [5].
Quality of life (QoL) is a concept used to indicate the general wellness of persons or societies, including wealth and employment elements, environment, physical and mental health, education, recreation and belonging to a social group [8]. For some authors it is a concept that could be compared to the paradigm of "happiness" [9].
Several authors studied the impact of ichthyosis on quality of life (QoL) [10, 11], and showed that congenital ichthyosis appears to affect several aspects of life negatively, and is responsible of lower scores in the used questionnaire. In literature there is not a review on this topic;the aim of our study was therefore to assess the quality of life in patients with ichtyosis, examining and summarizing all the peer reviewed literature published in PubMed on this field.

Methods

In July 2018 we performed a systematic search for original peer-reviewed papers in the electronic database PubMed (MEDLINE). The MESH term "quality of life" was combined, through the Boolean operator AND with the keyword "ichthyosis". We searched for

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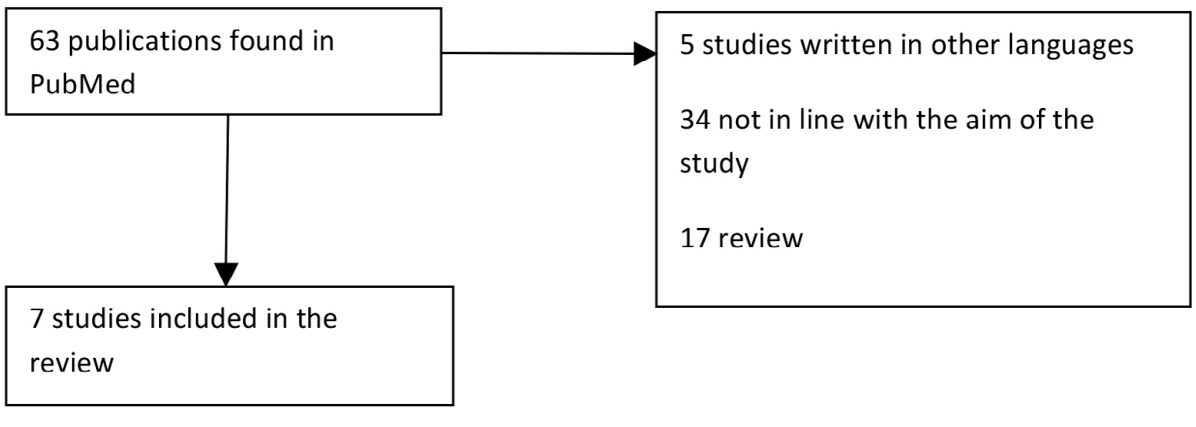
studies published without temporal limits, reporting information about the quality of life of patients suffering from ichthyosis.
We considered eligible for the review original articles that reported clear data on: i) number of involved patients; ii) tool (or tools) used to evaluate the quality of life; iii)setting. We considered eligible for the systematic review studies written in English.
Studies were selected in a 2-stage process. Titles and abstracts from electronic searches were first scrutinised. Then, full manuscripts and their citations list were analysed to retrieve missing articles and to select the eligible manuscripts according to the inclusion criteria.
The literature search yielded 63 publications. The titles and abstracts of these manuscripts were screened, resulting in 7 studies considered potentially eligible to be included in the review [10-16]: 17 were review, 2 studies were excluded because they were written in German, 1 study was excluded because written in Polish, 2 studies were excluded because they were written in French, and 34 because they were not in line with the aim of the study (Fig. 1).
Studies were published in 2003-2014: 3 were settled in Sweden, 1 in USA, 3 in France. They involved a minimum of 10 and a maximum of 235 patients. One study was focused only on a paediatric population.
Authors used 5 types of tools: Dermatology Life Quality Index (DLQI), Dermatitis Family Impact Questionnaire (DFI), Nottingham Health Profile (NHP) questionnaire, Short Form Questionnaire 36 and 12 (SF-36, SF-12).
The Dermatology Life Quality Index or DLQI, developed in 1994, was the first dermatology-specific Quality of Life instrument. It is a simple 10-question validated questionnaire that has been used in over 40 different skin conditions in over 80 countries and is available in over 90 languages. Its use has been described in over 1000 publications including many multinational studies [17].
The questionnaire DFI had 10 questions scoring 0-3, giving a maximum score of 30: the higher the score, the greater the impairment of QoL [18].
NHP I measures HRQoL and contains 39 questions in six areas (emotions, sleep, energy, pain, mobility and social isolation) [19].
NHP II contains seven yes/no questions about health-related problems in the following areas: paid employment, housework, social life, home life, sex life, hobbies and holidays [10].
The Short Form 36 (SF-36), is a validated questionnaire, which detects the health-related quality of life. It was developed in the '80s in the United States as a generic multi-dimensional questionnaire, with 36 questions that create 8 different scales: PA-physical activity, PR-role limitations due to physical health, ER -limitations due to emotional state, physical pain-BP, GH-perception of general health, vitality-VT, SA-social activities, MH mental health [20-22]. The shortened version is SF 12.
In addition, it should be mentioned that in 2013 Dreyfus et al. [14] created an innovative tool: the IQOL 32 specifically designed for ichthyosis. The questions are specifically dedicated to ichthyosis and explore all disease particularities such as skin pain/discomfort, ear- and eye-related problems, heat intolerance, skin odor, scalp involvement, restrictions related to the disease (dressings, sports, leisure), expenses, psychological aspects, and consequences of the treatment.

Results

The main results of the review are reported in Table I.
Ganemo in his three studies reported that patients' skin disease affected them negatively to varying degrees during their entire lives, and that the most problematic period was childhood.
Kamalpour reported that mean DLQI scores were significantly higher for adults than for children; the

Fig. 1. Flow diagram for identifying studies included in our review.



Tab. I. Main characteristics of the studies included in the systematic review.

Author, year	Setting	Sample size	Study design	Tool	Results
Ganemo, 2003	Sweden	10 (56-80 years old)	Cross sectional	Nottingham Health Profile (NHP) questionnaire	Individual overall NHP I scores varied from 0 to 427. Total scores for NHP II varied from 0% to 57% affirmative answers.
Ganemo, 2004	Sweden	122 (17-78 years old)	Cross sectional	DLQI and SF-36 (sent by email)	Median DLQI 5.0; SF-36 scores lower in 6 dimensions compared to normal population.
Ganemo, 2010	Sweden	15 (5-16 years old)	Cross sectional	Children's DLQI and DFI	The median score was 9.0 (range 2-19) for CDLQI; 9.0 for DFI (range 3-21)
Kamalpour, 2011	U.S.A.	235 (mean age 27.3 years old)	Cross sectional	DLQI (online)	Mean DLQI scores were significantly higher for adults (>18) (9.5 SD 6.6) than for children (7.7 SD 5.7) ($p = 0.04$). Mean DLQI scores were significantly higher for women (9.7 SD 6.6) than for men (7.4 SD 5.6, $p = 0.01$), regardless of age. Alopecia severity had a much weaker but still significant correlation with QOL ($r = 0.19$, $p < 0.01$).
Mazereeuw-Hautier, 2012	France	25 (21-67 years old)	Qualitative	Qualitative approach using focus groups	Not reported numerical results
Dreyfus, 2013	France	59 (17-70 years old)	n.r.	DLQI and SF-12 (sent by email)	Mean IQOL-32 score: 74.5 SD 21.1 Mean SF 12 score: 46.6 SD 11.1 (physical component) and 36.6 SD 10.9 (health component)
Dreyfus, 2014	France	158 (16-88 years old)	Multicentre Prospective Study	DLQI	Mean score of DLQI was 8.3 ± 6.5 (0-27).

mean DLQI scores were significantly higher for women than for men, regardless of age. Alopecia severity had a much weaker but still significant correlation with QOL.

Mazereeuw-Hautier observed that the acceptance of the disease and support from families or friends were considered as positively influencing patients' QOL. Difficulty in relationship with others was often reported by patients.

Dreyfus in his two studies showed that the clinical severity had strongest correlations with discomfort, pain, and social aspects. Females, patients who lived alone, patients suffering from cutaneous pain had the highest DLQI scores.

Discussion

The application of quality of life studies in dermatology is recent, but of great interest as skin diseases have a deep impact on psychological status and daily activities of patients [23].

Mazereeuw-Hautier et al. in 2012 [15] used an interesting approach. In their study they used in fact a qualitative approach, which let us better understand the impact of the disease on quality of life, without "measuring" it with a scale/score. Several patients referred physical problems related to pain (which negatively influenced the mobility). Daily cream application was considered to be time consuming and nasty and this practiced could negatively influence the possibility to travel. Although the patients didn't consider themselves to be handicapped (even if they realized they have limitations), the relationship with others represented an important point negatively influenced by the disease. The problem of stigmatization about certain diseases has been already described in literature: Norman Sartorius [24] affirmed that there are a number of diseases that are stigmatized such as mental disorders, AIDS, venereal diseases, leprosy, and certain skin diseases. People living with these diseases are discriminated in several settings and tend not to receive enough social support with consequent difficulties in organizing their life.

The element that mostly affected the quality of life in all the studies that we examined was pain. Pain is one of the most common medical complaints [25] and a another study [26] focused on other diseases showed that pain affects most domains of QOL, primarily physical and emotional functioning. The effect depends on the extent, duration, acuteness, intensity, affectivity, and meaning of the pain as well as on the underlying disease and the individual's characteristics. Many chronic pain sufferers reported that pain had deleterious effects on their mental health, employment status, sleep, and personal relationships [25].

Very interesting are the contrasting results deriving from the paediatric population. The study conducted by Ganemo et al in 2003 affirmed that the most problematic period for patients was childhood, and this evidence was confirmed by the study conducted in 2010 on children with congenital ichthyosis that

demonstrated that ichthyosis affected both the children and the families and caused greater impairment of QoL than other skin diseases in children. However, Kalmapour et al. in 2011 showed that children tend to have a better quality of life than adults. The impact of skin diseases on children was investigated also by other authors: Catucci et al in 2016 [27] published a study on vitiligo that showed that the median DLQI scores in children and especially in adolescents was very high (almost 11) and that the disease influenced several aspects of children's life.

The person with ichtyosis had been in hospital about 25 times during childhood and early adulthood, mainly for skin infections. All respondents described their skin as very problematic during childhood, with thick scaling, fissures, wounds and pain. The skin symptoms had improved in adulthood when medical treatments such as oral retinoids and new cream formulations containing salicylic acid, urea and alpha-hydroxy acids had became available [10].

In conclusion, this review highlighted that ichthyosis considerably impaired the QoL: the disease was responsible of negative effects on patients lives, and several studies showed that children had worse scores compared to adults. Several further factors (gender, acceptance of the disease, support from families or friends) could influence patients' QOL. Pain is one of the most important factors, so further studies and efforts should be done to manage and treat the pain deeply involved in the reduction of quality of life of these patients.

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Conflicts of interest statement

The authors declare no conflict of interest.

Authors' contributions

GT and GL had the idea of the article, collected data and wrote the article.

References

[1] Marukian NV, Choate KA. Recent advances in understanding ichthyosis pathogenesis. F1000Res 2016;5. https://doi.org/10.12688/f1000research.8584.1

[2] Thyssen JP, Godoy-Gijon E, Elias PM. Ichthyosis vulgaris: the filaggrin mutation disease. Br J Dermatol 2013;168:1155-66. https://doi.org/10.1111/bjd.12219

[3] De D, HandaS. Filaggrin mutations and the skin. Indian J Dermatol Venereol Leprol, 2012;78:545-51. https://doi.org/10.4103/0378-6323.100518

[4] Oji V, Akiyama M, Blanchet Bardon C, Bodemer C, Bourrat E. Revised nomenclature and classification of inherited ichthyosis: results of the first ichthyosis consensus conference in Soreze 2009 J Am Acad Dermatol 2010;63:607-41. https://doi.org/10.1016/j.jaad.2009.11.020.

[5] Hernandez-Martin A. A systematic review of clinical trials of treatments for the congenital ichthyoses, excluding ichthyosis vulgaris. J Am Acad Dermatol 2013;69:544-549e8. https://doi.org/10.1016/j.jaad.2013.05.017

[6] Vega Almendra N, Aranibar Duran L. Hereditary ichthyosis: A diagnostic and therapeutic challenge. Rev Chil Pediatr 2016;87:213-23. http://dx.doi.org/10.1016/j.rchipe.2015.07.025

[7] Yoneda K. Inherited ichthyosis: Syndromic forms. J Dermatol 2016; 43 (3): 252-63. https://doi.org/10.1111/1346-8138.13284

[8] Gregory D Jr, Pratt G, Watts M, Whatmore S. The Quality of Life. the dictionary of human geography. Oxford: John Wiley & Sons 2009.

[9] Veenhoven R. Quality of life and happiness: not quite the same. Salute e Qualità della Vita 2001;67-95.

[10] Ganemo A. Quality of life in adults with congenital ichthyosis. J Adv Nurs 2003;44:412-9. https://doi.org/10.1046/j.0309-2402.2003.02820.x

[11] Ganemo A. Health-related quality of life among patients with ichthyosis. Eur J Dermatol 2004;14:61-6.

[12] Ganemo A. Quality of life in Swedish children with congenital ichthyosis. Dermatol Reports 2010;2:e7. https://doi.org/10.4081/dr.2010.e7

[13] Kamalpour L. Resource utilization and quality of life associated with congenital ichthyoses. Pediatr Dermatol 2011;28:512-8. https://doi.org/10.1111/j.1525-1470.2011.01432.x

[14] Dreyfus I. IQoL-32: a new ichthyosis-specific measure of quality of life. J Am Acad Dermatol 2013;69: 82-7. https://doi.org/10.1016/j.jaad.2013.01.022

[15] Mazereeuw-Hautier J. Factors influencing quality of life in patients with inherited ichthyosis: a qualitative study in adults using focus groups. Br J Dermatol 2012;166:646-8. https://doi.org/10.1111/j.1365-2133.2011.10701.x

[16] Dreyfus I. Factors associated with impaired quality of life in adult patients suffering from ichthyosis. Acta Derm Venereol 2014;94:344-6. https://doi.org/10.2340/00015555-1710

[17] Dermatology CU-Do. Dermatology Quality of Life Index (DLQI) 20 July 2018; Available from: http://sites.cardiff.ac.uk/dermatology/quality-of-life/dermatology-quality-of-life-index-dlqi/

[18] Lawson VL-JM, Finlay AY. The family impact of childhood atopic dermatitis: the Dermatitis Family Impact Questionnaire. Br J Dermatol 1998;138:107-13. https://doi.org/10.1046/J.1365-2133.1998.02034.X

[19] NHP Manual IW. Svensk version av Nottingham Health Profile – ett fra°geformula¨r som ma¨ter ha¨lsorelaterad livskvalitet (Swedish version). Gothenburg: OFTA Grafiska 1992.

[20] Messina G. Italian medical students quality of life: years 2005-2015. Ann Ig 2016;28:245-51. https://doi.org/10.7416/ai.2016.2103

[21] Nante N. Quality of life in refugees and asylum seekers in Italy: a pilot study. Ann Ist Super Sanita 2016;52:424-7. https://doi.org/10.4415/ANN_16_03_14

[22] Levorato S. Health status of homeless persons: a pilot study in the Padua municipal dorm. Ann Ig 2017;29:54-62. https://doi.org/10.7416/ai.2017.2132

[23] Finlay AY. Quality of life assessments in dermatology. Semin Cutan Med Surg, 1998;17:291-6. https://doi.org/10.1016/s1085-5629(98)80026-6

[24] Sartorius N. Stigmatized illnesses and health care. Croat Med J 2007;48:396-7.

[25] McCarberg BH. The impact of pain on quality of life and the unmet needs of pain management: results from pain sufferers and physicians participating in an Internet survey. Am J Ther 2008;15:312-20. https://doi.org/10.1097/MJT.0b013e31818164f2

[26] Niv D, Kreitler S. Pain and quality of life. Pain Pract 2001;1:150-61. https://doi.org/10.1046/j.1533-2500.2001.01016.x

[27] Juliana Catucci Boza NG, Machado P, Horn R, Fabbrin A, Cestari T. Quality of life impairment in children and adults with vitiligo: a cross-sectional study based on dermatology-specific and disease-specific quality of life instruments. Dermatology 2016;232:619-25. https://doi.org/10.1159/000448656

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Correspondence: Gianmarco Troiano, Department of Molecular & Developmental Medicine, University of Siena, Italy - E-mail: gianmarco.troiano@student.unisi.it

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ORIGINAL ARTICLE

A comparative profile of oropharyngeal colonization of *Streptococcus pneumoniae* and *Hemophilus influenzae* among HealthCare Workers (HCW) in a tertiary care hospital and non-healthcare individuals

AMRITHA G.N[1], MEENAKSHI N[2], ALICE PEACE SELVABAI R[3], PRIYADARSHINI SHANMUGAM[4], PERUMAL JAYARAMAN[5]

[1] MBBS, Chettinad Hospital and Research Institute, Kancheepuram, India; [2] Department of Respiratory and Thoracic Medicine, Chettinad Hospital and Research Institute, Kancheepuram, India; [3] Department of Microbiology, Chettinad Hospital and Research Institute, Kancheepuram, India; [4] Department of Microbiology, Chettinad Hospital and Research Institute, Kancheepuram, India; [5] Chettinad Hospital and Research Institute, Kancheepuram, India

Keywords

Oropharyngeal carriage • *Streptococcus pneumoniae* • *Haemophilus influenzae* • *Health care workers*

Summary

Introduction. *Streptococcus pneumoniae and Hemophilus influenzae are two major bacterial human pathogens responsible for causing both acute respiratory tract and life threatening invasive infections. Oropharyngeal carriage of these isolates can lead to its transmission frequently in healthcare settings between patients and HealthCare workers (HCW) and also common among population living in crowded communities resulting in serious invasive infections. Furthermore, awareness about preventive measures including appropriate vaccination against these bacterial infections, oropharyngeal carrier status, prevalent serotypes and the antimicrobial susceptibility pattern these bacterial strains among HCW and Non-HCW in the community in India remains inadequate. Therefore the current study is aimed to understand the prevalence of oropharyngeal carrier status, prevalent serotypes and antimicrobial susceptibility profile of these organisms among HCW and non-HCW.*

Methods. *A total of 200 oropharyngeal swabs were collected from HealthCare Workers and 200 from Non-Health care individuals of age 18 to 70. Antimicrobial susceptibility profile was studied for Pneumococci and H. influenzae. Specific serotypes for the carrier isolates of Pneumococci were identified using primers appropriate to the prevalent serotypes by multiplex PCR.*

Results. *About 30% of the HCW were colonized with S.pneumoniae and H. influenzae ($P \leq 0.0001$). Out of which 19% of them were S.pneumoniae and 11% were H. influenzae. A total of 23% of the Non-HCW was colonized with S. pneumoniae and H. influenzae. Out of which 16% had pneumococcal carriage and 7% had H. influenzae. Individuals in the age group 56-70 years had significantly a greater prevalence rate when compared to young people ($P = 0.0014$). Thus in this study 30% of the HCW and 23% of the Non-HCW were colonized with S. pneumoniae and H. influenzae. Both Pneumococci and H. influenzae showed 100% susceptibility to Penicillin and other cephalosporins. However, Pneumococcal isolates from HCW showed better susceptibility towards erythromycin & clindamycin whereas isolates from Non- HCW showed better susceptibility towards ofloxacin and tetracycline. Serotypes detected in our study include 19F, 3, 1 and 5.*

Conclusions. *The present study gives a greater prevalence rate of S.pneumoniae and H. influenzae among HCW when compared to Non-HCW. This will definitely increase horizontal spread of infections and further accelerate the occupational risk. Increased carrier state prevalence among old age group underscores the importance of vaccination among these individuals.*

Introduction

Streptococcus pneumoniae (*S. pneumoniae*) and *Haemophilus influenzae* (*H. influenzae*) remain as the most important bacterial respiratory tract pathogens [1, 2]. Humans are the only known asymptomatic carriers/reservoir [3]. The clinical infection of these bacteria is preceded by asymptomatic colonization of the human pharynx [4]. The pharyngeal mucosa is considered as the ecological niche for *S. pneumoniae* and *H. influenzae* which can extend their entry into the respiratory tract and further lead to invasive infections, like pneumonia a major cause of death, particularly in the elderly population [5, 6].

In the current study we have selected *S. pneumoniae* and *H. influenzae* because they are members of the normal microflora as well as being etiologic agents of common respiratory tract and disseminated infections [7]. Transmission occurs through direct contact with respiratory droplets from asymptomatic pharyngeal carrier, patients, or indirectly through fomites [8, 9]. Transmission occurs frequently in healthcare settings between patients and HealthCare workers (HCW) and also common among crowded communities through droplets [10, 11]. Other risk factors for the invasive disease caused by these bacteria include extremes of age, immunocompromised states and viral infections of the respiratory tract [12]. Awareness about preventive measures including appropriate vaccination remains inadequate [10]. Limited data exists with respect to prevalence of oropharyngeal carrier status, prevalent

serotypes and resistant patterns in HealthCare Workers and Non-HealthCare individuals in the community in India.

Materials and methods

Study design

Prospective observational study. Place of study: Department of Respiratory and Thoracic medicine and Department of Microbiology, Chettinad Hospital and Research Institute, Kelambakkam. Duration of the study: This was carried out in during a period of 6 months, May to Oct 2018. Sample size and study population: sample size was calculated using this formula:

$$\text{Sample size (n)} = \frac{Z^2\ P\ Q}{d^2}$$

A total of 200 oropharyngeal swabs were collected from HealthCare Workers and 200 from Non-Health care individuals of age 18 to 70. Data such as demographic details, antibiotic use, smoking habits, immunization and other health related details were collected through individual questionnaires. Healthcare Workers include doctors, nurses, housekeeping staff and laboratory workers. Non-Healthcare Workers (not exposed to healthcare settings) include white collar workers, fishermen, farmers, construction workers, driver, sweeper, tailor and others (home makers and other subjects currently not pursuing any job).

Inclusion criteria: Age between 18-70 years, both male and female, Health care workers from a tertiary care hospital, non-health care individuals (not exposed to healthcare settings) of age 18 to 70. *Exclusion criteria:* recent respiratory infections within 8 weeks, recent exposure or contact with individuals having respiratory infections, preexisting respiratory diseases, antibiotic usage for more than 2 weeks before taking oropharyngeal swab and immunocompromised individuals. This study was initiated after obtaining the approval from Institutional Human Ethical committee (IHEC) (Ref: no: 110/IHEC/03-18).

Sample collection

All participants were informed in detail about the study and written consent was obtained before collecting the samples from individuals who participated in the study. All participants were subjected to a detailed questionnaire and clinical examination. Oropharyngeal swabs were collected using sterile cotton swabs and the swabs were transported in Amie's transport medium to microbiology department within 4 hours. Swabs were streaked onto trypticase soy agar supplemented with 5% sheep blood agar containing 5µg of gentamicin and chocolate agar. Plates were incubated at 37°C aerobically in the presence of 5-10% CO_2 enriched air.

Bacterial identification

Pneumococci growing as alpha hemolytic colonies on blood agar were identified according to standard microbiological procedures like Gram's stain, optochin susceptibility test, bile solubility test and *H. influenzae* colonies growing as translucent colonies on chocolate agar were identified by phenomenon of Satellitism.

Antimicrobial susceptibility testing

Antimicrobial susceptibility profile was studied for *Pneumococci* and *H. influenzae* by Kirby Bauer disc diffusion method as per Clinical and Laboratory Standards Institute. The antibiotics tested against the isolates include: penicillin, vancomycin, erythromycin, azithromycin, tetracycline, ofloxacin and cotrimoxazole for *S. pneumoniae*; ampicillin, cefotaxime, cefipime, meropenem and imipenem for *H. influenzae*.

Serotyping PCR for *Pneumococci*

Specific serotypes were identified using primers appropriate to the prevalent serotypes by multiplex PCR. Briefly multiplex PCR was set up in a 25 µl reaction mix. The thermal cycling condition is as follows: 95°C for 15 min (1 cycle), 94°C for 30 sec, 54°C for 90 sec, 72°C for 60 sec (35 cycles) and 72°C for 10 min (1 cycle). The primer sets included are given in (Tab. I).

Statistical analysis

The results were analyzed using IBM SPSS (version 21.0) software. Pearson's Chi Square test was done to analyze the association between the two groups (Healthcare and Non-Healthcare workers).

Results

A total of 400 oropharyngeal swabs 200 from each category i.e. Health care workers and Non-Healthcare workers were collected.

About 66% {132/200} of the HCW were in the age group of 18-35, 32% {64/200} of them in the age group of 36-55 and 2% {4/200} of them in the age group 56-70 (Fig. 1). Of the 200 HCW, 72% {144/200} were female and 28% {56/200} were male volunteers (Fig. 2). With respect to occupation wise distribution 51.5% {103/200} were doctors and nurses, 39.5% {79/200} were housekeeping staff and 9% {18/200} were laboratory workers (Fig. 3).

About 30% {60/200} of the HCW were colonized with *S.pneumoniae* and *H. influenzae* ($P \leq 0.0001$). Out of which, 19% {38/200}of HCW were *S.pneumoniae* and 11% {22/200} were *H. influenzae* (Fig. 4). About 10% {13.2/132} of HCW in the age group 18-35 and 9% {5.76/64} in the age group 36-55 had *pneumococcal* carriage whereas 3.5% {4.62/132} of HCW in 18-35 age group and 7.5% {4.8/64} of HCW in the age group 36-55 carried *H. influenzae* in their pharynx (Fig. 5). About 14% {20.16/144} of the females and 5% {2.8/56}of the males harbored *S. pneumoniae* in their oropharynx whereas 6.5% {9.36/144} of the females and 4.5% {2.52/56} of the males harbored *H. influenzae* (Fig. 6). Carrier state was found to be significantly higher in female volunteers when compared to males ($p = 0.0298$). Distribution of *S.pneumoniae* colonization among HCW

Tab. I. Set of primers included in the study.

Primers	Gen. bank accession n.	Primer sequence (5'-3')	Gene	Nucleotide position	Product size bp
1-f	CR931632	CTC TAT AGA ATG GAG TAT ATA AAC TAT GGT TA	*wzy*	9,935	280
1-r		CCA AAG AAA ATA CTA ACA TTA TCA CAA TAT TGG C		10,181	
3-f	CR931634	ATG GTG TGA TTT CTC CTA GAT TGG AAA GTA G	*gal U*	*9,020*	*371*
3-r		CTT CTC CAA TTG CTT ACC AAG TGC AAT AAC G		*9,360*	
5-f	CR931637	ATA CCT ACA CAA CTT CTG ATT ATG CCT TTG TG	*wzy*	6,123	362
5-r		GCT CGA TAA ACA TAA TCA ATA TTT GAA AAA GTA TG		6,450	
19F-f	CR931678	GTT AAG ATT GCT GAT CGA TTA ATT GAT ATC C	*wzy*	11,135	304
19F-r		GTA ATA TGT CTT TAG GGC GTT TAT GGC GAT AG		11,407	
8-f	CR931644	GAA GAA ACG AAA CTG TCA GAG CAT TTA CAT	*wzy*	11,193	201
8-r		CTA TAG ATA CTA GTA GAG CTG TTC TAG TCT		11,364	
14-f	CR931662	GAA ATG TTA CTT GGC GCA GGT GTC AGA ATT	*wzy*	7,959	189
14-r		GCC AAT ACT TCT TAG TCT CTC AGA TGA AT		8,119	

Fig. 1. Age wise distribution among HCW.

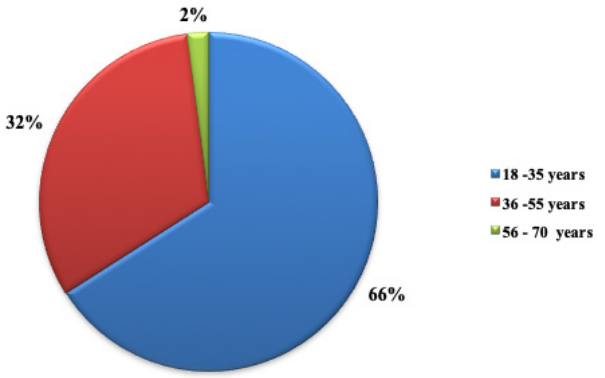


Fig. 2. Sex wise distribution of HCW.

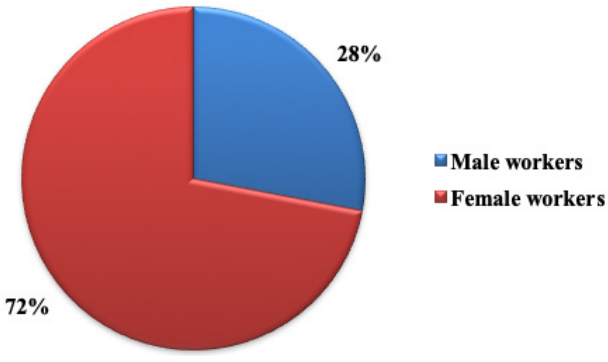


Fig. 3. Occupation status wise distribution among HCW.

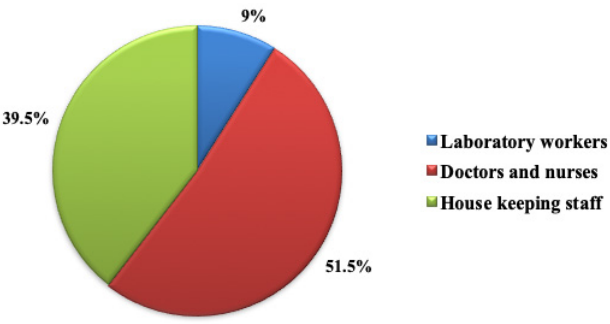


Fig. 4. Oropharyngeal colonization of *S. pneumoniae* and *H. influenzae* in HCW.

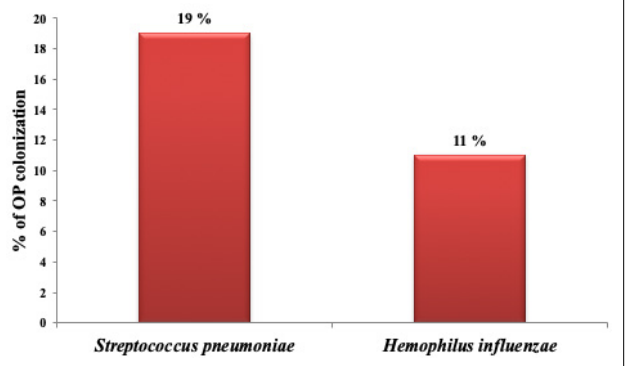
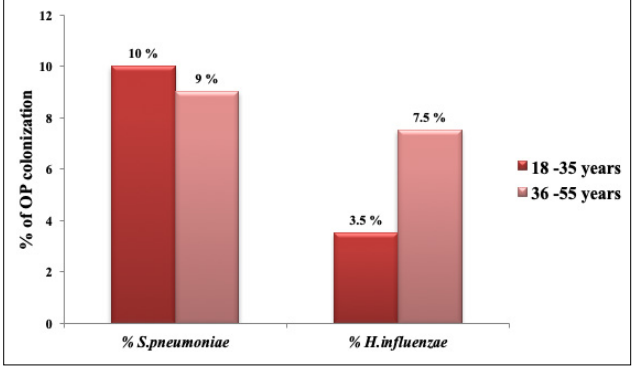


Fig. 5. Oropharyngeal colonization of *S. pneumoniae* and *H. influenzae* among different age groups of HCW.



belonging to different category include, 10% {10.3/103} of doctors and nurses, 8% {6.32/79} of housekeeping staff and 1% {0.18/18} of laboratory workers. Whereas distribution of *H. influenzae* include, 4.5%{4.635/103}, 6.5% {5.135/79} and 0% respectively (Fig. 7).

Among the Non-HCW 28% {56/200} of them belonged to the age group 18-35, 14% {28/200} were in the age group 36-55, 58% {116/200} in the age group 56-70 (Fig. 8). Out of the 200 Non-HCW, 59% {118/200} of the healthy volunteers were female and 41% {82/200} were male (Fig. 9). Among the non-HCW 42% {84/200} of them were white collar workers, 30% {60/200}

Fig. 6. Oropharyngeal colonization of *H. influenzae* and *S. pneumoniae* among male and female HCW.

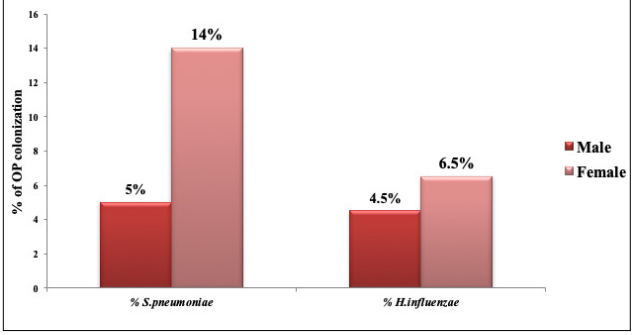


Fig. 7. % of Oropharyngeal colonization of *S. pneumoniae* and *H. influenzae* isolates among various category of HCW.

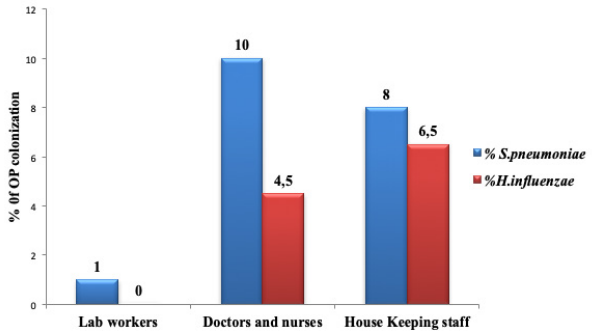


Fig. 8. Age wise distribution among Non-HCW.

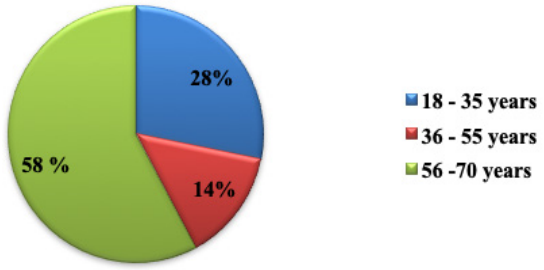
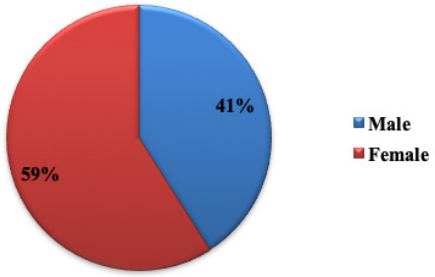
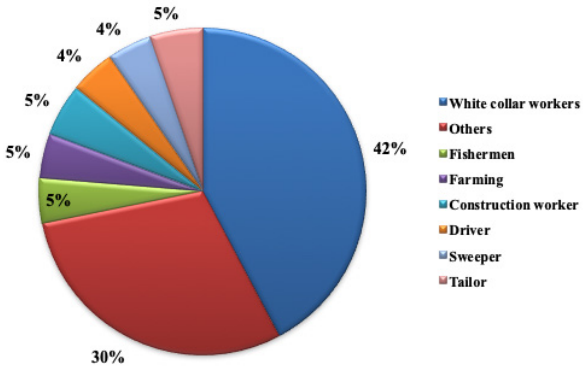


Fig. 9. Sex wise distribution of Non-HCW.



include home makers and other subjects not pursuing any job, 4% {8/200} fishermen, 4% {8/200} farmers, 5% {10/200} construction workers, 4%{8/200} drivers, 4% {8/200} sweepers and 5% {10/200} tailors (Fig. 10). A total of 23%{46/200} of Non-HCW were colonized with *S. pneumoniae* and *H. influenzae*. Out of which

Fig. 10. Occupation status wise distribution among Non-HCW.



16% {32/200} had pneumococcal carriage and 7% {14/200} had *H. influenzae* (Fig. 11).

In this study 3% {1.68/56} of Non-HCW in the age group 18-35, 4% {1.12/28} of Non-HCW in the age group 36-55 and 9% {10.44/116} of Non-HCW in the age group 56-70 harbored *Pneumococci*. Whereas 0.5% {0.28/56} of Non-HCW in age group 18-35, 2.0% {0.56/28} in the age group 36-55 and 4.5% {5.22/116} in the age group 56-70 had *H. influenzae* carriage (Fig. 12).

Individuals in the age group 56-70 years had significantly a greater prevalence rate when compared to young people

Fig. 11. Oropharyngeal colonization of *H. influenzae* and *S. pneumoniae* in Non-HCW.

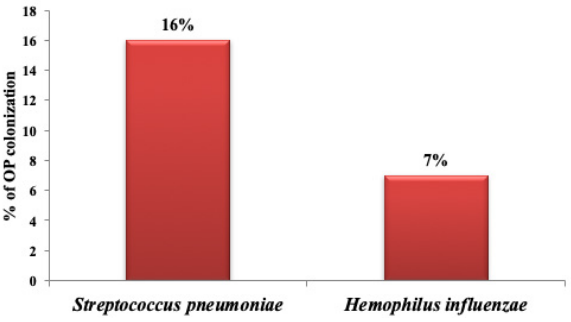
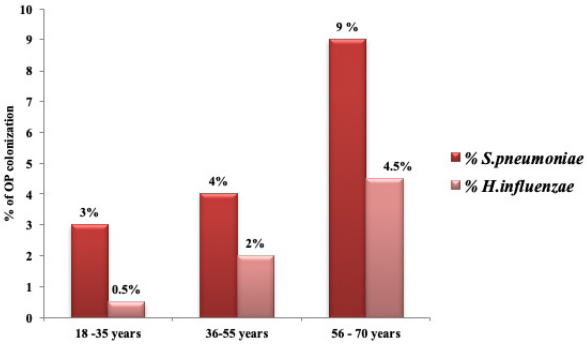


Fig. 12. Oropharyngeal colonization of *H. influenzae* and *S. pneumoniae* among different age groups of Non-HCW.



(P = 0.0014). About 9% {7.38/82} of the male and 7% {8.26/118} of the female Non-HCW was colonized with *Pneumococci* whereas 4.5%{3.69/82} of the male and 2.5% {2.95/118} of the females was colonized with *H. influenzae* (Fig. 13).

Colonization of *Pneumococci* among Non-HCW belonging to different occupation include white collar workers 5% {4.2/84}, others (home makers and other subjects not pursuing any job) 9% {5.4/60} , fishermen 2%{0.16/8} and sweepers nil. Non-HCW colonized with *H. influenzae* includes 1.5% {1.26/84}, 4.5% {2.7/60}, 0% and 1% {0.08/8} respectively (Fig. 14).

Thus in this study 30% {60/200} of the HCW and 23% {46/200} of the Non-HCW were colonized with *S. pneumoniae* and *H. influenzae* (Fig. 15).

Antimicrobial susceptibility pattern of *Pneumococci* and *H. influenzae* obtained from HCW & Non-HCW. The *Pneumococcal* isolates obtained from the healthcare workers showed 100% susceptibility to penicillin, 82% {31.16/38} to erythromycin, 73% {27.74/38} to clindamycin, 64% {24.32/38} to ofloxacin, 45% {17.1/38} to tetracycline and 100 % to vancomycin. Whereas the susceptibility pattern of *Pneumococcal* isolates from Non-HCW include 100% to penicillin, 47% {15.04/32} to erythromycin, 60% {19.2/32} to clindamycin, 87% {27.84/32} to ofloxacin, 67% {21.44/32} to tetracycline and 100 % to vancomycin (Fig. 16).

H. influenzae isolates obtained from Healthcare and Non-Healthcare workers showed 10% susceptibility to ampicillin, 85% to cefotaxime and 100% susceptibility to imipenem & meropenem (Fig. 17).

Representative *Pneumococcal* isolates obtained from Health care and Non-Healthcare workers were subjected to serotyping multiplex PCR. The serotypes obtained include 19F, 3, 1 & 5 (Fig. 18).

Fig. 13. Oropharyngeal colonization of *H. influenzae* and *S. pneumoniae* among male and female Non-HCW.

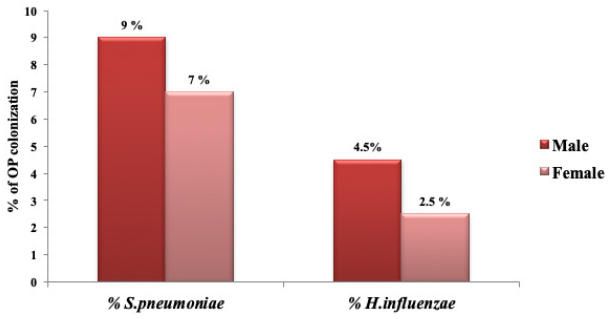


Fig. 14. Oropharyngeal colonization of *H. influenzae* and *S. pneumoniae* among Non-HCW belonging to different occupation.

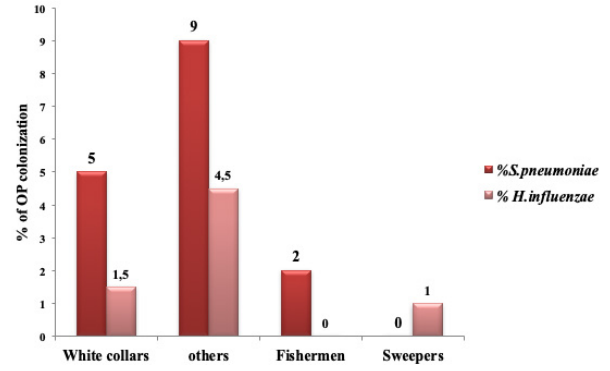


Fig. 15. *H. influenzae* and *S. pneumoniae* oropharyngeal carriage among HCW and Non-HCW.

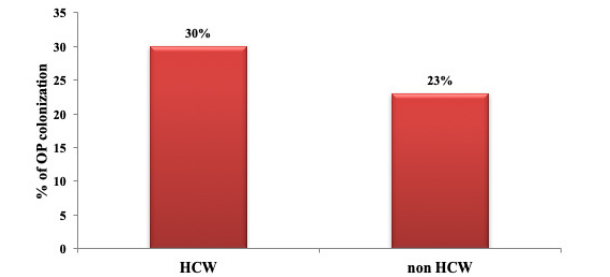


Fig. 16. Antibiotic susceptibility pattern of *Pneumococci* obtained from HCW and Non-HCW.

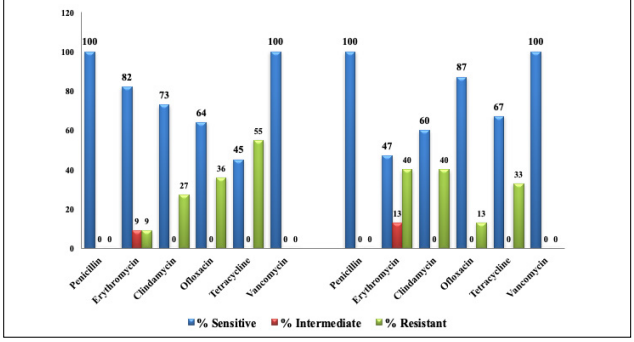


Fig. 17. Antibiotic susceptibility pattern of *H. influenzae* obtained from HCW and Non-HCW.

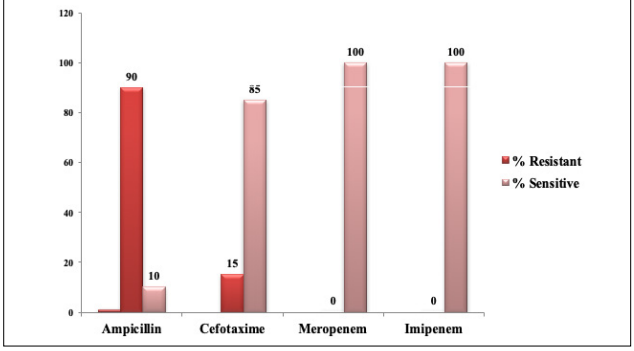
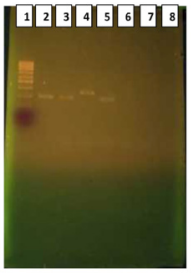


Fig. 18. Gel pictures representing different serotypes of *S. pneumoniae*.



Lane : 100bp Ladder, Lane 2 : serotype 19F, Lane 3: serotype 19F, Lane 4: serotype3, Lane 5: serotype1, Lane 6,7,8 -NIL

Discussion

The upper respiratory tract is the ecological niche for many bacterial species. Colonization of the pathogens like *S. pneumoniae* and *H. influenzae* usually result in the horizontal dissemination of these pathogens to individuals within the community. The rates of bacterial colonization mostly depend on the age, occupation, geographical area and socioeconomic conditions [13-16]. Current study shows that the people belonging to 56-70 years had more colonization of *S.pneumoniae* and *H. influenzae* when compared to people belonging to age groups between 18-55 years and this was concordant with few studies [17]. This could be due to decreasing immunity with increasing age and associated chronic conditions.

Our study shows that oropharyngeal colonization was found to be more predominant among female health care workers when compared to male health care workers. This may be due to the predominant female population among health care workers especially with nursing and housekeeping staff. In the present study the *S. pneumoniae* and *H. influenzae* carrier state was found to be higher in healthcare workers (30%) when compared to Non-Health care workers (23%). This was similar to the study report by Hosuru Subramaniya 2016 in which about 65% of HCW and 32% of Non-HCW harbored these pathogens.

Among the health care workers, doctors, nurses and housekeeping staff had higher carrier rate when compared to laboratory workers. This was consistent with other reports [10]. This clearly depicts that higher rate of carriage among healthcare workers especially among doctors, nurses and housekeeping personnel may be attributed due to frequent exposure to hospital environment.

In our study, *Pneumococcal* isolates from HCW showed 100% susceptibility to penicillin and vancomycin whereas resistance was noted against erythromycin, clindamycin, ofloxacin and tetracycline as 9%, 27%, 36% and 55% respectively. However, isolates from non-HCW showed 100% susceptibility to both penicillin and vancomycin but 40% of the isolates showed resistance towards erythromycin & clindamycin, 13% towards ofloxacin and 33% were resistant to tetracycline. In our study, isolates from HCW showed better susceptibility towards erythromycin & clindamycin whereas isolates from non-HCW showed better susceptibility towards ofloxacin and tetracycline. A report by Goyal gives 100% sensitivity to quinolones, macrolides and 61% resistance to tetracycline [18]. Whereas another report states that 90-100% of the isolates were found to be susceptible to penicillin and erythromycin which was found to be consistent with our report [10]. *H. influenzae* isolates from HCW and non-HCW showed 90% resistance towards penicillin whereas only 15% of the isolates were resistant towards cefotaxime. All the isolates were susceptible to imipenem and meropenem. A study by Yunusa Thairu states that majority of the *H. influenzae* isolates were resistant to ampicillin whereas all the isolates were sensitive to third generation cephalosporins like ceftriaxone [19]. Serotyping done for representative isolates revealed the following serotypes, which include 1, 5, 3 and 19F. Another study done by Molander gives a report that the most common serotypes obtained from invasive infections include 1,5, 19F, 6B, 14 and 3 [20]. However Felipe Piedade Gonçalves Neves reports that the prevalent serotype among carrier isolates include 6B, 19F, 6A, 14, 15C and 23F [21]. A study from India states that serotype 1 and 3 are the prevalent serotypes in all age groups [22]. Another report from India gives serotypes 1, 3, 5, 19F, 8, 14, 23F, 4, 19A and 6B as the predominant serotypes. Since the oropharyngeal colonizers are responsible for the spread of invasive infection a thorough knowledge about the prevalent colonizing serotypes can further aid in improving the efficacy of currently available pneumococcal vaccine.

Conclusions

The present study gives a greater prevalence rate of *S. pneumoniae* and *H. influenzae* among HCW when compared to Non-healthcare workers. This will definitely increase horizontal spread of infections and further accelerate the occupational risk. Thus carrier status is a concern for both HCW and non-HCW which can pave way for increased morbidity and mortality rate. Increased carrier state prevalence among old age group underscores the importance of vaccination among these individuals. Serotyping of the carrier strains definitely will give a greater insight for the inclusion of the serotype as a vaccine candidate and also help in identifying its clinical significance.

Acknowledgements

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

APSR, MN, AGN and PS designed the study. AGN, APSR and PJ collected, processed and identified the strains. AGN, APSR, MN and PS participated in the manuscript revision. All authors of the manuscript gave their complete approval.

References

[1] Otsuka T, Chang B, Shirai T, Iwaya A, Wada A, Yamanaka N, Okazaki M, SADO-study Working Group. Individual risk factors associated with nasopharyngeal colonization with Streptococcus pneumoniae and Haemophilus influenzae: a Japanese birth cohort study. Pediatr Infect Dis J 2013;32:709-14. https://doi.org/10.1097/INF.0b013e31828701ea

[2] Factor SH, LaClaire L, Bronsdon M, Suleymanova F, Altynbaeva G, Kadirov BA,Shamieva U, Dowell SF, Schuchat A, Facklam R, Schwartz B. Streptococcus pneumoniae and Haemophilus influenzae type b Carriage, Central Asia. Emerg Infect Dis 2005;11:1476. https://doi.org/10.3201/eid1109.040798

[3] Dhakal R, Sujatha S, Parija SC, Bhat BV. Asymptomatic colonization of upper respiratory tract by potential bacterial pathogens. Indian J Pediatr 2010;77:775-8. https://doi.org/10.1007/s12098-010-0118-x

[4] Faden H, Duffy L, Wasielewski R, Wolf J, Krystofik D, Tung Y. Relationship between nasopharyngeal colonization and the development of otitis media in children. J Infect Dis 1997;175:1440-5. https://doi.org/10.1086/516477

[5] Mukundan D, Ecevit Z, Patel M, Marrs CF, Gilsdorf JR. Pharyngeal colonization dynamics of Haemophilus influenzae and Haemophilus haemolyticus in healthy adult carriers. J Clin Microbiol 2007;45:3207-17. https://doi.org/ 10.1128/JCM.00492-07

[6] Janssens JP, Krause KH. Pneumonia in the very old. Lancet Infect Dis 2004;4:112-24. https://doi.org/10.1016/S1473-3099(04)00931-4

[7] Kim JO, Weiser JN. The respiratory tract microflora and disease. In: Medical importance of the normal microflora. Boston: Springer 1999, pp. 47-73. https://doi.org/10.1007/978-1-4757-3021-0_3

[8] Murphy TV, Clements JF, Petroni M, Coury S, Stetler L. Haemophilus influenzae type b in respiratory secretions. Pediatr Infect Dis J 1989;8:148-51.

[9] Smith-Vaughan H, Crichton F, Beissbarth J, Morris PS, Leach AJ. Survival of pneumococcus on hands and fomites. BMC Res Notes 2008;1:112. https://doi.org/10.1186/1756-0500-1-112

[10] Subramanya SH, Thapa S, Dwedi SK, Gokhale S, Sathian B, Nayak N, Bairy I. Streptococcus pneumoniae and Haemophilus species colonization in health care workers: the launch of invasive infections? BMC Res Notes 2016;9:66. https://doi.org/10.1186/s13104-016-1877-x

[11] Ueno M, Ishii Y, Tateda K, Anahara Y, Ebata A, Iida M, Inamura S, Takahata K, Suzuki Y, Chang B, Wada A. Prevalence and risk factors of nasopharyngeal carriage of Streptococcus pneumoniae in healthy children in Japan. Jpn J Infect Dis 2013;66:22-5. https://doi.org/10.7883/yoken.66.22

[12] Kolšek-šušteršič M, Krasnič AB, Mioč V, Paragi M, Rifel J. Nasopharyngeal carriage of Streptococcus pneumoniae and serotypes indentified among nursing home residents in comparison to the elderly and patients younger than 65 years living in domestic environment. Slovenian Journal of Public Health 2017;56:172-8. https://doi.org/ 10.1515/sjph-2017-0023

[13] Bogaert D, De Groot R, Hermans PW. Streptococcus Pneumoniae colonisation: the key to pneumococcal disease. Lancet Infect Dis 2004;4:144-54. https://doi.org/10.1016/S1473-3099(04)00938-7

[14] Jourdain S, Smeesters PR, Denis O, Dramaix M, Sputael V, Malaviolle X, et al. Differences in nasopharyngeal bacterial carriage in preschool children from different socio-economic origins. Clin Microbiol Infect. 2011;17(6):907–914. https://doi.org/10.1111/j.1469-0691.2010.03410.x.

[15] Mackenzie GA, Leach AJ, Carapetis JR, Fisher J, Morris PS. Epidemiology of nasopharyngeal carriage of respiratory bacterial pathogens in children and adults: cross-sectional surveys in a population with high rates of pneumococcal disease. BMC Infect Dis 2010;10:304. https://doi.org/10.1186/1471-2334-10-304

[16] Garcia-Rodriguez JA, Fresnadillo Martinez MJ. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. J Antimicrob Chemother 2002;50(Suppl S2):59-73. https://doi.org/10.1093/jac/dkf506

[17] Smith EL, Wheeler I, Adler H, Ferreira D, Abdullahi O, Adetifa I, Sa-Leao R, Becker-Dreps S, Esposito S, Farida H, Kandasamy R. Upper airways carriage of Streptococcus pneumoniae in over 60 year olds: a systematic review of prevalence and individual participant data meta-analysis of risk factors. Journal of Infection 2020;81:540-8. https://doi.org/10.1016/j.jinf.2020.06.028

[18] Goyal R, Singh NP, Kaur M, Talwar V. Antimicrobial resistance in invasive and colonising Streptococcus pneumoniae in North India. Indian J Med Microbiol 2007;25:256. https://doi.org/ 10.4103/0255-0857.34770

[19] Thairu Y, Nasiru IA, Kolade-Yunusa OH. Antimicrobial susceptibility pattern of *Haemophilus influenzae* among under-five children presenting at the Emergency Paediatric Unit (EPU) of two teaching hospitals in Jos, Nigeria. Br J Med Med Res 2014;4:4812. https://doi.org/10.9734/BJMMR/2014/10806

[20] Molander V, Elisson C, Balaji V, Backhaus E, John J, Vargheese R, Jayaraman R, Andersson R. Invasive pneumococcal infections in Vellore, India: clinical characteristics and distribution of serotypes. BMC Infect Dis 2013;13:532. https://doi.org/ 10.1186/1471-2334-13-532

[21] Neves FP, Pinto TC, Corrêa MA, dos Anjos Barreto R, Moreira LD, Rodrigues HG, Cardoso CA, Barros RR, Teixeira LM. Nasopharyngeal carriage, serotype distribution and antimicrobial resistance of Streptococcus pneumoniae among children from Brazil before the introduction of the 10-valent conjugate vaccine. BMC Infect Dis 2013;13:318. https://doi.org/ 10.1186/1471-2334-13-318

[22] Jayaraman R, Varghese R, Kumar JL, Neeravi A, Shanmugasundaram D, Ralph R,Thomas K, Veeraraghavan B. Invasive pneumococcal disease in Indian adults: 11 years'experience. J Microbiol Immunol Infect 2018;52:736-42. https://doi.org/ 10.1016/j.jmii.2018.03.004

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Correspondence: Alice Peace Selvabai R, Chettinad Hospital and Research Institute, Kelambakkam 603103 - Tel.: 7401098441 - E-mail: alice.peace@gmail.com

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ORIGINAL ARTICLE

HIV/AIDS knowledge among Iranian Health Care Workers

MEHDI MIRZAEI-ALAVIJEH[1], FARZAD JALILIAN[2], MOHAMMAD ESMAIEL MOTLAGH[3], ABDOLLAH SAADATFAR[4], MOHHAMAD FATTAHI[5]

[1] Social Development & Health Promotion Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran; [2] Lifestyle Modification Research Center, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran; [3] Department of Pediatrics, Faculty Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; [4] Department of Urology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran; [5] Social Development & Health Promotion Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran

Keywords

Sexually transmitted • Public Health • Health Care Workers

Summary

Background. *HIV/AIDS remains a major public health concern globally and Health Care Workers (HCWs) are in the frontline of preventing and providing care in the health care system. The aim of this study was to evaluate HIV/AIDS knowledge among Iranian HCWs.*

Methodology. *This cross-sectional study was conducted among 200 HCWs who were randomly selected from health care centers in Kermanshah city, west of Iran, 2018. HCWs filled out a self-administered questionnaire including the socio-demographic characteristics and HIV/AIDS knowledge items. Data were analyzed by SPSS version 16 using bivariate correlations, t-test, and ANOVA statistical tests.*

Results. *The mean score of HIV/AIDS knowledge was 29.73 [95% CI: 28.79, 30.67], ranged from 0 to 40 (74.3% of total percent). There was no significant association and correlation between HIV/AIDS knowledge and sex, education level, marital status, age and job history. Up to 50% had inadequate knowledge about HIV/AIDS status and transmission in Iran.*

Conclusions. *HCWs HIV/AIDS knowledge was average and it seems need to be educating regarding HIV/AIDS status and transmission in Iran.*

Introduction

HIV/AIDS remains a problem in many countries, especially in less developed countries [1]. According to the Global HIV & AIDS statistics, 37.9 million [32.7 million-44.0 million] million people live with HIV/AIDS worldwide [2]. Although, prevalence of HIV/AIDS in the Eastern Mediterranean Region (EMR) countries like Iran is lower than the western countries, but HIV/AIDS infection is becoming more prevalent in developing countries gradually, due to unprotected sexual intercourse and drug abuse behaviors [3]. In 2015, the rate of HIV/AIDS prevalence in Iran was 11.4 per 100,000 populations, a 10.5% increase from 1990 [4]. Of course, the real prevalence can be higher, due to the social, cultural, taboo and religious factors in the Middle East, reporting is underestimate [5]. Although people of all ages in both genders are susceptible to HIV/AIDS infection; nonetheless, gender roles and relations are increasingly known as one of the essential factors driving the rapid spread of HIV infection [6]. As well as, globally, differences in the number of new HIV infections between men and women are more pronounced at younger ages and statics indicated that the new infections among young women (aged 15-24 years) were higher than they were among men in the same age group [7]. Moreover, according to the World Health Organization (WHO) and the Joint United Nations Program on HIV/AIDS, younger people are more at risk of HIV [8]. The significant contributors for the spread of HIV/AIDS would be drug abuse (i.e. Injection Drug Users (IDUs) and needle and syringes sharing), unprotected and high-risk sexual activity (i.e. sex workers), inadequate health literacy (i.e. less HIV/AIDS knowledge), discriminatory attitude (i.e. HIV-positive patients may be rejected in a relationship) and anti-social behavior against infected people (i.e. HIV-positive patients may be separated from the society), and likewise HIV/AIDS is a rare social and also familial discussion [9].

A culture of silence surrounds HIV/AIDS and related issues, especially in developing countries like Iran, where people are still living in a conservative society, cannot help the health care system to reduce HIV/AIDS prevalence [10]. In the worst situation, HIV-related issues are still shadowed by cultural and social restrictions, discrimination, discounting, discrediting, denial, stigma, and prejudice directed at people living with HIV/AIDS; especially sex workers and IDUs, which leads to many HIV/AIDS prevention interventions are broken, and consequently the epidemic continues to spread [11]. For example, a study conducted in the United State (US) in 2016, illustrated most HIV-positive patients experienced stigma in their daily lives, as well as from their healthcare workers [12]. While based on the World Bank state-

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ment "waiting to intervene may be costly", indicates the need to take action immediately [13].

The knowledge of HCWs regarding HIV/AIDS infection is a major factor influencing their willingness to provide care for HIV-positive patients and also the willingness of people living with HIV to access care, and the quality of the care they receive [14]. Despite HIV-related medical education and experience in the last decades, some health care providers still have shown a reluctance to take care of HIV-positive patients, in particular in developing countries [15]. In addition, HCWs may be exposed to the risks associated with blood-borne viruses like HIV/AIDS via contact with blood (and other body fluids) during their work for example needle sticks; evidence shows that annually about 3 million percutaneous exposures with blood borne pathogens happen among 35 million HCWs across the world, leads to 200 to 5000 HIV/AIDS infections [16]. On the other hand, several studies noted that the use of determinants such as knowledge in planning health promotion programs could be useful in order to the promotion of healthy behavior [17]. HCWs are the milestone of medical care and also play a basic role in health areas and globalization health. Lack of knowledge may contribute to HCWs' attitudes and concerns about providing care and also may lead to occupational HIV/AIDS transmission. In addition, according to the Kermanshah University of Medical Sciences and Health Services, 3551 (11.76%) HIV/AIDS patients are identified in the Kermanshah province from 30183 HIV/AIDS patients in Iran [18].

With this background, HCWs are at serious risk of HIV/AIDS infection. Consequently, HIV may be transmitted from HCWs to the community if he/she does not have adequate HIV/AIDS Knowledge. Therefore, it is crucial to assess the knowledge of HCWs regarding HIV/AIDS infection if prevention is to be addressed appropriately. The aims of the study were to evaluation HIV/AIDS knowledge among HCWs in western Iran.

Methodology

Participants and sampling

This cross-sectional study was conducted among HCWs in Kermanshah, the west of Iran, during 2018, with the aim of providing knowledge for the planning health promotion program to increasing knowledge HCWs in west of Iran towards HIV/AIDS. The sample size was calculated at 95% significant level according to the results of a pilot study among 30 HCWs which that reported the standard deviation (SD) of HIV/AIDS knowledge among HCWs was 7.05 a sample of 200 was estimated. Of the population of 200, 191 (95.5%) signed the consent form and voluntarily agreed to participate in the study, that has been approved by institutional review board at the Kermanshah University of medical sciences. This research has been approved by the research ethics committees at the Kermanshah University of medical sciences, the west of Iran (IR.KUMS.REC.1398.469).

To select the participants and collect data the following stages were done. First, areas of the city were classified based on the division of the geographical region, next for each social class two health care centers were randomly selected (a total of sixteen health centers were selected). Then, among the primary health care workers on the health care centers, were enrolled into this study voluntarily. Finally, the volunteers were given the self-administration questionnaire, after we explained the main objective of the study.

Tool and data collection

Prior to conducting the main study, a pilot study was conducted to evaluate the validity and reliability of the instrument. The pilot study was conducted among 30 subjects, similar to those who participated in the main study. Questionnaire included two sections that comprised of 45 question and items: 5 questions for demographic factors, and 40 items for HIV-AIDS knowledge.

A: Demographics questionnaire

The background variables assessed in this study included: age (year), job history (year), sex (male, female), marital status (single, married), and educational level (technician, BSc, MSc and MD).

B: HIV-AIDS knowledge questionnaire

HIV-AIDS knowledge was evaluated by 40 questions standard scale in Persian [14]. Each question was measured on a multiple-choice question. Score ranged was 0 to 40. Example of the question is: "HIV/AIDS not transmitted by social relationships, so there isn't needed in isolation of HIV-positive patients from others". The reliability coefficient of HIV-AIDS knowledge questionnaire in our study was 0.84.

To collect data, we contacted the administrative staff in each health centers to find available times that we could complete the questionnaires among selected HCWs. Data collection was done in a face-to-face interview style, which took about 10-12 min to complete. Then, the eligible HCWs signed consent forms, were interviewed separately by two trained interviewers after we explained the main objective of the study.

Data analysis

The Statistical Package for the Social Sciences (SPSS) (ver. 16.0) was used for the purpose of data entry, manipulation, and analysis. Quantitative variables were expressed as means with CI, SDs, and qualitative/categorical ones as frequencies and percentages. Bivariate correlations were computed to ascertain the magnitude and direction of the associations between the knowledge with the age and job history. One-way ANOVA and t-test was performed to explain the association of sex, education level and marital status with HIV-related knowledge. In addition, split-half was used to estimate the internal consistency of the various measures in our study.

Results

The mean age of respondents was 37.04 years [95% CI: 36.11, 37.97], ranged from 22 to 54 years. The mean years of job history of respondents was 12.30 years [95% CI: 11.36, 13.25], ranged from 1 to 27 years. More details of demographic characteristics of the participants are shown in Table I.

The mean score of HIV-AIDS knowledge of respondents was 29.73 [95% CI: 28.79, 30.67], ranged from 0 to 40. Based on this finding, respondents were received 74.3% of total score of HIV-AIDS knowledge questioner.

As indicated in Table II HIV-AIDS knowledge was not significant association with sex, education level and marital status.

Our findings indicated knowledge was not significant correlation with the age (r = 0.082) and job history (r = 0.088).

Table III has been showed the correct and incorrect answer of HIV-AIDS knowledge items among participants.

Tab. I. Distribution of the demographic characteristics among the participants.

Percent	Number		Variables
13.1	25	20-29	Age group (year)
49.2	94	30-39	
35.6	68	40-49	
2.1	4	50-60	
18.3	35	1-5	Job history
25.7	49	6-10	
19.4	37	11-15	
29.3	56	16-20	
7.3	14	21-30	
25.7	49	Male	Sex
74.3	142	Female	
23.6	45	Technician	Education level
67.5	129	BSc	
8.9	17	MSs or MD	
25.7	49	Single	Marital status
74.3	142	Married	

Tab. II. Association of sex, education level and marital status with HIV-related knowledge.

Sig	Mean (SD)		Variables
0.272	28.73 (7.69)	Male	Sex
	30.07 (6.14)	Female	
0.850	30.22 (5.93)	Technician	Education level
	29.57 (6.77)	BSc	
	29.64 (6.99)	MSs or MD	
0.341	28.95 (7.16)	Single	Marital status
	30.00 (6.37)	Married	

Discussion

Our results showed that the total HIV/AIDS knowledge in our study was at a relatively moderate level (74.3%); showing gaps relating to HIV/AIDS infections exposures, HIV/AIDS risk prevention, and occupational HIV/AIDS transmission; and expressed the need for more education. The moderate level of HIV/AIDS knowledge in this study might be due to most HCWs were aged (30-49 years), and so may not have received a curriculum with update HIV/AIDS knowledge or may not have participated in HIV/AIDS in-service programs.

Our results concur with the results of some studies conducted in Iran and other countries. For instance, an international study conducted with the nurses in Finland, Estonia and Lithuania reported that the whole respondents showed moderate levels of HIV/AIDS knowledge in 2010 [19]. Also, Mulaudzi and colleagues from South Africa have reported 83.8% nurses had average level of HIV knowledge in 2011 [20]. Likewise, Delobelle and colleagues from South Africa have reported HIV/AIDS knowledge of nurses was moderate in 2009 [21]. Similarly, Saydkhani et al work conducted in Ahvaz, another state of Iran, showed 60% anesthesia staff had moderate knowledge regarding HIV/AIDS infection in 2010 [22]. However, many other researchers reported converse results. For example, research conducted by Kumar et al among paramedical staff working in private healthcare facilities in Delhi, showed that majority had poor knowledge regarding HIV/AIDS transmission in 2017 [23]. A study conducted in KwaZulu-Natal, South Africa also revealed that the health care providers had limited knowledge toward the reproductive needs and rights of individuals living with HIV in 2017 [24]. Similarly, Kawale et al, from Malawi have reported most health care providers had very poor knowledge about HIV and reproductive health, as well as prevention of mother-to-child transmission in 2015 [25].

Conversely, Desai and colleagues showed 80% health care providers were knowledgeable in term of pre-exposure prophylaxis for HIV infection in United Kingdom in 2016 [26]. Likewise, the results of Hughes's survey conducted in 2011, showed the favorable knowledge of HIV/AIDS with scores of 89% and 84% for physicians and nurses, respectively [27]. A similar study conducted by Jafari et al. in Tehran, capital city of Iran, showed that 83.8% participants had good knowledge of HIV/AIDS and its transmission [28].

Moreover, we found no significant association between sex, education level, marital status, age and years of work experience with HIV/AIDS knowledge. A study by Delobelle et al. also indicated HIV/AIDS knowledge was not associated with age, gender and years of experience [21]. To best our knowledge, the higher level of education was directly related to more favorable knowledge, however, we found no relationship between education level and HIV/AIDS knowledge, need to be more addressed. In contrast, the results of study conducted by Jafari et al. observed a significant association between sex, age, education level and job history with HIV/AIDS knowledge [28]. Conversely, Oppong et al reported that HIV/AIDS knowledge were significantly associated with marital status [29].

Tab. III. Correct and incorrect answer of HIV-AIDS knowledge items among participants.

No	Items	Correct	Incorrect
1	The third wave of HIV/AIDS in Iran is caused by unprotected sexual behaviors.	2 (1%)	189 (99%)
2	In Iran, mother-to-child and sexual transmission have rapidly increased in recent years.	61 (31.9%)	130 (68.1%)
3	Nowadays, sexual transmission is the most common HIV/AIDS transmission in Iran.	63 (33%)	128 (67%)
4	Those with high-risk behaviors should refer to counseling centers for behavioral diseases.	85 (44.5%)	106 (55.5%)
5	Usually, mother-to-child HIV transmission occurs during the first and last month of pregnancy or during delivery or breastfeeding.	87 (45.5%)	104 (54.5%)
6	Repeating the HIV/AIDS test every 3-6 months is important for at-risk peoples.	88 (46.1%)	103 (53.9%)
7	Psychedelic drug use, especially methamphetamines increase the risk of sexual transmission of HIV/AIDS.	95 (49.7%)	96 (50.3%)
8	Multiple sexual partners, sexuality and the stage of HIV-AIDS in patients lead to the increased likelihood of transmission.	114 (59.7%)	77 (40.3%)
9	The window period is time between infections with HIV/AIDS until the HIV antigen detected by standard HIV tests.	127 (66.5%)	64 (33.5%)
10	HIV/AIDS transmission through blood and contaminated blood products has been controlled in Iran.	131 (68.6%)	60 (31.4%)
11	Formula milk feeding is an effective prevention strategy to reduce mother-to-child HIV/AIDS transmission during breastfeeding.	134 (70.2%)	57 (29.8%)
12	Mother-to-child transmission of HIV/AIDS occurs during pregnancy, delivery, and breastfeeding.	135 (70.7%)	56 (29.3%)
13	Negative HIV test results immediately after high-risk behavior, it's not actually confident for HIV/AIDS transmission.	143 (74.9%)	48 (25.1%)
14	IDUs are only vulnerable to HIV/AIDS infection.	144 (75.4%)	47 (24.6%)
15	To know about the HIV/AIDS status, counseling and getting a test for HIV/AIDS is recommended.	146 (76.4%)	45 (23.6%)
16	Protected and safe sex is the most important strategy for prevention of HIV/AIDS in Iran.	151 (79.1%)	40 (20.9%)
17	Educational programs for youth regarding HIV/AIDS transmission is an effective preventive strategy, especially in sexual transmission.	152 (79.6%)	39 (20.4%)
18	People with high-risk behaviors are in prioritizing for consultation and testing of HIV/AIDS.	152 (79.6%)	39 (20.4%)
19	Antiretroviral treatment for the mother, prenatal care for HIV-positive women and cesarean delivery may prevent mother-to-child transmission of HIV/AIDS.	156 (81.7%)	35 (18.3%)
20	HIV/AIDS infection is becoming more prevalent in Iran.	157 (82.2%)	34 (17.8%)
21	Sexually transmitted diseases increase the risk of HIV/AIDS infection transmission.	159 (83.2%)	32 (16.8%)
22	HIV/AIDS may not be transmitted from a shared bathroom and toilet.	161 (84.3%)	30 (15.7%)
23	In testing HIV/AIDS, the private space of information should be kept confidential.	161 (84.3%)	30 (15.7%)
24	HIV/AIDS infection is transmitted by mosquito bites and social relationships like talking, coughing, shaking hands and or kissing.	162 (84.8%)	29 (15.2%)
25	Reducing stress and anxiety, decisions on the need for repeat testing and appropriate psychological support from people who have positive test results are the purpose of the consultation before and after the test.	162 (84.8%)	29 (15.2%)
26	HIV/AIDS can be transmitted through needle and syringes sharing.	165 (86.4%)	26 (13.6%)
27	People with unprotected and high-risk sexual activity are more vulnerable to HIV/AIDS infection.	165 (86.4%)	26 (13.6%)
28	HIV/AIDS is not transmitted by social relationships, so there isn't needed the isolation of HIV-positive patients from others.	165 (86.4%)	26 (13.6%)
29	An HIV positive patient may be asymptomatic in the early stages.	166 (86.9%)	25 (13.1%)
30	HIV infection transmitted by blood, breast milk and sexual secretions.	167 (87.4%)	24 (12.6%)
31	HIV/AIDS not transmitted by touching the infected things e.g. money, dress and etc.	167 (87.4%)	24 (12.6%)
32	Drug abuse, infected with sexually transmitted diseases (STDs) and intercourse with men who have sex with men (MSM) increased the risk of HIV transmission.	167 (87.4%)	24 (12.6%)
33	Early diagnosis of HIV/AIDS leads to control of the disease, treatment and reduces the prevalence.	168 (88%)	23 (12%)
34	Lack of loyalty to the spouse and sexual immorality is the predisposing factor for HIV/AIDS transmission.	169 (88.5%)	22 (11.5%)
35	Sexual restraint, loyalty to the husband, sexual intercourse protected by a suspect sex partner is recommended to prevent sexual transmission of HIV/AIDS.	171 (89.5%)	20 (10.5%)
36	Pregnant women that themselves and their husbands' had a history of high-risk behaviors and also the history of prison should be recommended to HIV/AIDS tests.	174 (91.1%)	17 (8.9%)
37	HIV, which causes AIDS weakens and destroys the immune system	175 (91.6%)	16 (8.4%)
38	People with high-risk behaviors (i.e. unprotected sexual activity or needle sharing and syringes) should be recommended for getting a consultation and HIV/AIDS test.	175 (91.6%)	16 (8.4%)
39	Education is the best strategy to control HIV/AIDS.	177 (92.7%)	14 (7.3%)
40	Adolescence and youth are more vulnerable to HIV/AIDS infection.	180 (94.2%)	11 (5.8%)

Our participants had little knowledge regarding HIV/AIDS status in Iran, for example about; "The third wave of HIV/AIDS in Iran is caused by unprotected sexual behaviors" and "In Iran, mother-to-child and sexual transmission have rapidly increased in recent years" also "Nowadays, sexual transmission is the most common HIV/AIDS transmission in Iran. This finding clearly shows our study population did not access to update HIV/AIDS knowledge, in particular regarding HIV/AIDS status in Iran.

Less than 50% of the participants had the correct answer to the following questions; "Those with high-risk behaviors should refer to counseling centers for behavioral diseases", "Usually, mother-to-child HIV transmission occurs during the first and last month of pregnancy or during delivery or breastfeeding", "Repeating the HIV/AIDS test every 3-6 months is important for at-risk peoples" and "Psychedelic drug use, especially methamphetamines increase the risk of sexual transmission of HIV/AIDS", such findings clearly observe that more effort needs to be done to improve HIV-related knowledge among HCWs in Western Iran. In this regard Mbanya carried out a study on 107 nursing staff in a rural hospital of Cameroon and indicated the 70.1% of the nurses who responded scored highly in the knowledge section [30]. Moreover, Ghorbani in their study among nurses of Baqiyatallah hospital in Tehran in the capital of Iran and reported about half of the nurses had moderate knowledge about HIV/AIDS [31]. These results indicated supplementary education toward HIV/AIDS is needed to strengthen the knowledge of HCWs.

The most important limitation of the current study, as a cross-sectional, was that we used self-reported data to examine the knowledge toward HIV/AIDS among HCWs; these types of data may have a lower accuracy rather than observational methods. Secondly, some further analysis directed to the associations did not apply due to the cross-sectional nature of the study design. Third, our study was conducted among HCWs in the west of Iran and the results of this study are not generalizable to the other place or population. Fourthly, some HCWs were unwilling to participating in the study.

Conclusions

In our study, HIV/AIDS knowledge of HCWs was average, and several gaps and misconceptions were found regarding HIV/AIDS status and transmission in Iran. Given the global HIV/AIDS epidemic and increasing numbers of HIV/AIDS patients, HCWs should be educated regarding HIV/AIDS.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

Study design: MM-A and FJ. Data analysis: FJ and MM-A. Study supervision: MM-A. Manuscript writing and revisions: MM-A, FJ, MEM, AS, and MF. All authors provided comments and approved the final manuscript.

References

[1] Mafigiri R, Matovu JK, Makumbi FE, Ndyanabo A, Nabukalu D, Sakor M, Kigozi G, Nalugoda F, Wanyenze RK. HIV prevalence and uptake of HIV/AIDS services among youths (15–24 Years) in fishing and neighboring communities of Kasensero, Rakai District, South Western Uganda. BMC Public Health 2017;17:251 https://doi.org/10.1186/s12889-017-4166-2

[2] Global HIV & AIDS statistics - 2019 fact sheet. Available at: https://www.unaids.org/en/resources/fact-sheet. Accessed on 17-02-2020.

[3] Berhan Y, Berhan A. A meta-analysis of risky sexual behaviour among male youth in developing countries. AIDS Res Treat 2015;2015. https://doi.org/10.1155/2015/580961

[4] Mokdad AH. Trends in HIV/AIDS morbidity and mortality in Eastern Mediterranean countries, 1990-2015: findings from the Global Burden of Disease 2015 study. Int J Public Health 2017:1-14. https://doi.org/10.1007/s00038-017-1023-0

[5] Kabbash IA, Felemban SM, Stephens GM, Al-Hakeem RF, Zumla AI, Memish ZA. HIV case notification rates in the Kingdom of Saudi Arabia over the past decade (2000–2009). PloS One 2012;7:e45919. https://doi.org/10.1371/journal.pone.0045919

[6] Lekalakala-Mokgele E. Exploring gender perceptions of risk of HIV infection and related behaviour among elderly men and women of Ga-Rankuwa, Gauteng Province, South Africa. Sahara J 2016;13:88-95. https://doi.org/10.1080/17290376.2016.1218790

[7] Girum T, Wasie A, Lentiro K, Muktar E, Shumbej T, Difer M, Shegaze M, Worku A. Gender disparity in epidemiological trend of HIV/AIDS infection and treatment in Ethiopia. Arch Public Health 2018;76:51. https://doi.org/10.1186/s13690-018-0299-8

[8] Badru T, Mwaisaka J, Khamofu H, Agbakwuru C, Adedokun O, Pandey SR, Essiet P, James E, Chen-Carrington A, Mastro TD, Aliyu SH. HIV comprehensive knowledge and prevalence among young adolescents in Nigeria: evidence from Akwa Ibom AIDS indicator survey, 2017. BMC Public Health 2020;20:45. https://doi.org/10.1186/s12889-019-7890-y

[9] White S, Chen J, Atchison R. Relationship of preventive health practices and health literacy: a national study. Am J Health Behav 2008;32:227-42. https://doi.org/10.5993/AJHB.32.3.1

[10] Swendeman D, Rotheram-Borus MJ, Comulada S, Weiss R, Ramos ME. Predictors of HIV-related stigma among young people living with HIV. Health Psychol 2006;25:501. https://doi.org/10.1037/0278-6133.25.4.501

[11] Helms CB, Turan JM, Atkins G, Kempf M-C, Clay OJ, Raper JL, Mugavero MJ, Turan B. Interpersonal mechanisms contributing to the association between HIV-related internalized stig-

ma and medication adherence. AIDS Behav 2017;21:238-47. https://doi.org/10.1007/s10461-016-1320-2

[12] Stringer KL, Turan B, McCormick L, Durojaiye M, Nyblade L, Kempf M-C, Lichtenstein B, Turan JM. HIV-related stigma among healthcare providers in the deep south. AIDS Behav 2016;20:115-25. https://doi.org/10.1007/s10461-015-1256-y

[13] Jenkins C, Robalino DA. HIV/AIDS in the Middle East and North Africa: the costs of inaction. The World Bank 2003 Sep 15. https://doi.org/10.1596/0-8213-5578-3

[14] Liljestrand P. HIV care: continuing medical education and consultation needs of nurses, physicians, and pharmacists. J Assoc Nurses AIDS Care 2004;15:38-50. https://doi.org/10.1177/1055329003252053

[15] Ahsan Ullah AK. HIV/AIDS-related stigma and discrimination: A study of health care providers in Bangladesh. J Int Assoc Physicians AIDS Care (Chic) 2011;10:97-104. https://doi.org/10.1177/1545109710381926

[16] Kermode M, Jolley D, Langkham B, Thomas MS, Crofts N. Occupational exposure to blood and risk of bloodborne virus infection among health care workers in rural north Indian health care settings. Am J Infect Control 2005;33:34-41. https://doi.org/10.1016/j.ajic.2004.07.015

[17] Kok G, Gottlieb NH, Peters GJ, Mullen PD, Parcel GS, Ruiter RA, Fernández ME, Markham C, Bartholomew LK. A taxonomy of behaviour change methods: an Intervention Mapping approach. Health Psychol Rev 2016;10:297-312. https://doi.org/10.1080/17437199.2015.1077155

[18] Kermanshah University of Medical Sciences (KUMS). Available at: https://vc-health.kums.ac.ir/fa/news/28761. Accessed on 7-12 -2016.

[19] Suominen T, Koponen N, Mockiene V, Raid U, Istomina N, Vänskä ML, Blek-Vehkaluoto M, Välimäki M. Nurses' knowledge and attitudes to HIV/AIDS. An international comparison between Finland, Estonia and Lithuania. Int J Nurs Pract 2010;16:138-47. https://doi.org/10.1111/j.1440-172X.2010.01822.x

[20] Mulaudzi MV, Pengpid S, Peltzer K. Nurses' knowledge, attitudes, and coping related to HIV and AIDS in a rural hospital in South Africa. Studies on Ethno-Medicine 2011;5:25-32. https://doi.org/10.1080/09735070.2011.11886388

[21] Delobelle P, Rawlinson JL, Ntuli S, Malatsi I, Decock R, Depoorter AM. HIV/AIDS knowledge, attitudes, practices and perceptions of rural nurses in South Africa. J Adv Nurs 2009;65:1061-73. https://doi.org/10.1111/j.1365-2648.2009.04973.x

[22] Saydkhani V, Albooghobeish M, Khosravi S, Banisakini H, Mousahosaini N. Evaluation of AIDS disease information between anesthesia practitioners in educational hospitals of Ahvaz Jundishapur Univercity of Medical Sciences in 2010. Jentashapir Journal of Health Research 2012;3(2). http://eprints.ajums.ac.ir/id/eprint/11823

[23] Kumar S, Jain P, Jangid N. HIV related knowledge and attitude among nurses and paramedical health care providers in Delhi. Adhyayan: A Journal of Management Sciences 2017;7:41-6. https://doi.org/10.21567/adhyayan.v7i02.10759

[24] Mindry D, Maharaj P, Letsoalo T, Munthree C, Crankshaw T. Knowing client rights and meeting their needs: provider knowledge and attitudes toward meeting the reproductive needs and rights of people living with HIV in South Africa. Global perspectives on women's sexual and reproductive health across the life course. Springer 2018. p. 141-58. https://doi org/10.1007/978-3-319-60417-6_9

[25] Kawale P, Mindry D, Phoya A, Jansen P, Hoffman RM. Provider attitudes about childbearing and knowledge of safer conception at two HIV clinics in Malawi. Reprod Health 2015;12:17. https://doi.org/10.1186/s12978-015-0004-0

[26] Desai M, Gafos M, Dolling D, McCormack S, Nardone A. Healthcare providers' knowledge of, attitudes to and practice of pre-exposure prophylaxis for HIV infection. HIV Med 2016;17:133-42. https://doi.org/10.1111/hiv.12285

[27] Hughes AK. HIV knowledge and attitudes among providers in aging: results from a national survey. AIDS Patient Care STDS 2011;25:539-45. https://doi.org/10.1089/apc.2011.0026

[28] Jafari F, Kholdi N, Falah N, Mahmoudpour A. Factors related to knowledge and attitude about AIDS. Daneshvar Medicine 2007;14:1-8. https://www.sid.ir/en/Journal/ViewPaper.aspx?ID=101182

[29] Oppong AK, Oti-Boadi M. HIV/AIDS knowledge among undergraduate university students: implications for health education programs in Ghana. Afr Health Sci 2013;13:270-7. https://doi.org/10.4314/ahs.v13i2.11

[30] Mbanya DN, Zebaze R, Kengne AP, Minkoulou EM, Awah P. Knowledge, attitudes and practices of nursing staff in a rural hospital of Cameroon: how much does the health care provider know about the human immunodeficiency virus/acquired immune deficiency syndrome? Int Nurs Rev 2001;48:241-9. https://doi.org/10.1046/j.1466-7657.2001.00090.x

[31] Ghorbani GhA, Mehrabi Tavana A, Ataee RA. Knowledge assessment in nurses of Baqiyatallah (a.s) hospital about AIDS. J Mil Med 2006;8:91-6 http://militarymedj.ir/article-1-204-en.html

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Correspondence: Farzad Jalilian, Lifestyle Modification Research Center, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran, 67198 - 51351 - Tel (Fax). +988338263048 - Email: f_jalilian@yahoo.com

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ORIGINAL ARTICLE

Cholera knowledge, socioeconomic and WaSH characteristics in Aden - Yemen, 2017: a community-based comparative survey

KHALED AL-SAKKAF[1], AWSAN BAHATTAB[1,2], HUDA BASALEEM[1]
[1] Department of Community Medicine and Public Health, Faculty of Medicine and Health Sciences, University of Aden, Aden, Yemen;
[2] CRIMEDIM - Research Center in Emergency and Disaster Medicine, Università del Piemonte Orientale, Novara, Italy

Keywords

Acute watery diarrhoea • Awareness • Environmental sanitation • Education •Yemen

Summary

Objectives. *To improve prevention and control response to the cholera outbreak, we sought to assess and compare the community's cholera awareness and needs in high and low epidemic areas in Aden.*
Methods. *A community-based comparative survey was conducted in 2017. We used multi-stage cluster sampling. Eligible household heads in high and low epidemic areas were interviewed. The data collected from both areas using a pretested questionnaire.*
Results. *Cholera cases and mortality were higher in high epidemic areas compared with low epidemic areas. Socioeconomic, water, sanitation, and hygienic conditions were poorer in high epidemic areas compared with low epidemic areas. Knowledge of cholera transmission and prevention was sub-optimal in both areas. We found a mismatch between the delivered education and distributed preventive materials.*
Conclusions. *Stakeholders should tailor the design, content, and implementation of future cholera prevention and control methods to meet the needs of the community. Future educational camping should focus on the transmission prevention, including vaccination. Education and sustainable interventions should be implemented to improve the water, sanitation, and hygiene.*

Introduction

Cholera is a fatal dehydrating diarrheal disease caused by *Vibrio cholera.* The disease acquired through the ingestion of an infective dose of contaminated food or water and spread through many mechanisms [1]. The improvement in water and sanitation system has eliminated the cholera transmission in Europe and other developed countries since the late nineteenth century. However, most developing countries failed to implement such measures [2, 3], and cholera remains a significant public health problem. Ali and colleagues have estimated about 2.86 million cholera cases and 95,000 associated deaths had occurred annually between 2008-2012, in 69 endemic countries [4]. However, only 5-10% of the actual cases reported to the World Health Organization (WHO) due to social, economic and political sensitivities, and inadequacies of laboratory and epidemiological surveillance systems of cholera-endemic countries [4].

Cholera is a synonym of poverty, inequity, and lack of social development. Risk factors for cholera – lack of safe water, poor hygiene, overcrowding, poor infrastructure, and improper environmental sanitation – exist in the underprivileged areas of many developing countries. The massive population displacements imposed by complex humanitarian emergencies also increase the risk of disease transmission, if the pathogen is present or introduced into the population [2, 5]. Yemen is one of the poorest countries in the Middle East. The country suffered from protracted political conflict for nearly a decade, which escalated into conflict in 2015. The violence displaced 3.34 million people and disrupted the fragile services of water, sanitation, and health. More than half of the 30.5 million Yemeni people lack safe drinking water and sanitation, and two-third of people have no or limited access to basic health care [6]. Cholera in Yemen became reportable to the WHO since the 1970s. Several outbreaks have been declared since then [7]. More recently, the cholera outbreak began first in October 2016 in the capital, Sana'a [8]. During this wave, over 70% of cases were reported from five governorates, including Aden [9]. By mid-March 2017, the outbreak was in decline [10]. The resurgence of cholera cases after 27 April 2017 marked the second wave with the unprecedented spread in 22 out of 23 governorates. By January 2019, almost 1.5 million cases and 2,906 associated deaths were reported [11], making the worst recorded cholera epidemic. More than half of the cases were among children under 15 years. The cases-fatality rate was as high as 0.76% in the elderly above 60 years [6].

As a response to the outbreak, the Yemen Ministry of Public Health and Population (MoPHP) along with "Health and Water, Sanitation, and Hygiene (WaSH)" clusters, developed an integrated cholera preparedness and response plan in October 2016 [12]. The plan was based on the WHO strategy for cholera prevention and control, a combination of surveillance, water, sanitation and hygiene, social mobilization, treatment, and oral cholera vaccines [12, 13]. Although the first release of the plan missed important components, including infection preventions, the following iterations of the plans were more comprehensive [12]. However, how to implement these interventions remains a

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challenge in the context of Yemen. In August 2017, MoPHP and its partners initiated a cholera awareness campaign as a strategy to control the outbreak. However, the oral cholera vaccine was not administrated until May 2018 [12].

To design community-tailored educational messages and methods for prevention and control activities, it is important to understand the community awareness and needs. This study assessed and compared cholera knowledge, socioeconomic, and WaSH characteristic between high epidemic areas (HEAs) and low epidemic areas (LEAs) in Aden city. We also suggest recommendations for stakeholders to prioritize future preventive actions and mobilization effort.

Methods

Study design, setting and target population

We conducted a community-based comparative cross-sectional survey between September-October 2017, in the southern port city of Aden. Aden – the officially Yemen's temporary capital – divided into 8 administrative urban districts. It has about 1.14 million inhabitants, in addition to 60,000 internally displaced (IDPs) and 138,000 refugees [6]. For a meaningful comparison, we targeted population in areas with the highest and the lowest suspected cholera cases. A suspected cholera case defined as *"a patient aged 5 years or more develops acute watery diarrhea, with or without vomiting"* [14]. We used the cholera case attack rate (AR), and the caseload to indicate the epidemic level in districts, and blocks within districts, respectively.

Sample size and sampling strategy

The multi-stage cluster sampling method was applied to reach the eligible households [15]. Based on the 34th epidemic week report – obtained from the Primary Health Care Directorate and WHO – the two districts with highest, and the two with lowest suspected cholera AR were selected purposively in the first stage (Fig. 1). In the second stage, blocks with the highest cumulative number of suspected cholera [high-epidemic areas (HEAs)] were selected from the two districts with the highest suspected cholera AR. In low AR districts [low-epidemic areas (LEAs)], blocks with the lowest caseload were included purposefully [16]. In the third stage, 30 clusters were selected with probability proportionate to the population size in each area. Finally, a random selection of the seven households within each of the 30 clusters was targeted. This resulted in a minimum sample size of 210 households in each area.

The questionnaire was administered either to the head of the household or in his absence to a responsible adult above 18 years of age, after obtaining their informed consent.

Study tool and data collection

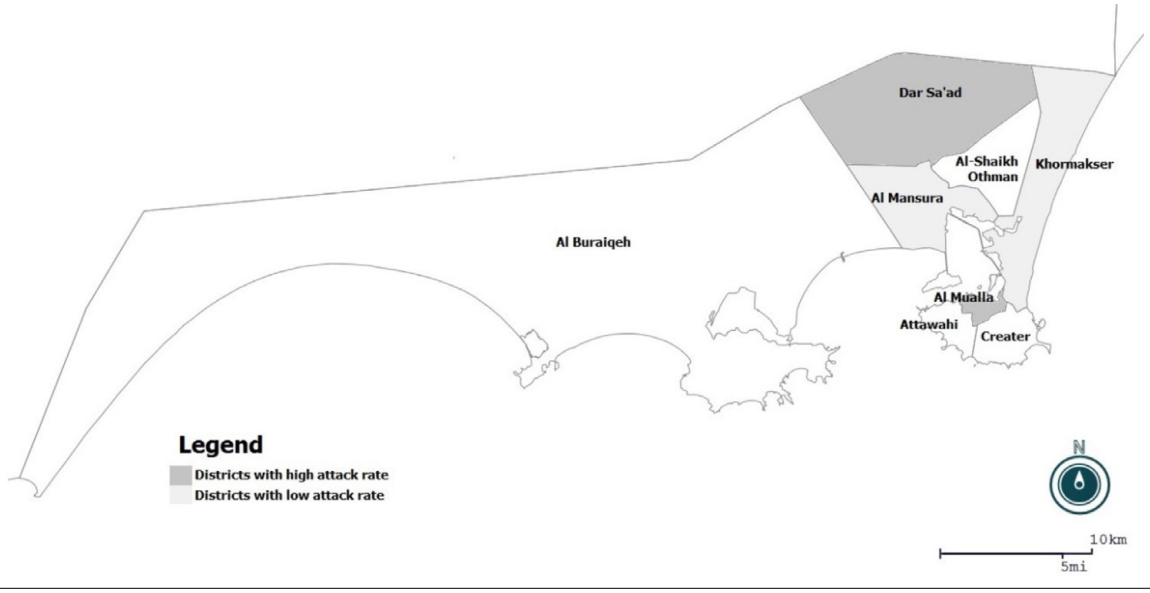
A translated version of a semi-structured questionnaire adapted from a previous study about cholera in Bangladesh [17] was administrated to the eligible household members through face-to-face interviews. Interviewers were trained before the survey through classroom and field training to ensure the questionnaire was well understood by the surveyors, avoiding the difference in the definitions and interpretations of concepts used.

Pre-testing of the questionnaire was also conducted on a small number of participants (n = 10) in a district not included in the study and their responses were not used in the final analysis. The final version of the questionnaire consists of six sections: 1) socio-demographic characteristics; 2) illness in the family; 3) water sources, storage and handling practices; 4) Hand-washing and sewage disposal; 5) cholera-related knowledge, and exposure to health communication message, and intervention.

Analysis of data

Data were entered and analyzed using the Statistical Package for Social Sciences software version 22 (SPSS

Fig. 1. Aden administrative map showing selected districts, drawn using Epi Info™ version 7.3.2. Data source: UN Office for the coordination of humanitarian affairs, 2019. The humanitarian data exchange. https://data.humdata.org/dataset/yemen-admin-boundaries



Incorporation, Chicago, IL, USA). Variables were presented as proportions or means. To measure knowledge of the respondents about cholera, a scoring system was used. Correct and incorrect answers for knowledge were given scores of "one" and "zero", respectively. Fourteen items were used in the calculation of the knowledge score, with a total score ranged from 0 to 14, as shown in Table I. The poor knowledge was defined as a score of ≤ 7, while good knowledge defined as a score of ≥ 8 [18]. The comparison between HEAs and LEAs characteristics was done by using the Chi-Square test or Fisher's exact (FE) test as appropriate. To identify the predictors of a high level of cholera epidemicity, a multivariate logistic regression for the relevant socioeconomic and WaSH variables, which were also significant by chi-square test, was done. Relevant socioeconomic variables are low educational level [18], low income, and crowding [19]. Relevant WaSH variables are water sources, water treatment, sanitation facilities and sanitary system, and handwashing. Crude and adjusted odds ratio (OR), 95 % confidence interval (CI) and P-value were reported. The statistical significance of all tests was considered when the P-value was < 0.05.

Ethical consideration

Approval for conducting the study was obtained from the MoPHP, and the ethical clearance was issued from the Ethics Research Committee, Faculty of Medicine and Health Sciences, University of Aden before conducting the study. Verbal consent was obtained from participants before conducting the interview. The agreement to take part in the study indicates participant's consent.

Tab. I. Knowledge score items.

Items	Given score
Cholera symptoms	
Watery diarrhoea	1
Vomiting	1
Dehydration	1
Transmission of cholera	
Eating polluted food	1
Drinking polluted water	1
Poor hygienic practices	1
Flies and insects	1
Person-to-person contact	1
Cholera prevention	
Adequate food safety: wash fruits and vegetables, cooking food thoroughly, cover food and keep it away from flies and insects, clean cooking utensils	1
Use of safe water treat water with chlorine tablet or boiling	1
Basic hygienic practice: washing hands with soap	1
Proper sanitation: adequate disposal of human wastes	1
Vaccine	1
Cholera treatment	
Diarrhoea treatment centre, hospital, clinic and ORS	1
Total score	**14**

Results

We interviewed members of 440 households in four targeted districts, 228 *vs* 212 in HEAs and LEAs, respectively. The self-reported prevalence of suspected cholera in the six months preceding the survey was high (44.8%). HEAs respondents reported a statistically significant higher prevalence of suspected cholera (49.6%) than LEAs respondent did (39.6%). Additionally, all five deaths attributed to suspected cholera were reported from the HEAs.

Socioeconomic characteristics

The age of respondents was similar in both areas with significantly more female from HEAs. HEAs and LEAs were significantly different in the other socioeconomic characteristics, except for family size, with the least favourable conditions such as low educational level, unemployment, low income are striking features for the HEAs respondents (Tab. II).

Tab. II. Socioeconomic characteristics.

Characteristic	Total (n = 440)	HEAs (n = 228)	LEAs (n = 212)	P-value
	%	%	%	
Sex				
Male	46.4	38.6	54.7	P < 0.001*
Female	53.6	61.4	45.3	
Age				
18-24	17.0	18.4	15.6	P = 0.842
25-34	22.7	23.2	22.2	
35-44	23.9	24.1	23.6	
45-54	18.9	17.1	20.8	
≥ 55	17.5	17.1	17.9	
Family size				
< 5	19.8	16.2	23.6	P = 0.059
5-10	69.1	70.2	67.9	
> 10	11.1	13.6	8.5	
Educational level				
Illiterate, read and write	23.9	36.8	9.9	P < 0.001*
Basic school	26.8	28.5	25.0	
Secondary school	21.8	19.7	24.1	
University	27.5	14.9	41.0	
Job				
Housewife	37.3	45.2	28.8	P < 0.001*
Public work	20.7	12.7	29.2	
Private work	18.4	12.7	24.5	
Unemployed	8.0	10.1	5.7	
Work on daily basis	5.7	9.6	1.4	
Students	5.7	3.9	7.5	
Others	4.3	5.7	2.8	
Monthly per capita income (YR)[a]				
< 5,000	20.9	35.1	5.7	P < 0.001*
5,000 - < 10,000	27.3	28.1	26.4	
10,000 - < 15,000	25.0	20.6	29.7	
≥ 15,000	26.8	16.2	38.2	

[a] YR: Yemeni Riyal; *: statistically significant.

Water, sanitation and hygiene (WaSH) related characteristics

More than 77% respondents drank untreated water, 66.2% of them claimed their current water source was safe. However, HEAs and LEAs had a significant difference in water sources and management. The LEAs respondents reported safer water sources for drinking and domestic use. For example, the piped water system is prevalent in 98% of LEAs households compared with 71.5% in HEAs households. The more efficient way of water treatment, such as filters was also significantly higher in LEAs. Similarly, LEAs respondents had reported significantly improved sanitary and sewerage system compared with HEAs respondents. In HEAs, although respondents claimed washing their hands significantly more than respondents from LEAs did (92.5% versus 84.9% respectively), a significantly higher percentage from LEAs respondents wash their hands before eating (98.1% *vs* 93.8%) and after using the toilet compared to HEAs respondents (93.4% *vs* 73.9%). More details about WaSH characteristics are shown in Table III.

Education about cholera prevention and treatment

Eighty-four percent of the respondents were exposed to education regarding cholera in the last six months.

Tab. III. Households' WaSH characteristics.

Characteristic	Total (n = 440)	HEAs (n = 228)	LEAs (n = 212)	P-value
	%	%	%	
Water characteristics				
Main source of drinking water for the household				
Piped water in house	53.9	51.3	56.6	P = 0.001*
Bottled water	38.6	36.4	41.5	
Others	7.3	12.3	1.9	
Main source of water used for other purposes such as cooking and handwashing				
Piped water in house	84.3	71.5	98.1	P = 0.001*
Others	15.7	28.5	1.9	
Using procedures to make water safer to drink	23.4	19.3	27.8	P = 0.035*
Procedures used to make water safer to drink (total = 103, HEAs = 44, LEAs = 59)				
Use water filter	68.9	43.2	88.1	P < 0.001*
Add chlorine tablets	18.4	29.5	10.2	P = 0.012*
Boil the water	11.7	22.7	3.4	P = 0.002*
Strain it through cloth	7.8	15.9	1.7	FEP = 0.020*
Others	3.9	9.1	0.0	FEP = 0.031*
Reasons for not treating drinking water before use (total = 337, HEAs = 184, LEAs = 153)				
Current water source is safe	66.2	68.5	63.4	P = 0.326
No money	13.1	17.4	7.8	P = 0.010*
No time	11.9	7.1	17.6	P = 0.003*
No chlorine	3.0	5.4	0.0	FEP = 0.002*
Cannot tolerate chlorine taste/smell	2.4	0.5	4.6	FEP = 0.018*
Other reasons	3.6	2.2	5.2	P = 0.113
Treatment of water tanks	53.9	53.7	54.1	P = 0.921
Sanitation characteristics				
Having flush toilet	44.6	28.2	62.3	P < 0.001*
Having public sewerage network	76.1	61.8	91.5	P < 0.001*
Hygiene characteristics				
Practising regular hand washing	88.9	92.5	84.9	P = 0.011*
Hand washing occasions				
Before eating	95.9	93.8	98.1	P = 0.023*
After eating	94.5	92.0	97.2	P = 0.018*
After using the toilet	83.3	73.9	93.4	P < 0.001*
Before cooking	36.3	37.6	34.9	P = 0.556
After cleaning the home	18.7	15.5	22.2	P = 0.073
After washing/cleaning tables	15.1	22.6	7.1	P < 0.001*
After cleaning the child	11.0	14.2	7.5	P = 0.027*
Others	8.7	5.3	12.3	P = 0.010*

*: statistically significant.

Community health workers (CHWs) (71.2%), mass media (32.3%), and health care providers (14.4%) were the main sources of information in both areas. Reporting CHWs and media varied significantly between HEAs and LEAs respondents. While most HEAs and LEAs respondents mentioned CHWs (80.0% and 60% respectively), LEAs respondents mentioned Radio and TV (49.1%) more frequently compared with HEAs respondents (17.4%). Respondents mentioned they were educated about many preventive ways against cholera, top of which were washing hands (81.2%) and washing fruits/vegetables (50.3%). A statistically significant difference between both localities was observed only for washing fruits/vegetables and covering food in favour of LEAs. Respondents from HEAs received more soap and oral rehydration solution (ORS). It is noteworthy respondents received chlorine more than they have been educated about it. Respondents also received printed educational materials, but fewer numbers reported it as a source of information as illustrated in Table IV.

Cholera knowledge

Most of the respondents (97.9%) claimed they heard about cholera, with a statistically insignificant difference between the two localities. Of them, 87% mentioned two symptoms for cholera, 72.7% mentioned two causes of cholera, and 75.7% mentioned two preventive methods correctly. However, 15.9% mentioned three symptoms, 0.5% mentioned five causes, and 4.3% mentioned five preventive measures against cholera.

Table V shows the respondents' knowledge about cholera.

Less than half of the respondents have an overall good knowledge score. Watery diarrhoea was the most recognized symptom of cholera by the participants (93.4%). Polluted food (65.5%) was the most frequently mentioned cholera causes. Similarly, adequate food safety (79.5%) was the most frequently mentioned preventive method. However, only 15.2% mentioned using safe water and 1.1% mentioned vaccines as methods of cholera prevention.

Tab. IV. Education received about cholera prevention.

Characteristic	Total (n = 440)	HEAs (n = 228)	LEAs (n = 212)	P-value
	%	%	%	
Exposed to education about cholera prevention and treatment in the last 6 months (total = 440, HEAs = 228, LEAs = 212)	83.6	85.5	81.6	P=0.266
Source of information about cholera (total = 368, HEAs = 195, LEAs = 173)				
Community health worker	71.2	80.0	61.3	P < 0.001*
Radio/TV	32.3	17.4	49.1	P < 0.001*
Healthcare providers	14.4	11.3	17.9	P = 0.070
Community meetings	3.8	3.1	4.6	P = 0.439
Internet/social media	3.5	3.6	3.5	P = 0.950
Neighbour/friend	3.0	4.1	1.7	P = 0.183
Printed materials	2.4	0.5	4.6	FEP = 0.015*
Family member	2.2	2.6	1.7	P = 0.586
Religious people	0.5	0.0	1.2	P = 0.132
Ways of preventing cholera they were educated about (total = 368, HEAs = 195, LEAs = 173)				
Wash hands with soap and water	81.2	79.0	83.8	P = 0.235
Wash vegetables/fruits	50.3	43.6	57.8	P = 0.006*
Cover food to keep away from flies	49.7	43.6	56.6	P = 0.012*
Cook food thoroughly	26.1	24.1	28.3	P = 0.357
Dispose of human waste properly	23.6	20.5	27.2	P = 0.134
Treat water with chlorine products	22.3	24.6	19.7	P = 0.254
Clean cooking utensils/vessels	20.4	23.6	16.8	P = 0.105
Boil water	10.6	11.8	9.2	P = 0.428
Personal and domestic hygiene	5.7	4.1	7.5	P = 0.159
Cholera vaccine	0.5	0.6	0.5	FEP = 0.720
ORS	3.3	2.1	4.6	P = 0.165
Received educational/preventive materials (total = 293, HEAs = 173, LEAs = 120)	66.6	75.9	65.6	P < 0.001*
Type of materials received (total = 293, HEAs = 173, LEAs = 120)				
Soap	66.9	71.7	60.0	P = 0.037*
Chlorine solution/tablets	48.8	47.4	50.8	P = 0.563
Printed materials (brochure, leaflet)	30.4	28.9	32.5	P = 0.510
ORS	18.4	27.7	5.0	P < 0.001*
Oral information	17.7	15.0	21.7	P = 0.144

*: statistically significant.

Tab. V. Knowledge about cholera.

Items	Total (n = 440)	HEAs (n = 228)	LEAs (n = 212)	P-value
	%	%	%	
Cholera symptoms				
Watery diarrhoea	93.4	93	93.9	P = 0.708
Vomiting	85.7	86.0	85.4	P = 0.860
Dehydration	18.2	14.9	21.7	P = 0.065
Causes of cholera				
Eating polluted food	65.9	64.5	67.5	P = 0.510
Drinking polluted water	61.6	59.6	63.7	P = 0.358
Poor hygienic practices	48.2	52.2	43.9	P = 0.081
Flies and insects	48.1	54.8	41.1	P = 0.005*
Person-to-person contact	6.6	6.1	7.1	P = 0.693
Cholera prevention				
Adequate food safety wash fruits and vegetables, cooking food thoroughly, cover food and keep it away from flies and insects, clean cooking utensils	79.5	77.6	81.6	P = 0.302
Basic hygienic practice: washing hands with soap	78.4	76.8	80.2	P = 0.382
Proper sanitation and adequate disposal of human wastes	24.5	28.5	20.3	P = 0.045
Use of safe water treat water with chlorine tablet or boiling	15.2	18.0	12.3	P = 0.095
Vaccine	1.1	0.9	1.4	P = 0.595
Cholera treatment				
Diarrhoea treatment centre, hospital, clinic and ORS	95.2	94.3	96.2	P = 0.343
Knowledge score				
Good knowledge score ≥ 8	51.1	52.2	50.0	P = 0.646

*: statistically significant.

Predictors of a high level of cholera epidemicity

Table VI presents the results of logistic regression analysis of HAEs as an outcome variable with different independent variables, such as socioeconomic and WaSH characteristics. The relevant variables associated with the outcome variable through Chi-square tests were family size, education, monthly income per capita, water sources, water treatment, sanitation facilities and regular hands-washing. Therefore, these variables were included in the regression model. In the adjusted regression model, only the lack of piped water for domestic use, and the lack of sewerage systems were significant positive predictors of being HEAs, while higher income and regular handwashing were significant negative predictors of HEAs.

Discussion

Educational interventions are essential for community mobilization effort [18, 20]. To the best of our knowledge, this is the first community-based study that assessed the community's cholera knowledge in the context of Yemen's recent cholera outbreak. The comparison between the HEAs and LEAs illuminated the reasons for such spread of cholera. The result of this study will enable stakeholders to adapt prevention and control strategies to the local's needs.

This study identified the respondents' cholera knowledge gaps. Most respondents from both areas had good knowledge of symptoms and treatment, but poor knowledge of transmission and prevention. Additionally, respondents from HEAs were educated about cholera more than those in LEAs (85.5 *% vs* 81.6%). They also showed slightly better knowledge (52.2%) than LEAs respondents (50.0%). A better knowledge of HEAs respondents could be a result of education and communication campaign that were conducted later in the outbreak and targeted HEAs.

Additionally, behavioral change is not a direct result of the knowledge per se [21]. Despite the Somalians' very good knowledge of cholera, disrupted water and sanitation services limited their preventive practices. The protracted conflict and the ignorance of infrastructure investment resulted in limited access to water, sanitation, and health services despite the locals' high demands for them [23]. In Yemen, the ongoing conflict had a devastating impact on water and sanitation services. Additionally, the economic crisis led to an upsurge in food and fuel prices, which rendered people unable to pay for safe water and food. In similar situations, people prioritize water and food availability over their sources and safety [22, 23].

Socioeconomic and WaSH characteristics are major determinants of cholera morbidity and mortality [18, 19, 24]. This study showed safer water sources for both drinking and domestic uses, water treatment, effective water treatment methods such as filters, and improved sanitary and sewerage systems were significantly reported more by the LEAs respondents. Additionally, the adjusted regression model revealed poverty, lack of piped water system, and the lack of sanitary systems as significant positive predictors of HEAs. Indeed, a higher burden of diseases affected the

Tab. VI. Logistic regression findings of cholera epidemicity by socioeconomic and WaSH characteristics.

	Crude			Adjusted		
Variable	**OR**	**95% CI**	**P-value**	**OR**	**95% CI**	**P-value**
Family size						
< 5 (reference)						
5-10	1.502	0.928-2.429	P < 0.001*	0.994	0.535-1.847	P = 0.986
>10	2.327	1.133-4.780	P < 0.001*	0.781	0.291-2.097	P = 0.624
Education						
Illiterate, read and write (reference)						
Basic school	0.307	0.168-0.559	P < 0.001*	0.571	0.277-1.178	P = 0.571
Secondary school	0.221	0.118-0.412	P < 0.001*	0.513	0.242-1.086	P = 0.513
University	0.098	0.052-0.182	P < 0.001*	0.299	0.140-0.635	P = 0.299
Monthly per capita income (YR)						
< 5,000 (reference)						
5,000 - < 10,000	0.171	0.085-0.347	P < 0.001*	0.213	0.093-0.488	P < 0.001*
10,000 - < 15,000	0.112	0.055-0.229	P < 0.001*	0.201	0.084-0.480	P < 0.001*
≥ 15000	0.0069	0.033-0.141	P < 0.001*	0.158	0.063-0.396	P < 0.001*
Main source of drinking water for the household						
Piped water in house (reference)						
Bottled water	0.967	0.653-1.434	P = 0.869	0.768	0.455-1.296	P = 0.323
Others	7.179	2.443-21.102	P < 0.001*	0.503	0.097-2.608	P = 0.413
Main source of water used for other purposes such as cooking and handwashing						
Piped water in house (reference)						
Others	20.736	7.401-58.100	P < 0.001*	14.770	3.941-55.356	P < 0.001*
Using procedures to make water safer to drink						
Yes						
No	1.613	1.033-2.518	P = 0.035*	1.197	0.660-2.168	P = 0.554
Having flush toilet						
Yes (reference)						
No	4.228	2.832-6.312	P < 0.001*	1.586	0.962-2.615	P = 0.070
Having public sewerage network						
Yes (reference)						
No	6.650	3.829-11.548	P < 0.001*	3.886	2.062-7.322	P < 0.001*
Practicing regular hand washing						
Yes (reference)						
No	0.453	0.244-0.843	P = 0.012*	0.198	0.077-0.511	P = 0.001*

*: statistically significant.

less privileged population more, continuing the cycle of disease, poverty, and inequity, and driving more conflict. In this study, although HEAs respondents reported slightly better cholera knowledge, higher self- reported incidence, and all the five cholera - related deaths were in this locality. Additionally, education becomes an insignificant predictor for cholera epidemicity in the adjusted regression model. The piped water for domestic use (and not for drinking), which indicated the availability of water network at home, suggest that water and sanitation facilities and infrastructures are the main determinants of cholera in urban settings. Still, the burden of cholera reported in this study was high in both areas.

The humanitarian response to control the ongoing cholera outbreak since 2016, focused on case management instead of early prevention [25]. The failure to contain the outbreak early in the first wave, where two-thirds of the cases were confined to five governorates [9], lead to widespread of the disease throughout the country. Moreover, no major intervention targeted the environmental determinants of the disease. Instead, water, sanitation, and hygiene interventions carried out were similar to those carried out to contain outbreaks in refugee camps, which is not suitable for a country wrapped up by protected conflict [25].

The study showed the respondents' lack knowledge about cholera vaccine [12, 25]. It is important to integrate vaccine education in future preventive activities to ensure public trust and intake.

The study also revealed discrepancies related to the current outbreak preventive and control measures. Examples include a higher number of respondents who received chlorine (48.8%) compared with those educated about (22.3%) or those who use it (18.4%). Besides, only 2.4% of the respondents considered the brochure as educative compared with those who received

it (30.4%). Such discrepancies necessitate improving the design, content, and implementation of education campaign, taking into consideration the educational/ cultural background of the beneficiaries. Stakeholders must understand and invest in the community accepted methods and materials for education and prevention. Thus, formative research to inform the design of the materials is required.

The study site was limited to Aden, so the representativeness is limited. Yet, the findings of this study could be relevant to similar settings. We could not attribute the respondents' knowledge to the educational camping as the population's baseline data were lacking. The study design is vulnerable to response bias. The respondents' knowledge may not represent their actual practices without validating that with observation method, is another limitation of this study.

Conclusions

We conducted this study on population from HEAs and LEAs in Aden city. We compared the knowledge level of cholera, socioeconomic, and WaSH characteristic between both areas. Findings revealed a strike difference between HEAs and LEAs economic level, and water and sanitary systems. It also identified knowledge gaps of both areas' respondents concerning cholera transmission and prevention. The study also identified a mismatch between education and prevention interventions.

The identified gaps must be addressed in future preventive activities and should be adapted to consider beneficiaries' needs without wasting resources. Future education camping should focus on community prevention and control methods, including detailed information on the cholera vaccine. The stakeholders should support the localities for sustainable water and sewerage systems, through long-term engagement rather than unsustainable WaSH activities. We also recommend further research to understand the community needs and stakeholder implementation barriers.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

KAZA and HOSB designed the study, train the data collectors, and designed and validated the questionnaire. KAZA and AASB supervised data collectors. HOSB and AASB analyzed data with the contribution of KAZA. AASB drafted the manuscript with the contribution of KAZA and HOSB. All authors reviewed and approved the final version of the manuscript.

References

[1] Heymann DL. The control of communicable diseases manual. 19th ed. An offical report of American Public Health Association. Washington DC: American Public Health Association 2008.

[2] Legros D. Global cholera epidemiology: opportunities to reduce the burden of cholera by 2030. J Infect Dis 2018;218(Suppl 3):137-40. Available at: https://academic.oup.com/jid/article/218/suppl_3/S137/5089194 - https://doi.org/10.1093/infdis/jiy486

[3] Clemens JD, Nair GB, Ahmed T, Qadri F, Holmgren J. Cholera. Lancet 2017;6736:1-11. Available at: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(17)30559-7/fulltext - https://doi.org/10.1016/S0140-6736(17)30559-7

[4] Ali M, Nelson AR, Lopez AL, Sack DA. Updated global burden of cholera in endemic countries. PLoS Negl Trop Dis 2015;9:1-13. Available at: https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0003832 - https://doi.org/10.1371/journal.pntd.0003832

[5] Kelly-Hope LA. Conflict and emerging infectious diseases on a global level. Emerg Infect Dis 2008;14:1004-5. Available at: https://wwwnc.cdc.gov/eid (accessed on 10/01/2019).

[6] Humanitarian country team and partners. Humanitarian needs overview 2019. UN office for the coordination of humanitarian Affairs. UN country team in Yemen. 2018. Available at: https://www.unocha.org/yemen (accessed on 10/01/2019).

[7] World Health Organization. WHO report on global surveillance of epidemic-prone infectious diseases. WHO 2000. Available at: https://www.who.int/csr/resources/publications/surveillance/WHO_CDS_CSR_ISR_2000_1/en (accessed on 19/01/2019).

[8] World Health Orgnization. Cholera cases in Yemen. World Health Organization Eastern Mediterranean Regional Office. WHO 2016. Epidemic and pandemic-prone diseases: archived outbreak. Available at: http://www.emro.who.int/pandemic-epidemic-diseases/cholera/cholera-cases-in-yemen.html (accessed on 19/01/2019).

[9] World Health Orgnization. Cholera situation in Yemen: November 2016. Cairo, Egypt: 2016. Available at: http://www.emro.who.int/images/stories/csr/documents/Infographics_cholera_YEM-situation_update_November_2016-_2.pdf (accessed on 19/01/2019).

[10] World Health Organization. Weekly update: cholera cases in Yemen, 21 March 2017. World Health Organization, Eastern Mediterranean Regional Office. WHO 2017. Epidemic and pandemic-prone diseases: archived outbreaks. Available at: http://

www.emro.who.int/pandemic-epidemic-diseases/cholera/update-cholera-cases-in-yemen-21-march-2017.html (accessed on 19/01/2019).

[11] World Health Organization. Cholera situation in Yemen: January 2019. Cairo, Egypt: 2019. Available at: http://applications.emro.who.int/docs/EMROPub_2019_EN_22332.pdf?ua=1 (accessed on 19/01/2019).

[12] Spiegel PB, Ratnayake R, Hellman N, Lantagne DS, Ververs M, Ngwa M, Wise PH. Cholera in Yemen: a case study of epidemic preparedness and response. Baltimore: 2018. Available at: https://www.jhsph.edu/news/news-releases/2018/lack-of-preparedness-and-insecurity-hampered-response-to-cholera-epidemic-in-yemen.html (accessed on 22/01/2019).

[13] World Health Orgnization. Cholera: key facts. 2019. Available at: https://www.who.int/en/news-room/fact-sheets/detail/cholera (accessed on 22/01/2019).

[14] Global task force on cholera control. Global task force on cholera control: prevention and control of cholera outbreaks: WHO policy and recommendations. World Health Orgnization 2019, p. 2. Available at: https://www.who.int/cholera/technical/prevention/control/en/index1.html (accessed on 22/01/2019).

[15] Davis R, Luna J, Rodriguez-Lainz A, Sarriot E. Rapid health survy: pinciples and design handbook. ICF Macro and Public health institute. Calverton, MD: 2009.

[16] Primary health Care (Aden) and World Health Organization. Cholera cases in Aden Week 34 (unpublished data). 2017.

[17] Working group on monitoring and evaluatio. Knowledge, attitudes, and practices (KAP) surveys during cholera vaccination campaigns : guidance for oral cholera vaccine stockpile campaigns " working copy." 2014. Available at: https://www.eccnetwork.net/resources/knowledge-attitudes-and-practices-kap-surveys-during-cholera-vaccination-campaigns (accessed on 22/01/2019)

[18] Wahed T, Shah S, Kaukab T, Saha NC, Khan IA, Khanam F. Knowledge of, attitudes toward, and preventive practices relating to cholera and oral cholera vaccine among urban high-risk groups: findings of a cross-sectional study in Dhaka, Bangladesh. 2013. Available at: https://bmcpublichealth.biomedcentral.com/articles/10.1186/1471-2458-13-24 - https://doi.org/10.1186/1471-2458-13-242

[19] Sur D, Deen JL, Manna B, Niyogi SK, Deb AK, Kanungo S, Sarkar BL, Kim DR, Danovaro-Holliday, MC, Holliday K, V K Gupta VK, Ali M, L von Seidlein L, Clemens JD, Bhattacharya SK. The burden of cholera in the slums of Kolkata, India: data from a prospective, community based study. Arch Dis Child 2005;90:1175-81. Available at: https://adc.bmj.com/content/90/11/1175 - https://doi.org/10.1136/adc.2004.071316 Accessed on 19/01/2019

[20] Quick RE, Gerber ML, Palacios ANAM, Beingolea L, Vargas R, Mujica O, Moreno D, Seminario L, Smithwick EB, Tauxe RV. Using a knowledge, attitudes and practices survey to supplement findings of an outbreak investigation : Cholera prevention measures during the 1991 Epidemic in Peru. Int J Epidemiol 1996;25:872–8. Available at: https://academic.oup.com/ije/article/25/4/872/655830 - https://doi.org/10.1093/ije/25.4.872

[21] Chandler C, Fairhead J, Kelly A, Leach M, Martineau F, Mokuwa E, Parker M, Richards P, Wilkinson A. Ebola: limitations of correcting misinformation. Lancet 2014;385:1275-7. Available at: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(14)62382-5/fulltext - https://doi.org/10.1016/S0140-6736(14)62382-5

[22] Ripoll S, Ayele S, Dirie Y, Scott-Villiers P, Lind J, Carruth L, Hammond L, Vecchione M, Catley A, Laurent P, Balfour N, Tiilikaanien M, Hailey P, Hussein MF, Livingstone J, Nori M. Contextual factors shaping cholera transmission and treatment-seeking in Somalia and the Somali region of Ethiopia. The social science in humanitarian action. 2017. Available at: https://pdfs.semanticscholar.org/d612/f96b6831f54ba6e37d4d12737886a7a8cc7a.pdf (accessed on 22/01/2019).

[23] World Health Organization. Community engagement contributes to control of acute watery diarrhea in Ethiopia's Somali region. WHO 2017, pp. 1-4. Available at: https://www.afro.who.int/news/community-engagement-contributes-control-acute-watery-diarrhea-ethiopias-somali-region (accessed on 22/01/2019).

[24] Merga N, Alemayehu T. Knowledge, perception, and management skills of mothers withunder-five children about diarrhoeal disease in indigenous and resettlement communities in Assosa district, Western Ethiopia. JHPN 2015;33:20-30. Available at: http://www.jhpn.net/index.php/jhpn (accessed on 22/01/2019).

[25] Federspiel F, Ali M. The cholera outbreak in Yemen: lessons learned and way forward. BMC Public Health 2018;18:1338-46. Available at: https://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-018-6227-6. https://doi.org/10.1186/s12889-018-6227-6

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Correspondence: Awsan Bahattab, CRIMEDIM – Research Center in Emergency and Disaster Medicine, Università del Piemonte, vicolo Monte Ariolo 4, 28100 Novara, Italy - E-mail: awsan.bahattab@med.uniupo.it

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ORIGINAL ARTICLE

Mothers' knowledge of breastfeeding and infant feeding types affect acute respiratory infections

SUSIANA JANSEN[1,2], WIDYA WASITYASTUTI[3], FAJAR DWI ASTARINI[4], SRI HARTINI[5]
[1] PELNI Nursing Academy of Jakarta, Indonesia; [2] Master in Nursing Program, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia; [3] Department of Physiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia; [4] Master in Biomedical Sciences, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia; [5] Department of Pediatric Nursing and Maternity, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia

Keywords

Acute Respiratory Infections • Exclusive Breastfeeding • Feeding practice • Mothers' knowledge

Summary

Introduction. *World Health Organization (WHO) recommends exclusive breastfeeding for new-borns until 6 months of age. However, exclusive breastfeeding in Indonesia only reached 52.3% in 2014 and 65.16% in 2018. It is known that administration of infant formula and non-formula supplements to infants aged less than 6 months increases the risk of Acute Respiratory Infections (ARIs). In addition, the high prevalence of ARIs in infants in Sleman Regency, Indonesia indicates the need of optimal early prevention. Therefore, we conducted this study to confirm that mothers' knowledge of breastfeeding and infant feeding types affect the prevalence of Acute Respiratory Infections (ARIs).*
Methods. *Data were collected through questionnaires from 50 mothers with infants aged 7-12 months who had experienced ARIs in the last 3 months (case group) and 50 mothers with healthy infants (control group). Collected data were then analysed using Chi-Square, Logistic Regression, Lambda, and Somers' D tests.*
Results. *The results showed that types of infant feeding are associated with the prevalence of ARIs. Non-breastfed infants were 14 times riskier to contract ARIs. Mothers' knowledge of exclusive breastfeeding influenced their preferences of feeding practice. However, their attitude towards breastfeeding did not appear to significantly affect their choices of feeding practice.*
Conclusions. *Exclusive breastfeeding during the first 6 months of an infant's life can lower the prevalence of ARIs for when they are older. Mothers' good knowledge of breastfeeding is associated with its practice.*

Introduction

Acute respiratory infections (ARIs) are acute infections in one or more parts of the respiratory tract extending from the nose to the alveoli in the lungs [1]. ARIs are caused by various pathogens such as bacteria or viruses [2]. ARIs produce several symptoms such as fever, cough, sore throat, flu, shortness of breath, wheezing or difficulty of breathing [3].
More than 10 million children under five years of age die every year, and one of the most common causes is ARIs [4]. Less developed countries have 2-6 times higher percentage of deaths caused by ARIs than developed countries [5]. In Indonesia, ARIs have become one of the main causes of infant death and often rank first on the morbidity rate of children under five years of age with a percentage of 20-30% [6].
The prevalence of ARIs is associated with ventilation condition, kitchen location, population density, socio-economic status, nutritional status and immunization status [5, 7]. Age is an independent risk factor for ARIs, and the risk of contracting ARIs is lower when we get older [8]. Exclusive breastfeeding, nutritional status and young age are associated with ARIs prevalence in infants and toddlers [8-10]. Exclusive breastfeeding prevents infants from broader exposure to pathogens [11] while good nutritional status improves the immune system [12], resulting in lower risk of ARIs prevalence. On the other hand, infants at the age of less than 23 months old are riskier to contract ARIs [13], hence supporting the importance of breastfeeding up to two years of age.
Appropriate and timely feeding practices of breast milk, infant formula, and non-breast supplements in infants and toddlers can support their growth and development [14]. United Nations Children's Fund (UNICEF) and World Health Organization (WHO) recommend exclusive breastfeeding for new-borns until 6 months of age. Breastfeeding practice is encouraged to continue until 2 years of age with the addition of non-breast supplements after the first 6 months [15].
Based on the data acquired from the Health Profile of Indonesia in 2014, exclusive breastfeeding in Indonesia only reached 52.3% in 2014 and 65.16% in 2018 of the 80% target, showing that the targeted number has not been reached yet [10]. Maternal education on feeding practice increases mothers' knowledge on exclusive breastfeeding and its practice [4].
Organ systems of infants under 6 months of age are still immature, including their pulmonary, genitourinary, and gastrointestinal (GI) systems [16]. The epithelial layers, mucus secretions, and the mucosal immunologic system from those organ systems provide protection against

pathogens [17, 18]. In neonates, the mucosal immune system is still immature which makes them vulnerable to infections. At the same time, infants are at high risk of various antigen exposure shortly after birth [19]. The ingestion of breast milk helps regulate the development of the immune system in infants [20]. It also has been known that breast milk contains nutrition, anti-pathogenic and anti-inflammatory factors. These factors will provide passive defense mechanisms against pathogens [21]. In response to that, the WHO and UNICEF, as well as Indonesian Government, recommend mothers to exclusively breastfeed their infants for the first 6 months. Infant formula and non-breast supplements can be given after the infants reach 6 months old [10]. However, not all mothers practice exclusive breastfeeding. Therefore, to emphasize the importance of exclusive breastfeeding in relation to ARIs, we conducted this study to investigate the association between types of infant feeding and the prevalence of ARIs, as well as determining the association between mothers' knowledge and their attitude towards exclusive breastfeeding.

Methods

Study design, site and ethical clearance

This case-control study was conducted using retrospective techniques to study the association between feeding practice in infants aged 0-6 months and the prevalence of ARIs in infants aged 7-12 months in relation to mothers' knowledge and attitude towards exclusive breastfeeding. Data were collected from Maternal and Child Health Services in the area of 3 Community Health Centres (Mlati I, Godean I, and Gamping I) in Sleman Regency, Special Region of Yogyakarta, Indonesia. Those Community Health Centres were preferred because of the high ARIs prevalence on infants under 1 year of age in the last 4 years (2013-2016). The study was conducted in April-May 2017. It has acquired research permit from Regional Development Planning Department (Badan Perencanaan Pembangunan Daerah) of Sleman Regency (Ref. No.: 070/Bappeda/1632/2017) and has been approved by Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada (Reference no. KE/FK/0457/EC/2017).

Participants

The participants of this study were 50 mothers of 7-12 months old infants with ARIs (case group) and other 50 mothers with healthy infants (control group). Calculation of sample size was performed using Odds Ratio sample size formula [22] for case control study, and the results showed that the study required at least 47 (n = 46.27) participants. Participants who met the inclusion criteria were selected using consecutive sampling technique. The inclusion criteria for the case group were mothers and their 7-12 months old infants who suffered from ARIs with non-pneumonia cough condition and were not being infected by other infections. For the control group, mothers and their healthy 7-12 months old infants who had not been infected with ARIs in the last 3 months were chosen. The participants were either recruited at the Community Health Centres through a direct interview or selected by screening through the sign-up forms. For the case group, respiratory rate (number of breaths per minute) of the infants was recorded, and presence of stridor in infants was also observed. After the selection of participants, the mothers completed informed consent forms before the data collection using questionnaires was conducted.

Data collections and instrument assessment

Participants who consented were requested to provide demographic data consisting of mother and infant personal information and asked additional data to ensure that the chosen participants were appropriate for the study through direct interviews. The mothers were asked if their infants were born with normal weight, not having measles, diarrhoea, and asthma condition. Infant's nutritional status was measured by considering the body weight and height of the infants. Completeness of mandatory vaccinations (BCG, Hepatitis B, Polio, and DTP) in infants was also confirmed by the mothers. After the above screening process, data of feeding practice during the first 6 months of the infant's life were collected. Feeding practice was categorized into 6 types: exclusive breastfeeding, breast milk plus infant formula, breast milk plus non-formula supplement, infant formula, infant formula plus non-formula supplement, and mixed feeding (mix of breast milk, infant formula and non-formula supplement). The mothers were also asked to fill in the questionnaire related to their knowledge and attitude towards exclusive breastfeeding.

All of the questionnaires used in this study have been tested for instrument validity and reliability. ARIs screening questionnaire, adapted from Integrated Management of Childhood Illness-World Health Organization (IMCI-WHO), was used to select the participants. Feeding practice questionnaire was used to determine the type of feeding practice that the infants had been given during the first 6 months of life. The questionnaire of mothers' knowledge and attitudes towards exclusive breastfeeding was adapted from the Modules of Early Initiation of Breastfeeding Activities and 6 Months Exclusive Breastfeeding from the Ministry of Health of the Republic of Indonesia in 2008. The questionnaire of mothers' knowledge on exclusive breastfeeding consisted of 16 questions in the form of multiple choices (a, b, c and d) covering 5 major themes: definitions, benefits, procedures, when to breastfeed and how to manage problems related to breastfeeding. The questionnaire of mothers' attitude towards exclusive breastfeeding consisted of 12 attitude statements in the form of a Likert scale: Strongly Agree (SS), Agree (S), Disagree (TS) and Strongly Disagree (STS). The questionnaires of mothers' knowledge and attitude towards exclusive breastfeeding were then graded and classified into 3 groups: good (> 70%), enough (51-69%), and poor (< 50%). Data collection was conducted for approximately 45 minutes for each participant.

Data analysis

The acquired data went through 5 steps of data analysis, which were editing, coding, data entry, processing, and cleaning. Coded data were then inputted to a data analysis software and processed. Data analysis was performed using univariate, bivariate, and multivariable analysis. Chi-Square Test (univariate analysis) was used to determine the distribution of participants' characteristics between case and control groups (mother's education level, mother's employment status, sex of the infants, mother's age, and number of children) and association between feeding practice and the prevalence of ARIs. Bivariate analysis, with Lambda tests, was used to observe the association between feeding practice during the first 6 months of infant life and the prevalence of ARIs when they were 7-12 months old. The same analysis was also used to see the association between mothers' knowledge and attitude towards exclusive breastfeeding and the type of feeding practice they were given to their infants during the first 6 months. The multivariable analysis using logistic regression was performed to determine the extent of the association of types of feeding practice, ARIs prevalence, and other variables involved in this study. The last step of the analysis was the cleaning process which served to double check if there were any errors with the inputted data.

Results

Demographic data of the participants are shown in Table I. It shows that there were no significant differences between the participants in both control and case groups in 5 aspects, which were mother's education background (P = 0.29), mother's employment status (P = 0.30), sex of the infants (P = 0.69), mother's age (P = 0.27), number of children (P = 0.18), mothers' knowledge in breastfeeding (P = 0.30), and mothers' attitude towards breastfeeding (P = 0.80). The association between feeding practice and ARIs prevalence in infants is shown in Table II. The results showed that the association was statistically significant with P = 0.001. Based on the performed Lambda test, the l value was 0.50 (moderately positive), indicating that infants with mixed feeding were more likely to contract ARIs than breastfed infants. Table III shows the results of multivariable analysis on types of feeding practice in infants compared to exclusive breastfeeding to see which type of feeding practice highly affects ARIs prevalence (model 1). Mixed feeding had the highest risk of causing ARIs with an odds ratio 14 times higher than exclusive breastfeeding. Infant formula and infant formula plus non-formula supplement types of feeding also had 14 times higher risk to cause ARIs compared to exclusive breastfeeding. Mothers' knowledge (model 2), mothers' attitude (model 3) or both combined (model 4) did not affect the prevalence of ARIs. The analysis of all variables altogether (model 5) also did not significantly affect ARIs prevalence.

Table IV shows the association between feeding practice in infants and mother's knowledge and attitude towards exclusive breastfeeding. The percentage of exclusive breastfeeding in both groups is the highest with 36% in case group and 86% in control group. Mothers' knowledge was significantly associated with types of infant feeding (P = 0.016) with moderately positive correlation (G = 0.38). However, insignificant association between types of feeding practice in 0-6 months old infants and mothers' attitude towards exclusive breastfeeding was observed with P = 0.17.

Tab. I. Characteristics of mothers and infants (n case = 50, n control = 50).

Characteristics		Groups		χ^2
		Case n (%)	Control n (%)	
Mother's education	Elementary school Junior high school Senior high school College	0 (0,0) 8 (16) 31 (62) 11 (22)	3 (6) 10 (20) 29 (58) 8 (16)	0.29
Mother's employment status	Housewife Work	33 (66) 17 (34)	37 (75.5) 12 (24.5)	0.30
Sex of the infants	Male Female	25 (50) 25(50)	23 (46) 27 (54)	0.69
Mother's age	< 25 years 25-35 years > 35 years	16 (32) 30 (60) 4 (8)	9 (18) 36 (72) 5 (10)	0.27
Number of children	≤ 2 > 2	47 (94) 3 (6)	43 (86) 7 (14)	0.18
Knowledge	Good Enough Bad	24 (48) 24 (48) 2 (4)	28 (56) 22 (44) 0 (0)	0.30
Attitude	Good Enough	41 (82) 9 (18)	40 (80) 0 (0)	0.80

N: number of samples; χ^2: chi-square compared test; if Sig. < 0.05: characteristic distribution between two groups is different (heterogenous); %: percentage.

Tab. II. The association between feeding practice and ARIs prevalence in infants (n case = 50, n control = 50).

Feeding practice	Groups				P	λ
	Case		Control			
	N	(%)	N	%		
Exclusive breastfeeding	18	36.0%	43	86.0%	0.001	0.500
Breast milk plus non-formula supplement	7	14.0%	2	4.0%		
Breast milk plus infant formula	9	18.0%	4	8.0%		
Infant formula	5	10.0%	0	0.0%		
Infant formula plus non-formula supplement	5	10.0%	0	0.0%		
Mixed feeding	6	12.0%	1	2.0%		

N: number of samples; p-χ^2: the data were statistically significant if $p < 0.05$; λ: strength of Lambda test, the closer to 1, the correlation becomes stronger; %: percentage.

Tab. III. Multivariable analysis on the prevalence of ARIs (n case = 50, n control = 50).

	Model 1 ARIs	Model 2 ARIs	Model 3 ARIs	Model 4 ARIs	Model 5 ARIs
Exclusive breastfeeding	1.00 [1.00, 1.00]	1.00 [1.00, 1.00]	1.00 [1.00, 1.00]	1.00 [1.00, 1.00]	1.00 [1.00, 1.00]
Breast milk plus non-formula supplement	8.36* [1.58, 44.19]	8.37* [1.58, 44.31]	9.25* [1.69, 50.67]	9.46** [1.73, 51.76]	10.07* [1.67, 60.67]
Breast milk plus infant formula	5.37* [1.46, 19.72]	5.36* [1.42, 20.14]	5.93** [1.56, 22.50]	5.66* [1.47, 21.80]	7.46* [1.55, 35.89]
Infant formula	13.89** [1.56, 123.45]	13.89** [1.56, 123.45]	13.89** [1.56, 123.45]	13.89** [1.56, 123.45]	13.89** [1.56, 123.45]
Infant formula plus non-formula supplement	13.89** [1.56, 123.45]	13.89** [1.56, 123.45]	13.89** [1.56, 123.45]	13.89** [1.56, 123.45]	13.89** [1.56, 123.45]
Mixed feeding	14.33* [1.61, 127.73]	14.23* [1.50, 135.12]	19.05* [1.93, 187.73]	17.59* [1.72, 179.37]	20.50* [1.77, 236.80]
Mothers' knowledge		1.01 [0.41, 2.51]		1.24 [0.47, 3.32]	1.15 [0.38, 3.52]
Mothers' attitude			0.46 [0.12, 1.72]	0.42 [0.10, 1.69]	0.44 [0.10, 2.01]
Mothers' education					1.44 [0.63, 3.29]
Mothers' employment status					1.58 [0.52, 4.75]
Sex of the infants					0.92 [0.32, 2.66]
Mothers' age					0.94 [0.35, 2.53]
Number of children					0.54 [0.08, 3.63]
N	100	100	100	100	100
Pseudo R-squared (R^2)	0.148	0.148	0.159	0.161	0.202
AIC	113.3	115.3	113.9	115.7	119.7

N: number of samples; data presented as OR: Odds Ratio; CI (in brackets): Confidence Interval, max and min of OR; AIC: Akaike information Criterion; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Tab. IV. The association between feeding practice in infants and mother's knowledge and attitude towards exclusive breastfeeding (n = 100).

		Types of feeding practice						P	G
		A1	A2	A3	A4	A5	A6		
		N	N	N	N	N	N		
Knowledge	Good	36	6	5	2	2	1	0.016	0.38
	Enough	25	3	7	3	3	5		
	Bad	0	0	1	0	0	1		
Attitude	Good	52	7	10	3	5	4	0.17	0.19
	Enough	9	2	3	2	0	3		
	Bad	0	0	0	0	0	0		

A1: exclusive breastfeeding; A2: breast milk plus non-formula supplement; A3: breast milk plus infant formula; A4: exclusive infant formula; A5: infant formula plus non-formula supplement; A6: mixed feeding; n: number of samples; p: statistically significant if $p<0.05$; %: percentage.

Discussion

This study provides a more general conclusion on the relationship between exclusive breastfeeding and ARIs prevalence in infants. Firstly, we identified 5 characteristics of participants to ensure that control and case groups had similar profile. The results showed that the participants of both groups were not different in the areas of mother's education, mother's employment status, sex of the infants, mother's age and number of children. The results also revealed that mothers' knowledge in breastfeeding and their attitude towards breastfeeding did not affect the prevalence of ARIs. However, mothers' knowledge in breastfeeding did affect their preference of feeding practice, especially exclusive breastfeeding.

ARIs often rank first as the cause of infant death in Indonesia. Exclusive breastfeeding for the first 6 months of life as recommended by WHO has been known to lower the risk of respiratory infections, especially in female infants [23-28]. The percentage of infections in infants with exclusive breastfeeding was lower than non-exclusive breastfeeding in the first year of life until they reached the age of 4 years [29]. According to another study, exclusive breastfeeding also contributes to the protection from common infections during infancy by reducing the frequency and severity of episodes. Partial breastfeeding appears to have no such protective effect [30]. The results of this study were in line with those studies. The Lambda test showed that mixed feeding correlates with the prevalence of ARIs, suggesting that exclusive breastfeeding is less likely to increase the chance of ARIs.

Breast milk, other than being an important source of nutrients, contains various bioactive factors that protect against infection and inflammation in infancy, and also contribute to organ development and the maturation of immune system [31-34]. The major bioactive factors found in human breast milk are cells, immunoglobulins, cytokines, chemokines, cytokine inhibitors, growth factors, hormones, anti-microbial, metabolic hormones, oligosaccharides and mucins [32]. The cell components consist of stem cells and macrophages, and the latter gives protection against infection and assists T-cell activation [35-37]. Immunoglobulins such as IgA/sIgA, IgG and IgM also contribute to the inhibition of pathogen binding, serve as anti-microbial and anti-inflammatory agent, and respond to allergens [38-40]. Lactoferrin and lactadherin act as anti-microbial components in breast milk [41-43]. Those bioactive components protect infants from infection, hence the lower prevalence of ARIs in infants who are exclusively breastfed during the first 6 months of life.

Infants aged 0-6 months are strongly encouraged to get exclusive breastfeeding unless they have specific conditions that require otherwise [4]. Infants who do not get exclusive breastfeeding usually get the other types of infant feeding such as exclusive infant formula, early non-formula supplements, or combination of both [44]. The results of this study showed that infants who were fed with infant formula, infant formula plus non-formula supplement, and mixed feeding during the first 6 months of life had 14 times higher risk to contract ARIs than those who were exclusively breastfed. This likely occurred due to the lack of dynamic composition of breast milk [31, 33]. Standardized composition of infant formula still cannot mimic the composition or performance of breast milk. Addition of new ingredients to infant formula is often performed to enhance its composition but the risks still persist [45]. Infants fed with formula also have a higher prevalence of respiratory illnesses especially in the first year of life than infants with exclusive breastfeeding [46]. Non-formula supplements such as liquid and solid food also have different compositions of nutrients and bioactive molecules than breast milk. The administration of non-formula supplement decreases breast milk consumption and increases the risk of choking and allergic reaction [47-51]. Besides, early introduction of formula and non-formula supplement increases the risk of pathogen exposure in infants. Infectious agents may contaminate the bottles, teats, formula milk, unclean water used to prepare formula, liquid food and solid food [4, 25] while in breastfeeding, exposure to external pathogens can be minimalized. That is why complementary foods are recommended to be given when the infants reach around 6 months of age [52].

The other risk factors for ARIs such as Low Birth Weight (LBW), incomplete immunization, nutritional intake and age were controlled in this study. However, Clean and Healthy Behaviour (CHB) and environment were factors that have not been controlled and reviewed more deeply in this study. It has been known that Sleman Regency has a fairly high percentage of smoking (26.2%) which may affect the incidence of ARIs in infants. Furthermore, some people still use inadequate water resources such as using unprotected well water (53.1%) and rainwater storage for household use (0.5%) which can increase the risk of infections and infant diseases [6].

Based on the results of Gamma test, mothers' knowledge was associated with their attitude towards exclusive breastfeeding which is in line with studies by Rachmaniah [53] and Yuliarti [54]. Factors influencing exclusive breastfeeding knowledge in this case are education, experience, socioeconomic and culture [55]. In this study, only 52% of participants had good knowledge of exclusive breastfeeding which is lower than similar studies conducted in Dabat Health Centre Northwest Ethiopia (69.8%), Abha City Saudi Arabia (55.3%), Guba Lafto Woreda Ethiopia (65.1%), Calabar Nigeria (80%) and Bedele Town Ethiopia (87.3%) [56-60]. The lower percentage of mothers' knowledge might be due to the limited distribution or access to information about exclusive breastfeeding. Furthermore, as it has been mentioned before, education affects mothers' knowledge about exclusive breastfeeding. Most of the participants of this study were high school graduates or less, and only 20% of the participants went to college. The knowledge about exclusive breastfeeding will influence mothers' attitude towards its practice [61, 62]. However, even

though the percentage of mothers' knowledge was low, 81% of participants had good attitudes towards exclusive breastfeeding.

Knowledge and attitude are predisposing factors for mothers in practicing exclusive breastfeeding [63]. However, in this study, Gamma test results indicated that mothers' knowledge was significantly associated with the type of feeding practice while their attitude towards exclusive breastfeeding did not affect their preferences of feeding practice. For instance, working mothers are less likely to give exclusive breastfeeding due to their busy schedules [64]. Some working mothers also prefer to express and freeze their breast milk for future infant feeding. Heat treatment and freeze-thaw processes can degrade many milk proteins and reduce the bioactivity of its components [32].

Conclusions

In conclusion, exclusive breastfeeding during the first 6 months of an infant's life can lower the prevalence of ARIs when they are older. This study revealed that mothers' knowledge of exclusive breastfeeding affected mothers' preferences of feeding types. Further exploration on why mothers with good knowledge and attitude towards exclusive breastfeeding did not implement their knowledge needs to be conducted to formulate better strategies in emphasizing the importance of exclusive breastfeeding during infant's early life.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection, data analysis, and manuscript writing were performed by all authors. All authors read and approved the final manuscript.

References

[1] Departemen Kesehatan RI. Pedoman pemberantasan penyakit infeksi saluran pernafasan akut untuk penanggulangan pneumonia pada balita. Dirjen P2M dan PLP 2004.

[2] Bulla A, Hitze KL. Acute respiratory infections: a review. Bull World Health Organ 1978;56:481-98. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2395579

[3] World Health Organization (WHO). Infection prevention and control of epidemic-and pandemic-prone acute respiratory disease in health care. Geneva: WHO; 2017.

[4] Hengsterman S, Mantaring JBV, Sobel HL, Borja VE, Basilio MD, Iellamo AD, Nyunt-US. Formula feeding is associated with increased hospital admissions due to infections among infants younger than 6 months in Manila, Philippines. J Hum Lact 2010;26:19-25. https://doi.org/10.1177/0890334409344078

[5] Oyejide CD. Review of epidemiological risk factors affecting the pathogenesis of acute respiratory infections. Niger J Paediatr 1988;15:1-9.

[6] Kementerian Kesehatan RI. Riset Kesehatan Dasar: riskesdas Dalam Angka Daerah Istimewa Yogyakarta Tahun 2013. Yogyakarta: Kementerian Kesehatan RI; 2013.

[7] Islam F, Sarma R, Debroy A, Kar S, Pal R. Profiling acute respiratory tract infections in children from assam, India. J Glob Infect Dis 2013;5:8-14. https://doi.org/10.4103/0974-777X.107167

[8] Chen Y, Williams E, Kirk M. Risk factors for acute respiratory infections in the Australian community. PLoS One 2014;9:1-8. https://doi.org/10.1371/journal.pone.0101440

[9] Benediktsdóttir B. Upper airway infections in preschool children - frequency and risk factors. Scand J Prim Health Care 1993;11:197-201. https://doi.org/10.3109/02813439308994830

[10] Kementerian Kesehatan RI. Profil Kesehatan Indonesia Tahun 2014. Kementerian Kesehatan RI; 2014.

[11] Arifeen S, Black RE, Antelman G, Baqui A, Caulfield L, Becker S. Exclusive breastfeeding reduces acute respiratory infection and diarrhea deaths among infants in Dhaka slums. Pediatrics 2001;108:E67. https://doi.org/10.1542/peds.108.4.e67

[12] Kaushik PV, Singh JV, Bhatnagar M, Garg SK, Chopra H. Nutritional correlates of acute respiratory infections. Indian J Matern Child Health 1995;6:71-2. https://www.ncbi.nlm.nih.gov/pubmed/12346500

[13] Imran MIK, Inshafi MUA, Sheikh R, Chowdhury MAB, Uddin MJ. Risk factors for acute respiratory infection in children younger than five years in Bangladesh. Public Health 2019;173:112-9. https://doi.org/10.1016/j.puhe.2019.05.011

[14] Ikatan Dokter Anak Indonesia. Makanan Terbaik untuk Bayi. Ikatan Dokter Anak Indonesia; 2006.

[15] World Health Organization (WHO). Infant and young child feeding. WHO International Nutrition. Geneva: WHO; 2009.

[16] Wong DL, Hockenberry-Eaton M, Wilson D. Wong's essentials of pediatric nursing (6th ed.). St. Louis, Missouri: Mosby Year Book; 2000.

[17] Berg RD. The indigenous gastrointestinal microflora. Trends Microbiol 1996;4:430-5. https://doi.org/10.1016/0966-842x(96)10057-3

[18] Gill N, Wlodarska M, Finlay BB. The future of mucosal immunology: studying an integrated system-wide organ. Nat Immunol 2010;11:558-60. https://doi.org/10.1038/ni0710-558

[19] Jakaitis BM, Denning PW. Human breast milk and the gastrointestinal innate immune system. Clin Perinatol 2014;41:423-35. https://doi.org/10.1016/j.clp.2014.02.011

[20] Garofalo R. Cytokines in human milk. J Pediatr 2010;156(Suppl 2):S36-40. https://doi.org/10.1016/j.jpeds.2009.11.019

[21] Goldman AS. The immune system of human milk: antimicrobial, antiinflammatory and immunomodulating properties. Pediatr Infect Dis J 1993;12:664–71. https://doi.org/10.1097/00006454-199308000-00008

[22] Lemeshow S, David J. Besar Sampel dalam Penelitian. Yogyakarta: Gadjah Mada University Press; 1997.

[23] Mihrshahi S, Oddy WH, Peat JK, Kabir I. Association between infant feeding patterns and diarrhoeal an respiratory illness: a cohort study in Chittagong, Bangladesh. Int Breastfeed Jl 2008;3:28. https://doi.org/10.1186/1746-4358-3-28

[24] Gorlanova O, Thalman S, Proietti E, Stern G, Latzin P, Kuhni C, Röösli M, Frey U. Effects of breastfeeding on respiratory symptoms in infancy. J Pediatr 2016;174:111-7. https://doi.org/10.1016/j.jpeds.2016.03.041

[25] Quigley MA, Carson C, Sacker A, Kelly Y. Exclusive breastfeeding duration and infant infection. Eur J Clin Nutr 2016;70:1420-7. https://doi.org/10.1038/ejcn.2016.135

[26] Bartick MC, Jegier BJ, Green BD, Schwarz EB, Reinhold AG, Stuebe AM. Disparities in breastfeeding: impact on maternal and child health outcomes and costs. J Pediatr 2017;181:49-55. https://doi.org/10.1016/j.jpeds.2016.10.028

[27] Tromp I, Kiefte-de Jong J, Raat H, Jaddoe V, Franco O, Hofman A, de Jongste J, Moll H. Breastfeeding and the risk of respiratory tract infections after infancy: the Generation R Study. PloS One 2017;12:e0172763. https://doi.org/10.1371/journal.pone.0172763

[28] Wang J, Ramette A, Jurca M, Goutaki M, Beardsmore CS, Kuehni CE. Breastfeeding and respiratory tract infections during the first 2 years of life. ERJ Open Res 2017;3:00143-2016. https://doi.org/10.1183/23120541.00143-2016

[29] Al-Sharbatti SS, Aljumaa LI. Infant feeding patterns and risk of acute respiratory infections in Baghdad/Iraq. Ital J Public Health 2012;9:1-9. https://doi.org/10.2427/7534

[30] Ladomenou F, Moschandreas J, Kafatos A, Tselentis Y, Galanakis E. Protective effect of exclusive breastfeeding against infections during infancy: a prospective study. Arch Dis Child 2010;95:04-8. https://doi.org/10.1136/adc.2009.169912

[31] Lawrence RA, Lawrence RM. Breastfeeding: a guide for the medical professional (7th ed.). Philadelphia, Pennsylvania: Saunders; 2010.

[32] Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. Pediatr Clin North Am 2013;60:49-74. https://doi.org/10.1016/j.pcl.2012.10.002

[33] Gregory KE, Walker WA. Immunologic factors in human milk and disease prevention in the preterm infant. Curr Pediatr Rep 2013;1(4). https://doi.org/10.1007/s40124-013-0028-2

[34] Cacho NT, Lawrence RM. Innate immunity and breast milk. Front Immunol 2017;8:584. https://doi.org/10.3389/fimmu.2017.00584

[35] Jarvinen KM, Suomalainen H. Leucocytes in human milk and lymphocyte subsets in cow's milk-allergic infants. Pediatr Allergy Immunol 2002;13:243-54. https://doi.org/10.1034/j.1399-3038.2002.00087.x

[36] Ichikawa M, Sugita M, Takahashi M, Satomi M, Takeshita T, Araki T, Takahashi H. Breast milk macrophages spontaneously produce granulocyte-macrophage colony-stimulating factor and differentiate into dendritic cells in the presence of exogenous interleukin-4 alone. Immunol 2003;108:189-95. https://doi.org/ 10.1046/j.1365-2567.2003.01572.x

[37] Yagi Y, Watanabe E, Watari E, Shinya E, Satomi M, Takeshita T, Takahashi H. Inhibition of DC-SIGN-mediated transmission of human immunodeficiency virus type 1 by Toll-like receptor 3 signalling in breast milk macrophages. Immunol 2010;130:597-607. https://doi.org/10.1111/j.1365-2567.2010.03264.x

[38] Van de Perre P. Transfer of antibody via mother's milk. Vaccine 2003;21:3374–6. https://doi.org/10.1016/s0264-410x(03)00336-0

[39] Agarwal S, Karmaus W, Davis S, Gangur V. Immune markers in breast milk and fetal and maternal body fluids: a systematic review of perinatal concentrations. J Hum Lact 2011;27:171-86. https://doi.org/10.1177/0890334410395761

[40] Castellote C, Casillas R, Ramirez-Santana C, Perez-Cano FJ, Castell M, Moretones MG, Lopez-Sabater MC, Franch A. Premature delivery influences the immunological composition of colostrum and transitional and mature human milk. J Nutr 2011;141:1181-7. https://doi.org/10.3945/jn.110.133652

[41] Peterson JA, Hamosh M, Scallan CD, Ceriani RL, Henderson TR, Mehta NR, Armand M, Hamosh P. Milk fat globule glycoproteins in human milk and in gastric aspirates of mother's milk-fed preterm infants. Pediatr Res 1998;44:499-506. https://doi.org/10.1203/00006450-199810000-00006

[42] Sherman MP, Bennett SH, Hwang FF, Yu C. Neonatal small bowel epithelia: enhancing anti-bacterial defense with lactoferrin and Lactobacillus GG. Biometals 2004;17:285-9. https://doi.org/10.1023/b:biom.0000027706.51112.62

[43] Adamkin DH. Mother's milk, feeding strategies, and lactoferrin to prevent necrotizing enterocolitis. J Parenter Enteral Nutr 2012;36:25S-9S. https://doi.org/10.1177/0148607111420158

[44] Loss G, Depner M, Ulfman LH, Neerven JV, Jose AJ, Genuneit J, Karvonen AM, Hyvärinen A, Kaulek V, Roduit C, Weber J, Lauener R, Pfefferle PI, Pekkanen J, Vaarala O, Dalphin JC, Riedler J, Braun-Fahrländer C, von Mutius E, Ege MJ; PASTURE study group. Consumption of unprocessed cow's milk protects in from common respiratory infections. J Allergy Clin Immunol 2015;135:56-62. https://doi.org/10.1016/j.jaci.2014.08.044

[45] Institute of Medicine (US). Infant formula: evaluating the safety of new ingredients. Washington DC: National Academic Press; 2004.

[46] Jackson AA. Feeding the normal infant, child and adolescent. Paed Nutr 2014;43:127-31. https://doi.org/10.1016/j.mpmed.2014.11.005

[47] Drewett R, Amatayakul K, Wongsawasdii L, Mangklabruks A, Ruckpaopunt S, Ruangyuttikarn C, Baum D, Imong S, Jackson D, Woolridge M. Nursing frequency and the energy intake from breast milk and supplementary food in a rural Thai population: a longitudinal study. Eur J Clin Nutr 1993;47:880-91.

[48] Altmann AE, Ozanne-Smith J. Non-fatal asphyxiation and foreign body ingestion in children 0-14 years. Inj Prev 1997;3:176-82. https://doi.org/10.1136/ip.3.3.176

[49] American Academy of Pediatrics. Committee on nutrition hypoallergenic infant formulas. Pediatr 2000;106:346-9. https://doi.org/10.1542/peds.106.2.346

[50] UNICEF. Complementary foods and feeding: nutritional companion to breastfeeding after 6 months. https://www.unicef.org/programme/breastfeeding/food.htm (accessed February 5 2019).

[51] Karmaus W, Soto-Ramírez N, Zhang H. Infant feeding pattern in the first six months of age in USA: a follow-up study. Int Breastfeed J 2017;12:48. https://doi.org/10.1186/s13006-017-0139-4

[52] World Health Organization (WHO). Complementary feeding: report of the global consultation. Summary of guiding principles. Geneva: WHO; 2003.

[53] Rachmaniah N. Hubungan tingkat pengetahuan ibu tentang ASI dengan tindakan ASI eksklusif. Universitas Muhammadiyah Surakarta Repository; 2014.

[54] Yuliarti ID. Hubungan pengetahuan dan sikap ibu dengan perilaku pemberian ASI eksklusif. Universitas Sebelas Maret Thesis Repository; 2008.

[55] Zeller CL. Effect of education on breastfeeding knowledge and attitudes among middle school students. Health Educ J 2016;75:501-10. https://doi.org/10.1177/0017896915597531

[56] Essien NC, Samson-Akpan PE, Ndebbio TJ, John ME. Mothers' knowledge, attitudes, beliefs and practices concerning exclusive breastfeeding in Calabar, Nigeria. Afr J Nurs Midwifery 2009;11:65-75. https://journals.co.za/content/ajnm/11/1/EJC19316

[57] Ayed A. Knowledge, attitude and practice regarding exclusive breastfeeding among mothers attending primary health care centers in Abha city. Int J Med Sci Public Health 2014;3:1355. https://doi.org/10.5455/ijmsph.2014.140820141

[58] Mengesha MG. Knowledge, attitude, and practice of mothers towards EBF and mixed feeding, Gubalafto Wereda, Amhara

regional state of Ethiopia: a descriptive cross sectional study. Addis Ababa University Thesis Repository; 2014.

[59] Wolde T, Diriba G, Wakjira A, Misganu G, Negesse G, Debela H, Tadesse, Ejeta E. Knowledge, attitude and practice amongst lactating mothers in Bedele town, southwestern Ethiopia: descriptive cross sectional study. Researcher 2014;6:91-7. https://www.semanticscholar.org/paper/Knowledge%2C-Attitude-and-Practice-of-Exclusive-Among-Wolde-Diriba/f7c4df4634f094f2b2fefedd2b49a79df97564ff

[60] Alamirew MW, Bayu NH, Birhan Tebeje N, Kassa SF. Knowledge and attitude towards exclusive breast feeding among mothers attending antenatal and immunization clinic at Dabat Health Center, Northwest Ethiopia: a cross-sectional institution based study. Nurs Res Pract 2017:6561028. https://doi.org/10.1155/2017/6561028

[61] Mbada CE, Olowookere AE, Faronbi JO, Oyinlol-Aromoralan FC, Faremi FA, Ogundele A, Awotidebe TO, Ojo AA, Augustine OA. Knowledge, attitude and techniques of breastfeeding among Nigerian mothers from a semi-urban community. BMC Res Notes 2013;6:552. https://doi.org/10.1186/1756-0500-6-552

[62] Pound CM, Williams K, Grenon R, Aglipay M, Plint AC. Breastfeeding knowledge, confidence, beliefs, and attitudes of Canadian physicians. J Hum Lact 2014;30:298-309. https://doi.org/10.1177/0890334414535507

[63] Notoatmodjo S. Pendidikan dan perilaku kesehatan. Jakarta: Rineka Cipta; 2007.

[64] Astari N. Hubungan pemberian susu formula dengan kejadian diare pada bayi usia 0-6 bulan. Repository Universitas Diponegoro; 2013.

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Correspondence: Widya Wasityastuti, Department of Physiology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Jl. Farmako, Senolowo, Sekip Utara, Mlati, Kabupaten Sleman, Daerah Istimewa Yogyakarta 55281, Indonesia - Tel. +62-274-6492492 - Fax +62-274-631185 - E-mail: wasityastuti@ugm.ac.id

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ORIGINAL ARTICLE

Determinants of diarrhoeal diseases and height-for-age *z*-scores in children under five years of age in rural central Tanzania

ELPIDIUS RUKAMBILE[1-3*], GARY MUSCATELLO[1], VITALI SINTCHENKO[2,4], PETER C. THOMSON[1], WENDE MAULAGA[3], RICHARD MMASSY[5], JULIA DE BRUYN[6], RICHARD KOCK[7], IAN DARNTON-HILL[8], ROBYN ALDERS[9-12]
[1] School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Australia; [2] Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Australia; [3] Tanzania Veterinary Laboratory Agency, Dar es Salaam, Tanzania; [4] Centre for Infectious Diseases and Microbiology - Public Health, Westmead Hospital and New South Wales Health Pathology, Sydney, Australia; [5] University of Dar es Salaam, Dar es Salaam, Tanzania; [6] Natural Resources Institute, University of Greenwich, United Kingdom; [7] The Royal Veterinary College, University of London, United Kingdom; [8] The University of Sydney, Faculty of Medicine and Health; [9] Kyeema Foundation, Brisbane, Australia; [10] Centre for Global Health Security, Chatham House, London, United Kingdom; [11] Development Policy Centre, Australian National University, Canberra, Australia; [12]Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine, Tufts University

Keywords

Gastrointestinal infection • Linear growth • Mpwapwa • Manyoni • Stunting

Summary

Introduction. *Childhood diarrhoeal diseases and stunting are major health problems in low- and middle-income countries (LMICs). Poor water supply, sanitation services and hygiene, frequently encountered in resource-poor settings, contribute to childhood diarrhoea and stunting.*
Methods. *Data on demographic characteristics, hygiene practices, sanitation and human-animal interactions (predictors) and child height-for-age z-scores (HAZ) (outcome) were collected once, while diarrhoea incidences were collected fortnightly for 24 months (outcome).*
Results. *Drinking water from public taps (OR = 0.51, 95% CI. 0.44 - 0.61; p < 0.001) and open wells (OR = 0.46, 95% CI. 0.39 - 0.54; p < 0.001) and older age of children (OR = 0.43, 95% CI. 0.27 - 0.67; p < 0.001) were protective against diarrhoea. Inappropriate disposal of children's faeces (OR = 1.15, 95% CI. 1.02 - 1.31; p = 0.025), sharing water sources with animals in the dry season (OR = 1.48, 95% CI. 1.29 - 1.70; p < 0.001), overnight sharing of houses with cats (OR = 1.35, 95% CI. 1.16 - 1.57; p < 0.001) and keeping chickens inside the house overnight regardless of room (OR = 1.39, 95% CI. 1.20 - 1.60; p < 0.001) increased the risk of diarrhoea. The Sukuma language group (p = 0.005), washing hands in running water (p = 0.007), access of chickens to unwashed kitchen utensils (p = 0.030) and overnight sharing of the house with sheep (p = 0.020) were associated with higher HAZ in children.*
Conclusions. *Until a more precise understanding of the key risk factors is available, these findings suggest efforts towards control of diarrhoea and improved linear growth in these areas should be directed to increased access to clean and safe water, handwashing, sanitation, and improved animal husbandry practices.*

Introduction

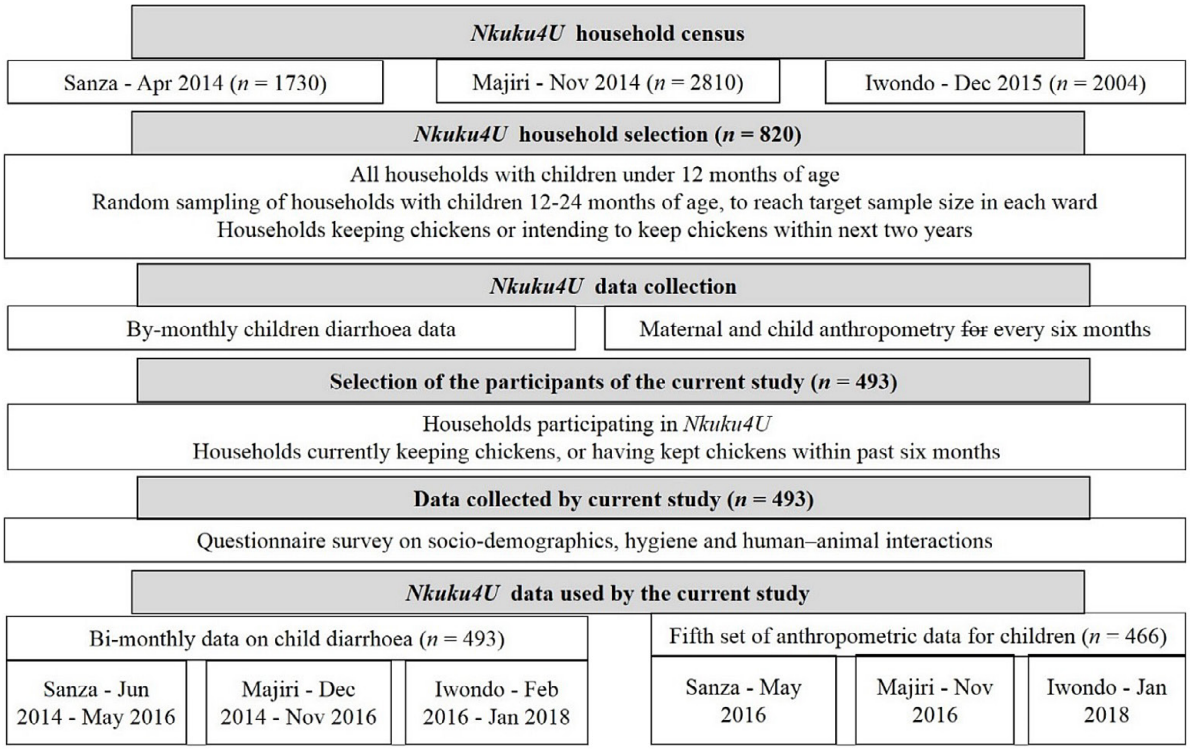
Diarrhoeal diseases and retarded linear growth are highly prevalent in children under five years old in many low- and middle-income countries (LMICs), particularly in South Asia and sub-Saharan Africa. Globally, diarrhoea and stunting are estimated to have affected 957.5 and 151 million children under five, respectively, from 2015 to 2017. Despite a 28.6% reduction in the incidence of diarrhoea in children under five between 2005 and 2015 [1], and a 10.0% decrease in stunting from 2005 to 2015, 12.0% and 34.4% of children under five in Tanzania were still affected by diarrhoea and stunting, respectively, during that period [2, 3].
Stunting or retarded linear growth in children occurs as the result of an inadequate dietary intake and repeated infections. It has immediate negative consequences such as an increased risk of death and longer term effects including increased subsequent risk of noncommunicable disease [4]. It has also been related to the increased risk of obstructed labour in women, due to the smaller pelvic size in shorter women, and to giving birth to babies with low birth weight [5]. Apart from nutritional inadequacy, chronic exposure to a variety of pathogens from human and animal faeces, as is mostly observed in children exposed to unsanitary environments, may induce pathological changes in the gastrointestinal tract that increase nutrient losses and reduce nutrient absorption [6]. Measures of growth, including wasting, low weight and stunting, have been reported to have a close association with childhood diarrhoea in children under five years old [7]. A study conducted in seven LMICs in Asia and Africa between 2000 and 2012 attributed over 30% of child mortality to persistent diarrhoea. In addition, 40% of the same population had severe undernutrition, which indicates a feasible strong relationship between the two [8].
A classification of risk factors for stunting using studies from 137 LMICs has reported that foetal growth restriction and preterm birth are the most important risk

factors associated with stunting in children under five years of age globally. This is followed by environmental factors (e.g. unimproved water supply, unimproved sanitation and use of biomass fuel), and maternal and child nutrition and infection [9]. Poor sanitation and hygiene, including lack of access to improved toilet facilities and a failure to wash hands with soap before eating and after toilet use, significantly increase the risk of diarrhoea in children under five years of age [10].

In rural communities of LMICs, dependency on domestic animals and peridomestic wildlife for livelihood and food is often very high, which increases the level of human-animal interactions. Free-roaming animals may have access to rooms within the house during the day and are often confined within a specific part of the house at night as a physical security measure. Reports associated with this close interaction and occurrence of childhood diarrhoeal diseases have mixed results to date. Recent attention has focused on the close contact between animals and children in such settings, where exposure to livestock faeces presents a risk of children acquiring animal-associated diarrhoeal pathogens [11]. A study conducted in Kenya reported an increased incidence of diarrhoea in children under five years of age with increases in the number of sheep owned by households and with children's participation in providing drinking water to chickens [12]. In contrast, a longitudinal study that included fortnightly records of diarrhoea in children under five years of age in one of the two districts where the current study was conducted showed no significant association with chicken ownership or keeping chickens inside the house overnight with child diarrhoea [13]. Also, a study in Peru reported a higher rate of *Campylobacter*-related diarrhoea in children from households with confined chickens compared to those from households keeping chickens under an extensive system [14]. This serves as an indication that relationships between animals and people and frequency of diarrhoea are undoubtedly complex.

This quantitative study explored associations between variables related to socio-demographic characteristics, hygiene practices and human-animal interactions with the frequency of diarrhoea and height-for-age *z*-scores (HAZ) in children under five years of age in three wards of central Tanzania. Height-for-age *z*-score (HAZ) expresses linear growth, whereby low HAZ is an indication of failure to reach linear growth potential (retarded linear growth). Low HAZ does not necessarily mean stunting, which in children is a subset of retarded growth [5]. This study is a sub-study associated with a broader cluster randomised controlled trial titled 'Strengthening food and nutrition security through family poultry and crop integration in Tanzania and Zambia' (Nkuku4U) [15]. An understanding of factors associated with diarrhoea and HAZ is an essential step

Fig. 1. Data collected by Nkuku4U project and this study and selection criteria of the participating households, the months and years indicates the time of collection of respective data set and ***n*** indicates the number of households.



towards formulating effective strategies for diarrhoea control and for improving growth in children.

Materials and methods

Study area

Iwondo Ward in the Mpwapwa District and Sanza and Majiri Wards in the Manyoni District are situated within the Great Rift Valley in Tanzania. These areas form part of the semi-arid area of the Central Zone, experiencing low, short-lived and often erratic rainfall (approximately 600 mm per annum) in a unimodal pattern, typically from November to April, with reasonably widespread drought occurring approximately one year in four [16]. Low and unpredictable rainfall is associated with chronic food and nutritional insecurity in the study area due to water and pasture shortage, reduced crop production, livestock deaths, and the sale of livestock and crops at suboptimal prices to meet immediate household needs. Village chickens are kept by more than 50% of the households throughout the year, mostly under an extensive production system, and are the livestock least affected by these unpredictable climatic conditions in terms of feed availability [17].

Selection of participating households

The Nkuku4U project team conducted a census of all households in Sanza Ward in April 2014, Majiri Ward in October 2014, and Iwondo Ward in December 2016, as part of a staggered implementation within the broader study design, giving a total of 1730, 2810 and 2004 households, respectively. The criteria used for inclusion of households in the broader Nkuku4U study were having at least one child less than two years of age, and either currently owning chickens or expressing an intention to keep chickens within two years. Few households were excluded based on the latter criterion. Two-stage sampling was used to reach the study target of 240 households in Sanza Ward, 280 in Majiri Ward and 300 in Iwondo Ward. First, all eligible households with a child under 12 months of age were enrolled, and then random selection through lottery draw using household identification numbers deployed to select additional households with children aged 12-24 months to give a required number of children. In cases where more than one child under two years of age was present in the household, information on diarrhoea and anthropometry was collected for the younger child.

Households in the present study are a subset of those participating in the Nkuku4U project: encompassing all households either currently owning chickens or who owned chickens within the six months before administration of the questionnaire in February 2018. A total of 493 out of 711 households participating in the larger project fulfilled this criterion and were included. The number of households participating in this study from each ward was 153, 153 and 187 for Sanza, Majiri and Iwondo Wards, respectively.

Data collection

Information on parental reports of diarrhoea in children was collected twice per month by trained male and female community members ('Community Assistants') over 24 months, starting in June 2014, December 2014 and February 2016 in Sanza, Majiri and Iwondo Wards, respectively. Community Assistants visited each household to record the occurrence of diarrhoea within the preceding fortnight, based on information provided by the mother or primary caretaker of the enrolled child. Diarrhoea was defined as the passage of loose or liquid stools three or more times per day [18]. At each household visit, children reported as having diarrhoea for one or more days during the preceding two weeks were documented as a single positive count. A questionnaire that was initially tested and validated using the sub-population from the same study population was administered in February 2018 to 493 mothers or caregivers of enrolled children in participating households by trained male and female enumerators recruited from within each ward. Survey questions were in Swahili, but enumerators were encouraged to make use of the languages of the two predominant language groups (Gogo and Sukuma) where appropriate to aid in communication. Information collected spanned three key areas: socio-demographic characteristics, hygiene practices and human-animal interactions. Anthropometric data were taken at six-monthly intervals during the Nkuku4U project by trained personnel from the Tanzania Food and Nutrition Centre, Ministry of Health and respective district hospitals. Recumbent length was recorded for children up to 24 months of age, and standing height for children aged 24 months of age or above, using UNICEF portable baby/child length-height measuring boards. Measurements were recorded to the nearest 1 mm. The weights of the mother and child were measured to the nearest 0.1 kg using TANITA HD355 digital scales. Mother/caregiver and child were weighed together and maternal weight subtracted from the combined weight to give the weight of the child; this method eliminated the difficulties of handling children alone on a digital scale. The fifth anthropometry data set in May 2016, November 2016 and January 2018 for Sanza, Majiri and Iwondo, respectively, were used in this study (Fig. 1). Anthropometry was recorded for 466 children (out of the total number of 493 children enrolled in this study) as 27 children (12 boys, 15 girls) were not available for measurements, therefore, *z*-scores were calculated for 466 children, 223 boys and 243 girls.

Defining variables

These particular diarrhoea and anthropometry data sets were selected for analysis because they were collected during the time when all enrolled children were less than five years of age and data collection was the closest to the time of the questionnaire survey conducted in February 2018. HAZ were calculated from children's measurements using the Emergency Nutrition Assessment for SMART software (http://www.nutrisurvey.net/ena/ena.html) and WHO child growth standards [19]. A HAZ of less than -2 was classified as stunting. Longitudinal diar-

rhoea data (episodes) were collected fortnightly for 24 months (48 trials) through household visits. The number of successful visits (i.e. household informant present and able to provide information on the occurrence of diarrhoea in the enrolled child) was taken as the binomial event. For each successful visit, the enrolled child was recorded as having (yes) or not having (no) diarrhoea in the previous fortnight. The incidence of diarrhoea was calculated as a proportion i.e. the numbers of positive diarrhoea records divided by number of successful visits. The dependent and independent variables evaluated by this study are described in Table I.

Data analysis

Descriptive statistics

Data was first entered into an Excel 2007 spreadsheets and then transferred to STATA® software version 14.2

Tab. I. Description of the variables evaluated in the study categorised into socio-demographic characteristics, hygiene practices and human-animal interactions.

Variable	Definition or description of categories
Socio-demographic characteristics	
Height-for-age ***z***-score (HAZ)	Calculated based on WHO child growth standards [19]
Incidence of diarrhoea	Positive records of diarrhoea in the number of successful fortnightly visits collected over 24 months
Sex of enrolled child	Male or female
Age of enrolled child	Months as a continuous variable
Age group of enrolled child	Months grouped as 24-34, 35-45 and 46-56
Number of children under five in household	≤ 2 children or > 2 children
Maternal age	Years as a continuous variable
Maternal level of education	None, some primary school, or post-primary school
Sex of the head of the household	Male or female
Language group of the head of household	Gogo, Sukuma or others
Type of house floor	Unimproved (sand/soil) or improved (cement, concrete, tiles etc.)
Primary sources of drinking water in dry or rainy seasons	Stream, river, pond or dam, open well or public tap; separate variables for dry season and rainy season
Time spent fetching water	≤ 1 hour or > 1 hour, separate variables for dry season and rainy season
Hygiene practices	
Storage of kitchen utensils (unwashed, washed and with food)	On the floor, raised surface (e.g. table, shelf etc.) or hanging by a rope
Treatment of drinking water	Daily, occasionally (e.g. during gastrointestinal disease outbreaks, in the dry or rainy season) or never
Method of hand washing before child feeding	All household members in the same container, or one at a time using running water
Use of dried utensils for serving food*	Yes or No
Sharing latrine facilities among households	Yes or No
Handwashing with soap before feeding the child	Yes or No
Maternal handwashing with soap after toilet use	Yes or No
Latrine disposal of child faeces	Yes or No
Access of chickens to human faeces	Yes or No
Received hygiene education	Yes or No
Human-animal interactions	
Water source sharing with animals	Yes or No, separate variables for dry season and rainy season
Access of chickens to drinking water container	Yes or No
Chicken roosting locations	Inside (any room in house) or outside (chicken house or outside with no specific area)
Chicken roosting locations	Kitchen, bedroom, chicken house, outside with no specific place, or in separate room in the house
Frequency of cleaning chicken roosting location	Daily or occasional
Access of chickens to house during the day	Yes or No
Access of chickens to dirty and washed kitchen utensils	Yes or No
Keeping cattle inside the house overnight	Yes or no
Keeping goats inside the house overnight	Yes or no
Keeping sheep inside the house overnight	Yes or no
Keeping dogs inside the house overnight	Yes or no
Keeping cats inside the house overnight	Yes or no

*The utensils used while wet after washing or the washed utensils are rinsed and used while wet

for analysis [20]. Descriptive statistics were used to characterise the study population and explore differences in explanatory variables among the three study wards. Proportions were used to present categorical variables, and means, range and standard deviations for quantitative variables. Differences between wards and groups were determined by using *t*-tests and chi-square tests for continuous and categorical variables, respectively, and the variables were considered statistically significant at $p \leq 0.05$.

Univariable and multivariable models

Univariable linear regression and logistic regression models were fitted to determine the independent variables unconditionally associated with HAZ and diarrhoea outcome variables, respectively. Independent variables that showed suggestive associations ($p \leq 0.2$) were retained for construction of multivariable models. Candidate variables for multivariable models were categorised into three groups associated with: (1) socio-demographic characteristics, (2) hygiene practices and (3) human-animal interactions. The individual multivariable linear regression model (HAZ outcome variable) and logistic regression model (diarrhoea outcome variable) were run to test the association of independent variables significantly and suggestively associated with the outcomes in univariable models from each category and the outcomes variables under this study. The variables that were significant associated with the outcomes in each multivariable model were run in a single model to generate a final multivariable model. Stepwise backward elimination was used to eliminate the variables with *p*-values higher than 0.05 to reach the final model in each of the three multivariable models and in final combined multivariable models. The models were fitted by R studio software version 3.6.0 using data stored in the STATA® spreadsheets [21].

Ethical approval

The study design, protocols and research tools for this program were approved by the National Institute for Medical Research ethics committee (NIMR/HQ/R.8a/Vol.IX/1690) in Tanzania, and The University of Sydney Human Research Ethics Committee (2014/209). Informed consent was obtained from all questionnaire survey respondents via signature or thumb print, with the assurance of confidentiality, anonymity and voluntary participation.

Results

Demographic characteristics

The mean maternal and children's age in this study was 32 ± 7.5 years and 32.5 ± 5.3 months, respectively. The percentage of mothers with education above primary school level ranged from 1.1% in Iwondo to 9.8% in Sanza, and the variation in the level of education across the three wards was significant ($p < 0.001$). Gogo was the predominant language spoken by more than 80% of the heads of households, whereas Sukuma speakers ranged from 13.0% of households in Majiri to 1.1% of households in Iwondo. The mean number of diarrhoea incidents collected fortnightly for 24 months were 2.3, 1.7, 2.8 and 2.4 over 24 months for the overall sample, Sanza, Majiri and Iwondo wards, respectively. The difference in mean diarrhoea incidence between Gogo and Sukuma language group households was not significant ($p = 0.29$). The prevalence of stunting was 46.9% (CI. 42.3-51.6), 49.8% (CI. 43.0-56.5) and 44.4% (CI. 38.1-50.9) in the overall sample, boys and girls, respectively, with no significant difference between sex ($p = 0.25$) and among wards ($p = 0.13$). The proportion of stunted children among the 24-34, 35-45 and 46-56 month age groups was 47.3% (CI. 41.8-52.8), 48.3% (CI. 38.9-57.7) and 33.3% (CI. 13.3-59.0), respectively. Stunting rates in Gogo, Sukuma and other language groups were 47.9% (CI. 43.1-52.7), 32.1% (CI. 15.9 -52.3) and 45.5% (CI. 16.7-76.6) respectively with no significant difference ($p = 0.27$); however, the height-for-age *z*-scores were significantly different in children from the Gogo (-1.9) and Sukuma (-1.1) language groups ($p = 0.004$). The details of the demographic characteristics in this study are in Table II.

Univariable and multivariable models

Diarrhoea

Due to the relatively large number of predictive variables tested, individual multivariable models (i.e. socio-demographic characteristics, hygiene practices, human-animal interactions) and final models were fitted to test their association with diarrhoea. The variables suggestive and significant associated diarrhoea in the univariable model are presented in Tables III, IV and V. In a univariable model, age of children as a continuous variable ($p < 0.001$), maternal education ($p = 0.008$), time spent fetching water ($p = 0.007$) and age of children ($p = 0.007$) were significantly associated with diarrhoea but not significant in the final socio-demographic characteristic multivariable model (Tab. III). Male children (OR = 1.17, 95% CI. 1.04-1.32; $p = 0.001$) were associated with increased diarrhoea incidence in the socio-demographic characteristics multivariable model. The older age of children (OR = 0.21, 95% CI. 0.07-0.67; $p < 0.004$), use of open wells (OR = 0.41, 95% CI. 0.35-0.48; $p < 0.001$) and public taps (OR = 0.47, 95% CI. 0.40-0.55; $p < 0.001$) in the dry season and public taps (OR = 0.70, 95% CI. 0.53-0.93; $p = 0.025$) in the rainy season as the household's primary source of drinking water were associated with low risk of childhood diarrhoea in the socio-demographic characteristic multivariable model.

Storage of washed utensils on raised surfaces reduced the risk of diarrhoea in children in the hygiene practices multivariable model (OR = 0.80, 95% CI. 0.68-0.93; $p = 0.004$), whereas inappropriate disposal of child faeces (OR = 1.17, 95% CI. 1.03-1.32; $p = 0.015$), access of chickens to human faeces (OR = 1.16, 95% CI. 1.02-1.32; $p = 0.026$) and respondents untrained in hygiene

Tab. II. Demographic characteristics of the studied populations expressed in percentage, mean, range and standard deviation (SD) depending on the type of variable presented.

Variable	Sanza (*n* = 153)	Majiri (*n* = 153)	Iwondo (*n* = 187)	Overall study sample (*n* = 493)
Age of children (months)				
Mean (SD)	34.6 (6.5)	31.0 (4.0)	31.8 (4.6)	32.4 (5.3)
Range	25.7-52.5	24.3-46.0	25.2-47.5	24.3-52.5
Age group of children (%)				
24-34 months	58.2	82.4	72.2	71.0
35-45 months	33.3	15.7	25.7	24.9
46-56 months	8.5	1.31	1.6	3.65
Age unknown	0	0.65	0.53	0.41
Sex of the enrolled children (%)				
Male	53.6	46.4	43.9	47.7
Female	46.4	53.6	56.2	52.3
Number of children under five years				
Mean (SD)	1.7 (0.7)	1.7 (0.6)	1.5 (0.6)	1.7 (0.70
Range	1–4	1–4	1–4	1-4
Number of diarrhoea incidents				
Mean (SD)	1.7 (1.6)	2.8 (3.0)	2.4 (2.8)	2.3 (2.6)
Range	0-8	0-16	0-14	0-16
Height-for-age *z*-scores (HAZ)				
Mean (SD)	-1.8 (1.0)	-1.9 (0.9)	-1.9 (1.1)	-1.9 (1.0)
Range	-4.4-0.4	-4.0-0.6	-4.7-1.5	-4.7-1.5
Missing	4	18	5	27
Maternal age (years)				
Mean (SD)	33.9 (7.4)	31.5 (8.1)	30.9 (7.0)	32.0 (7.5)
Range	18-56	17–54	18–53	17 - 56
Maternal level of education (%)				
Primary school	75.8	66.7	71.7	71.4
Above primary school	9.8	3.9	1.1	4.7
None	14.4	29.4	27.3	23.9
Sex of head of household (%)				
Male	74.5	80.4	86.6	80.9
Female	25.5	19.6	13.4	19.1
Language of head of household (%)				
Gogo	88.9	84.3	97.9	90.9
Sukuma	7.2	13.1	1.1	6.7
Other	3.9	2.6	0.5	2.2
Unspecified	0.0	0.0	0.5	0.2

(OR = 1.35, 95% CI. 1.19-1.53; $p < 0.001$) were associated with higher risk of childhood diarrhoea. Overnight sharing of housing with sheep (OR = 1.30, 95% CI. 1.02-1.66; $p = 0.034$) and cats (OR = 1.13, 95% CI. 1.12-1.52; $p < 0.001$), keeping the chickens inside the house overnight regardless of which room (OR = 1.49, 95% CI. 0.77-1.72; $p < 0.001$) or outside as compared to kitchen (OR = 1.50, 95% CI. 1.09-2.05; $p = 0.012$) and sharing water sources with animals in the dry season (OR = 1.55, 95% CI. 1.37-1.75; $p < 0.001$) were significantly associated with increased diarrhoea incidence in the human-animal interactions model (Tab. VI).

In the final multivariable model, children from the households depending on open wells (OR = 0.46, 95% CI. 0.39-0.54; $p < 0.001$) and public tap (OR = 0.51, 95% CI. 0.44-0.61; $p < 0.001$) as their primary source of drinking water in the dry season were less likely to report diarrhoea, as compared to those from households using water from a stream, river, pond or dam. Male children (OR = 1.24, 95% CI. 1.09-1.39; $p < 0.001$), not disposing of child faeces in latrines (OR = 1.15, 95% CI. 1.02-1.31; $p = 0.025$), sharing water sources with animals in the dry season (OR=1.48, 95% CI. 1.29-1.70; $p < 0.001$), overnight sharing of houses with cats (OR = 1.35, 95% CI. 1.16-1.57; $p < 0.001$) and chickens roosting in house regardless of the room (OR = 1.39, 95% CI. 1.20-1.60; $p < 0.001$) were associated with an increase in diarrhoea incidence in children. Older children (46-56 months)

Tab. III. Univariable models evaluating the significance of socio-demographic characteristic related independent variables showing *p*-values of all suggestive ($p \leq 0.2$) and significant ($p \leq 0.05$) variables associated with diarrhoea and height-for-age *z*-scores and the coefficient of association.

	Diarrhoea[a]			Height-for-age *z*-scores[b]		
Variable	Coef.*	*p*-value	Overall *p*-value	Coef.*	*p*-value	Overall *p*-value
Children age group (months)			< 0.001			0.314
24-34	Ref.*					
35-45	-0.08	0.236		0.10	0.456	
46-56	-0.83	< 0.001		-0.06	0.643	
Age of children (months)	-0.03	< 0.001	< 0.001	-0.00	0.662	0.661
Sex of child, male	0.16	0.007	0.007	-0.14	0.126	0.125
Number of children under 5 years			0.197			0.168
≤ 2 children	Ref.					
> 2 children	0.14	0.19		0.24	0.169	
Age of mothers (years)	0.00	0.441	0.442	-0.00	0.659	0.658
Maternal education			0.008			0.483
At most primary school	Ref.					
Above primary school	-0.28	0.095		0.26	0.248	
Never attended school	0.17	0.017		0.05	0.640	
Sex of head of household, male	-0.17	0.024	0.026	-0.05	0.694	0.693
Children diarrhoea incidence	NA	NA	NA	-1.34	0.78	0.088
Language group of head of household			0.577			0.010
Gogo	Ref.					
Sukuma	0.08	0.480		0.52	0.007	
Other	0.16	0.407		0.45	0.135	
Type of house floor			0.511			0.627
Unimproved	Ref.					
Improved	-0.06	0.514		0.05	0.627	
Source of drinking water						
Dry season			< 0.001			0.136
Stream/river/pond/dam	Ref.					
Open wells	-0.91	< 0.001		0.22	0.047	
Public tap	-0.84	< 0.001		0.12	0.297	
Rainy season			< 0.001			0.989
Stream/river/pond/dam	Ref.					
Open wells	-0.35	0.002		0.00	0.978	
Public tap	-0.63	< 0.001		-0.02	0.886	
Time spent to fetch water						
Dry season			0.007			0.322
≤ 1 hour	Ref.					
> 1 hour	0.17	0.007		-0.10	0.323	
Rainy season			0.571			0.959
≤ 1 hour	Ref.					
> 1 hour	0.06	0.568		0.01	0.959	

Coef.* = Regression coefficient; Ref.* = Reference category; [a] Logistic Regression Model; [b] Linear Regression Model

were less likely to have diarrhoea compared to younger children (24-34 months) (OR = 0.43, 95% CI. 0.27-0.67; $p < 0.001$) (Tab. VI).

Height-for-age z-score

In the multivariable model based on socio-demographic characteristics, only language group ($p = 0.019$) and house floor ($p = 0.028$) were significantly associated with HAZ, and in the final combined model, only the language group remained significant. Of variables relating to hygiene practices, handwashing with running water ($p = 0.009$) and storing washed utensils by hanging ($p = 0.007$) were positively and negatively associated with HAZ, respectively. Human-animal interaction-related variables significantly associated with HAZ were access of chickens to unwashed utensils ($p = 0.033$) and keeping sheep inside the house overnight ($p = 0.015$) which remained significant even in the final combined model (Tab. VII). The final combined multivariable model indicated that children from households headed

Tab. IV. Univariable models evaluating the significance of hygiene practices related independent variables showing *p*-values of all suggestive ($p \leq 0.2$) and significant (≤ 0.05) variables associated with diarrhoea and height-for-age *z*-scores and the coefficient of association.

	Diarrhoea[a]			Height-for-age *z*-scores[b]		
Variable	Coef.*	*p*-value	Overall *p*-value	Coef.*	*p*-value	Overall *p*-value
Maternal handwashing methods			0.780			0.017
All in the same container	Ref.					
One at time in running water	-0.02	0.780		0.24	0.017	
Handwashing with soap before feeding the child, Yes	0.19	0.031	0.034	0.05	0.723	0.722
Use of dry utensils for serving food, Yes	-0.16	0.041	0.044	0.08	0.573	0.572
Storage of utensils before washing			0.141			0.150
On the floor	Ref.					
On raised surface	0.15	0.058		0.00	0.979	
Hanging	0.08	0.274		-0.22	0.060	
Storage of utensils after washing			< 0.001			0.042
On the floor	Ref.					
On raised surface	-0.24	0.002		-0.12	0.314	
Hanging	0.04	0.597		-0.28	0.013	
Storage of utensils with food			0.003			0.080
On the floor	Ref.					
On raised surface	-0.24	0.005		0.22	0.076	
Hanging	0.00	0.999		-0.02	0.888	
Treatment of drinking water			0.135			0.634
Boiling always	Ref.					
Occasional boiling	-0.28	0.086		-0.10	0.679	
No treatment	-0.05	0.662		-0.17	0.370	
Latrine shared among households, Yes	0.07	0.277	0.277	-0.16	0.092	0.091
Disposal of child faeces in latrine, No	0.18	0.004	0.005	-0.08	0.404	0.403
Access of chicken to human faeces, Yes	0.12	0.066	0.065	0.01	0.878	0.878
Maternal handwashing with soap after toilet use, No	-0.07	0.261	0.2608	-0.15	0.098	0.097
Received hygiene education, No	0.31	< 0.001	< 0.001	-0.03	0.731	0.731

Coef.* = Regression coefficient; Ref.* = Reference category; [a] Logistic Regression Model; [b] Linear Regression Model.

by Sukuma speaking individuals have higher HAZ as compared to the Gogo headed households ($p = 0.005$). Washing hands in running water ($p = 0.007$), chickens gaining access to unwashed utensils ($p = 0.031$) and keeping sheep inside the house ($p = 0.020$) overnight were associated with higher HAZ.

Discussion

In this study we found that the rate of stunting in children under five was relative high in all three wards regardless of language group and gender of the children under study compared to the current national stunting rate which is at 34% [3]. This finding reflects the challenging agro-ecological conditions in the project area. The proportion of stunted children slightly decreased with increase in child age in contrast with other studies [22, 23]. The difference may be accounted for by the effects of diarrhoea, which was negatively related to age in the univariable model in the present study. Demographic and Health Survey data from Bangladesh indicates that stunting in children aged 0-59 months increases rapidly between 12 and 23 months of age, after which it levels out with minor variations [24]. An extrapolation of these data into the current study means that the minimum age of enrolled children in the current study was at the peak of the stunting prevalence, which may be the reason for the observed results of stunting rate of 47.3%, 48.3% and 33.3% at age groups 24-34, 35-45 and 46-56 months, respectively. On the other hand, the decrease in prevalence of stunting observed in the current study amongst children in the oldest age may be reflecting recovery, which has been reported to be as high as 45% in a recent longitudinal study in Kenyan children, especially those becoming stunted at less than 18 months of age [25]. Although anthropometry was conducted at different times in the three wards (May 2016 in Sanza, November 2016 in Majiri and January 2017 in Iwondo), the variation in stunting rate among wards did not vary significantly between wards.

It has been reported that the incidence of diarrhoea in children under five year of age decreases with increasing age [26]. The probability of developing diarrhoea in

Tab. V. Univariable models evaluating the significance of human-animal interactions related independent variables showing *p*-values of all suggestive ($p \leq 0.2$) and significant (≤ 0.05) variables associated with diarrhoea and height-for-age *z*-scores and the coefficient of association.

	Diarrhoea[a]			Height-for-age *z*-scores[b]		
Variable	Coef.*	*p*-value	Overall *p*-value	Coef.*	*p*-value	Overall *p*-value
Water source sharing with animals			< 0.001		0.534	
Dry season, Yes	0.47	< 0.001		-0.06		0.533
Rainy season, Yes	0.02	0.765	0.765	0.09	0.421	0.420
Access of chickens to water containers, Yes	-0.13	0.031	0.031	0.07	0.451	0.450
Chicken roosting location			0.003			0.116
Kitchen	Ref.*					
Bedroom	0.12	0.283		0.09	0.589	
Chicken house	-0.10	0.531		0.34	0.029	
Outside, no specific place	0.47	0.003		-0.11	0.712	
Separate room in the house	-0.07	0.409		0.25	0.054	
Chicken roosting location, Inside	0.31	< 0.001	< 0.001	0.15	0.192	0.190
Frequency of cleaning chicken roosting location			0.010			0.883
Daily	Ref.					
Occasional	0.24	0.008		-0.02	0.883	
Access of chickens to house during day, Yes	-0.07	0.356	0.359	0.13	0.254	0.253
Access of chickens to kitchen utensils						
Before washing, Yes	0.06	0.347	0.345	0.22	0.032	0.031
After washing, Yes	-0.03	0.604	0.6038	0.03	0.715	0.714
Other animals kept inside house overnight						
Cattle, Yes	0.21	0.10	0.109	0.11	0.600	0.599
Goat, Yes	0.06	0.601	0.604	0.39	0.031	0.030
Sheep, Yes	0.31	0.008	0.011	0.51	0.015	0.014
Dogs, Yes	0.02	0.932	0.932	0.09	0.449	0.448
Cats, Yes	0.29	< 0.001	< 0.001	0.07	0.581	0.580

Coef.* = Regression coefficient; Ref.* = Reference category; [a] Logistic Regression Model; [b] Linear Regression Model.

this study was lower in children aged between 46 and 56 months compared with those aged between 24 and 34 months, which is consistent with the literature. A higher incidence of diarrhoea in children aged between 6 and 11 months was reported compared to children aged 48-59 months [27]. This was attributed to declining levels of maternal immunity, introduction of complementary foods and mouthing of potentially contaminated objects by young children, and to strengthened immunity and environmental adaptation in older children.

Drinking water from open wells and public taps appeared to be protective against diarrhoea in children in the dry season. The scarcity of water sources and the time-intensive nature of sourcing water in the study area settings often led to close proximity between water accessed by livestock and that collected for household use, exposing humans to microbial contamination by animal faeces. The likelihood of animal faecal contamination of water sources was suggested by the results of the current study, which reported increases in the risk of developing diarrhoea in children from households sharing water sources with animals in the dry period. Poor microbial quality of drinking water is well documented as a cause of diarrhoea, sometimes in the form of a disease outbreaks [28]. Although water treatment was not a significant variable in the current study, boiling [29] and use of sodium hypochlorite (liquid bleach) [30] have been proven successful in lowering childhood diarrhoea in other studies. However, the latter method may be difficult to implement in the study area settings due to financial constraints. Treatment of drinking water should be accompanied by proper handling and storage to prevent in-house re-contamination from the users as has been reported in other studies [31-33].

Improper disposal of child faeces including discarding it in the field, leaving in open spaces to dry or covering with soil, was associated with an increased risk of diarrhoea in children, compared with latrine disposal. Similar results were reported in a study conducted in children under five years of age in Iraq, in which children from households leaving children's faeces on the ground were more likely to develop diarrhoea compared to those from households disposing of children's faeces in latrines [34]. In resource-poor settings, poor faecal disposal may result in direct contamination of already-prepared food and indirect contamination of kitchen utensils, particularly if the household is keeping chickens under an extensive production system with free access to every part of the house. Nonetheless, in the current study, access of chickens to human faeces was

Tab. VI. Socio-demographic characteristics, hygiene practices and human-animal interactions multivariable models[a] built using variables showing significant ($p \leq 0.05$) or suggestive association ($p \leq 0.2$) with diarrhoea incidence in univariable models, and final combined model[a] fitted using combination of significant variables from all three multivariable models.

Variable	Odd ratio	Odd ratio 95% Conf. Interval*		*p*-value	Overall *p*-value
Socio-demographic characteristics					
Sex of child, Male	1.17	1.04	1.32	0.001	0.001
Child age group	Ref.				0.004
24-34					
35-45	0.83	0.69	1.00	0.671	
46-56	0.21	0.07	0.67	0.005	
Source of drinking water					
Dry season					< 0.001
Stream/river/pond/dam	Ref.				
Open wells	0.41	0.35	0.48	< 0.001	
Public tap	0.47	0.40	0.55	< 0.001	
Rainy season					0.045
Stream/river/pond/dam	Ref.				
Open wells	1.10	0.85	1.42	0.363	
Public tap	0.70	0.53	0.93	0.025	
Hygiene practices					
Storage of washed utensils					0.004
On the floor	Ref.				
On raised surface	0.80	0.68	0.93	0.004	
Hanging	0.99	0.85	1.14	0.862	
Latrine disposal of children faeces, No	1.17	1.03	1.32	0.015	0.015
Access of chickens to human faeces, Yes	1.16	1.02	1.32	0.026	0.026
Training on hygiene, No	1.35	1.19	1.53	< 0.001	< 0.001
Human-animal interactions					
Sharing water source with animal in dry season, Yes	1.55	1.37	1.75	< 0.001	< 0.001
Chicken roosting location					
Kitchen	Ref.				
Bedroom	1.15	0.93	1.72	0.208	0.005
Chicken house	0.90	0.73	1.43	0.324	
Outside, no specific place	1.50	1.09	1.11	0.012	
Separate room in the house	0.91	0.77	2.05	0.308	
Chicken roosting location, Inside	1.49	1.29	1.09	< 0.001	< 0.001
Sheep inside house overnight, Yes	1.30	1.02	1.66	0.034	0.039
Cats inside house during night, Yes	1.31	1.12	1.52	< 0.001	< 0.001
Final model					
Drinking water source in dry season					< 0.001
Stream/river/pond/dam	Ref.				
Open wells	0.46	0.39	0.54	< 0.001	
Public tap	0.51	0.44	0.61	< 0.001	
Sex of child, male	1.24	1.09	1.39	< 0.001	< 0.001
Child age group (months)					< 0.001
24-34	Ref.				
35-45	0.97	0.84	1.12	0.666	
46-56	0.43	0.27	0.67	< 0.001	
Latrine disposal of child faeces, No	1.15	1.02	1.31	0.025	0.026
Training on hygiene, No	1.16	1.01	1.33	0.030	0.030
Sharing water source with animals in dry season, Yes	1.48	1.29	1.70	< 0.001	< 0.001
Chicken roosting location, Inside	1.39	1.20	1.60	< 0.001	< 0.001
Cats inside house overnight, Yes	1.35	1.16	1.57	< 0.001	< 0.001

OR = Odd ratio ; 95% Conf. Interval.* = 95% Confidence interval for odd ratio; Ref.* = Reference category; [a] Logistic Regression Model.

Tab. VII. Socio-demographic characteristics, hygiene practices and human-animal interactions multivariable models[b] built using variables showing significant ($p \leq 0.05$) or suggestive association ($p \leq 0.2$) with age-for-age *z*-scores in univariable models, and final combined model[b] built from combination of significant variables from all three multivariable models.

Variable	Coef.*	Std. error*	*p*-value	Overall *p*-value	95% Conf. Interval*	
Socio-demographic characteristics						
Language group				0.019		
Gogo	Ref.					
Sukuma	0.53	0.19	0.006		0.15	0.91
Others	0.22	0.32	0.495		-0.41	0.85
House floor, Improved	0.33	0.15	0.028	0.028	0.04	0.62
Hygiene practices						
Handwashing				0.009		
All on the same container	Ref.					
One at a time in running water	0.26	0.10	0.009		0.07	0.46
Storage of washed utensils				0.025		
On the floor	Ref.					
Raised surface	-0.13	-0.13	0.256		-0.35	0.09
Hanging	-0.30	-0.30	0.007		-0.53	-0.08
Human-animal interactions						
Chickens access to unwashed utensils, Yes	0.22	0.10	0.033	0.033	0.02	0.42
Sheep inside house overnight, Yes	0.51	0.21	0.015	0.015	0.10	0.91
Combined model						
Language group				0.011		
Gogo	Ref.					
Sukuma	0.54	0.19	0.005		0.16	0.91
Others	0.34	0.30	0.261		-0.25	0.93
Handwashing				0.007		
All in the same container	Ref.					
One at a time in flowing water	0.27	0.10	0.007		0.07	0.47
Chickens access to unwashed utensils, Yes	0.22	0.10	0.031	0.031	0.02	0.42
Sheep inside house overnight, Yes	0.48	0.21	0.020	0.020	0.08	0.89

Coef.* = Regression coefficient; Std. Err.* = Standard error; 95% Conf. Interval* = 95% Confidence interval; Ref.* = Reference category; [b] Linear Regression Model.

significantly associated with increased incidence of diarrhoea in the hygiene practices model but non-significant in the final combined multivariable model.

Children from the Sukuma language group households had significantly higher HAZ compared with children from the Gogo language group households. Similar results were reported in another study involving Sukuma and Pimbwe language groups conducted in the Southern Highland Zone of Tanzania [35]. People belonging to the Sukuma language group have been reported as having greater asset accumulation and practising sound agricultural and livestock production, all regarded as important predictors of food security. This may explain the better growth rates of Sukuma children compared to those of other ethnic groups in Tanzania including Gogo speakers [36]. Associations of poor growth with low wealth index have been reported in a number of studies indicating its importance in determining childhood nutrition and growth performance [23, 37-39].

Maternal handwashing during critical times, including before feeding children and after toilet use, is important in the control of gastrointestinal infections [40, 41] and stunting [42, 43]; however, handwashing should be properly executed. The current study shows that handwashing one at a time with running water was associated with increased HAZ in children, compared to one or more persons washing hands in a shared bowl of water. Improper handwashing, including submerging hands in a bowl of water used by multiple people or on multiple occasions, should be discouraged as it increases the risk of pathogen transfer [44]. Establishment of dedicated areas for handwashing within a house, providing water and soap, and availability of locally-made handwashing facilities may promote proper handwashing in resources-poor settings. Wood ash has been proven to have antimicrobial activities, therefore it can be used as an alternative for hand washing in the households that cannot afford to have soap constantly available [45].

We did not find any significant association between sanitation, water source and hygiene variables with HAZ in children under five years of age, which contradicts observations in other studies [46, 47]. The lack of a true control group, having participating households with similar characteristics (all from resource-poor settings), and a relatively small sample size of the current study make it more difficult to assess such associations, com-

pared to those involving socio-economically diverse study populations and larger sample sizes [48, 49]. Use of a small sample size from a localised area has been mentioned as a potential reason for non-significant results from improved toilet and water sources, compared to other studies that used larger sample size and more than one population from different settings [37].

The practice of keeping cats and chickens inside the house overnight was associated with an increased risk of child diarrhoea. Domestic animals including cats and chickens have been implicated in harbouring gastrointestinal pathogens that may also infect humans [50]. Similar strains of pathogens have been isolated in asymptomatic animals and symptomatic humans, highlighting the potential importance of animal-derived pathogens to public health [51, 52]. However, a clonal difference of *Salmonella* isolated in humans and animals in high human-animal interaction settings was reported, indicating that not every infected animal presents a risk to humans [53]. Unexpectedly, keeping sheep inside the house overnight and allowing chickens to access unwashed kitchen utensils for leftover food were associated with increased HAZ in children. Pre- and postnatal exposure to pet animals (dogs and cats) have been associated with increased abundance of beneficial gut microbiota in children, reducing pathogenic bacteria population in the gut [54], which may diminish any negative impact on child growth. A study from Ethiopia that involved poultry production as an intervention to improve nutrition in children aged at 0-36 months reported increasing HAZ and weight for age *z*-score (WAZ). Also, there was no statistically significant association between the intervention and anaemia, fever, vomiting or diarrhoea in children – even in households keeping the chickens in their house overnight [55].

In the current study, overnight sharing of the house with chickens and cats was found to be associated with an increased risk of child diarrhoea, while overnight sharing of the house with sheep and allowing chickens access to unwashed kitchen utensils was associated with higher HAZ; this presents a complex picture. The significance and direction of associations between human-animal interactions and child health and growth outcomes therefore warrants further investigation. Screening for gastrointestinal pathogens in children and all animal species kept in the study areas accompanied by genomic analysis may help to clarify the public health risks that may emerge from extensive human-animal interactions.

Proper handwashing during critical times has been proven effective in different studies in controlling diarrhoea and improving HAZ in children. Therefore, the importance of effective handwashing should be emphasised and introduced to the community through evidence- and theory-based, user- and resource-friendly interventions in relation to the community being targeted [56]. Safe water supplies are lacking in the study areas and may remain a challenge for quite some time due to inadequate community and local government resources. Home drinking water treatment by boiling, using chlorine tablets or some emerging simple, effective and cheap technologies including use of a bio-sand water filter [57] are the only immediate and effective interventions in controlling diarrhoea in children under five years of age in areas using unsafe sources of water. Sharing the house with animals, especially chickens, to overcome predation and theft, is commonly practised in the area. Building chicken houses close to the home, or having a designated room within the main house for keeping chickens overnight, which is cleaned before being accessed by children and other household members, may reduce the health impacts resulting from a shared dwelling, while still reducing chicken theft and predation risks. Childhood diarrhoea and stunting is determined by a complex array of risk factors that vary from one community to another, requiring collective action to be properly addressed. The nature and extent of interventions to address childhood diarrhoea and stunting in this study setting, and in similarly resource-limited communities, can be guided by findings from this and other similar studies. The present study highlights the complexity of associations between humans and domestic animals, in which potential positive contributions of livestock ownership and inter-species variation in the risk of zoonotic disease, requires further investigation. In this setting, results suggest that access to safe and clean water, improved sanitation and proper hand washing should be the first priority in improving the nutrition and health of young children.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contribution

Conceptualization: ER, GM, RA, RK; VS; Data curation: ER, WM, and RM; Formal analysis: ER, PCT and RM; Funding acquisition: GM, R; Investigation: ER, GM; Methodology: ER, GM, ID-H and PCT; Project administration: ER, WM; Supervision: RA, GM; Validation: WM, ER and ID-H; Visualization: ER and RK; Writing original draft: ER; Writing, review & editing: ER, GM, CT, PCT, WM, RM, JdB, RK, ID-H, RA.

References

[1] Troeger C, Forouzanfar M, Rao P C, Khalil I, Brown A, Reiner

RC, Fullman N, Thompson RL, Abajobir A, Ahmed M, Alemayohu MA, Alvis-Guzman N, Amare AT, Antonio CA, Asayesh H, Avokpaho E, Awasthi A, Bacha U, Barac A, Betsue BD, Beyene AS, Boneya DJ, Malta DC, Dandona L, Dandona R, Dubey M, Eshrati B, Fitchett JRA, Gebrehiwot TT, Hailu GB, Horino M, Hotez PJ, Jibat T, Jonas JB, Kasaeian A, Kissoon N, Kotloff K, Koyanagi A, Kumar GA, Rai RK, Lal A, El Razek HA, Mengistie MA, Moe C, Patton G, Platts-Mills JA, Qorbani M, Ram U, Roba HS, Sanabria J, Sartorius B, Sawhney M, Shigematsu M, Sreeramareddy C, Swaminathan S, Tedla B, Jagiellonian RT, Ukwaja K, Werdecker A, Widdowson M, Yonemoto N, El Sayed Zaki M, Lim, SS, Naghavi M, Vos T, Hay SI, Murray CJL, Mokdad AH. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. TLancet Infect Dis 2017;17:909-48. https://doi.org/10.1016/S1473-3099(17)30276-1

[2] UNICEF WHO World Bank. Monitoring the situation of children and women. UNICEF, WHO, World Bank: Joint Child Malnutrition Estimates. 2018 [cited 2019 28/3]; Available from: https://data.unicef.org/topic/nutrition/malnutrition/

[3] MoHCDGEC MoH NBS OCGS and ICF. Tanzania Demographic and Health Survey and Malaria Indicator Survey (TDHS-MIS) 2015-16. Ministry of Health, Community Development, Gender, Elderly and Children (MoHCDGEC) [Tanzania Mainland], Ministry of Health (MoH) [Zanzibar], National Bureau of Statistics (NBS), Office of the Chief Government Statistician (OCGS), and ICF. 2016. Dar es Salaam, Tanzania, and Rockville, Maryland, USA. Dar es Salaam: MoHCDGEC 2016:630. https://dhsprogram.com/pubs/pdf/FR321/FR321.pdf

[4] Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, Ezzati M, Grantham McGregor S, Katz J, Martorell R, Uauy R. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet 2013;382:427-51. https://doi.org/10.1016/S0140-6736(13)60937-X

[5] Leroy JL, Frongillo EA. Perspective: What Does Stunting Really Mean? A Critical Review of the Evidence. Adv Nutr 2019;10:196-204. https://doi.org/10.1093/advances/nmy101

[6] Humphrey JH. Child undernutrition, tropical enteropathy, toilets, and handwashing. Lancet 2009;374:1032-35. https://doi.org/10.1016/S0140-6736(09)60950-8

[7] El Samani EFZ, Willett WC, Ware JH. Association of malnutrition and diarrhea in children aged under five years: A prospective follow-up study in a rural Sudanese community. Am J Epidemiol 1988;128:93-105. https://doi.org/10.1093/oxfordjournals.aje.a114963

[8] Rahman AE, Moinuddin M, Molla M, Worku A, Hurt L, Kirkwood B, Mohan SB, Mazumder S, Bhutta Z, Raza F, Mrema S, Masanja H, Kadobera D, Waiswa P, Bahl R, Zangenberg M, Muhe L. Childhood diarrhoeal deaths in seven low- and middle-income countries. Bull World Health Organ 2014;92:664-71. https://doi.org/10.2471/BLT.13.134809

[9] Danaei G, Andrews KG, Sudfeld CR, Fink G, McCoy DC, Peet E, Sania A, Smith F, Mary C, Ezzati M, Fawzi WW. Risk factors for childhood stunting in 137 developing countries: a comparative risk assessment analysis at global, regional, and country levels. PLoS Med 2016;13:e1002164-e. https://doi.org/10.1371/journal.pmed.1002164

[10] Oloruntoba EO, Folarin TB, Ayede AI. Hygiene and sanitation risk factors of diarrhoeal disease among under-five children in Ibadan, Nigeria.Afr Health Sci 2014;14:1001-2. https://doi.org/10.4314/ahs.v14i4.32

[11] Zambrano LD, Levy K, Menezes NP, Freeman MC. Human diarrhea infections associated with domestic animal husbandry: a systematic review and meta-analysis. Trans R Soc Trop Med Hyg 2014;108: 313-25. https://doi.org/10.1093/trstmh/tru056

[12] Conan A, O'Reilly CE, Ogola E, Ochieng JB, Blackstock AJ, Omore R, Ochieng L, Moke F, Parsons MB, Xiao L, Roellig D, Farag TH, Nataro JP, Kotloff KL, Levine MM, Mintz ED, Breiman RF, Cleaveland S, Knobel DL. Animal-related factors associated with moderate-to-severe diarrhea in children younger than five years in western Kenya: a matched case-control study. PLoS Negl Trop Dis 2017;11:e0005795. https://doi.org/10.1371/journal.pntd.0005795

[13] de Bruyn J, Thomson PC, Darnton-Hill I Bagnol B, Maulaga W, Alders Robyn RG. Does Village chicken-keeping contribute to young children's diets and growth? A longitudinal observational study in Rural Tanzania. Nutrients 2018;10:1799. https://doi.org/10.3390/nu10111799

[14] Oberhelman RA, Gilman RH, Sheen P, Cordova J, Zimic M, Cabrera L, Meza R, Perez J. An intervention-control study of corralling of free-ranging chickens to control Campylobacter infections among children in a Peruvian periurban Shantytown. Am J Trop Med Hyg 2006;74:1054-9. https://doi.org/10.4269/ajtmh.2006.74.1054

[15] Alders R, Aongola A, Bagnol B, de Bruyn J, Kimboka S, Kock R, Li M, Maulaga W, McChonchie R, Mor. S, Msami H, Mulenga F, Mwale M, Mwale S, Pengely B, Rushton J, Simpson J, Victor R, Yongolo C, Young M. Using a one health approach to promote food and nutrition security in Tanzania and Zambia. Planet@Risk 2014;2:187-90. https://doi.org/https://planet-risk.org/index.php/pr/article/view/67

[16] Lema MA, Majule AE. Impacts of climate change, variability and adaptation strategies on agriculture in semi-arid areas of Tanzania: the case of Manyoni District in Singida Region, Tanzania. Afr J Environ Sci Tech 2009;3:206-18. https://doi.org/10.5897/AJEST09.099

[17] de Bruyn J, Thomson PC, Bagnol B, Maulaga W, Rukambile E, Alders RG. The chicken or the egg? Exploring bi-directional associations between Newcastle disease vaccination and village chicken flock size in rural Tanzania. PLoS One 2017;12:e0188230. https://doi.org/10.1371/journal.pone.0188230

[18] WHO. Diarrhoeal disease (Fact sheet No. 330). 2013 [cited 2018 1/10]; Available from: http://www.who.int/mediacentre/factsheets/fs330/en/.

[19] WHO. World Health Organisation Multicentre Growth Reference Study Group. WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. Geneva: World Health Organization 2006 ed. Geneva: WHO 2006:312. https://www.who.int/childgrowth/standards/technical_report/en/

[20] StataCorp. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP. 14. 2 ed. Texas, USA: StataCorp 2015. http://www.stata.com

[21] R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing. 2019 [cited 2019 10/05]; Available from: https://www.R-project.org/.

[22] Kang Y, Aguayo VM, Campbell RK, Dzed L, Joshi V, Waid JL, Gupta SD Haselow NJ, West Jr. KP. Nutritional status and risk factors for stunting in preschool children in Bhutan. Matern Child Nutr 2018;14:e12653. https://doi.org/10.1111/mcn.12653

[23] Nshimyiryo A, Hedt-Gauthier B, Mutaganzwa C, Kirk CM, Beck K, Ndayisaba A, Mubiligi J, Kateera F, El-Khatib Z. Risk factors for stunting among children under five years: a cross-sectional population-based study in Rwanda using the 2015 Demographic and Health Survey. BMC Public Health 2019;19:175. https://doi.org/10.1186/s12889-019-6504-z

[24] Hong R, Banta JE, Betancourt JA. Relationship between household wealth inequality and chronic childhood under-nutrition in Bangladesh. Int J Equity Health 2006; 5: 15-.https://doi.org/10.1186/1475-9276-5-15

[25] Faye CM, Fonn S, Levin J. Factors associated with recovery from stunting among under-five children in two Nairobi informal settlements. PLoS One 2019;14:1-17. https://doi.org/10.1371/journal.pone.0215488

[26] Bbaale E. Determinants of diarrhoea and acute respiratory infec-

tion among under-fives in Uganda. Australas Med J 2011;4:400-9. https://doi.org/10.4066/AMJ.2011.723

[27] Ahmed SF, Farheen A, Muzaffar A, Mattoo GM. Prevalence of diarrhoeal disease, its seasonal and age variation in under- fives in Kashmir, India. Int J Health Sci 2008;2:126-33. https://doi.org/https://www.ncbi.nlm.nih.gov/pubmed/21475494

[28] Bessong PO, Odiyo JO, Musekene JN, Tessema A. Spatial distribution of diarrhoea and microbial quality of domestic water during anoutbreak of diarrhoea in the Tshikuwi Community in Venda, South Africa. J Health Popul Nutr 2009;27:652-9. https://doi.org/10.3329/jhpn.v27i5.3642

[29] Diouf K, Tabatabai P, Rudolph J, Marx M. Diarrhoea prevalence in children under five years of age in rural Burundi: an assessment of social and behavioural factors at the household level. Glob Health Action 2014;7:24895. https://doi.org/10.3402/gha.v7.24895

[30] Mengistie B, Berhane Y, Worku A. Household water chlorination reduces incidence of diarrhea among under-five children in rural Ethiopia: A cluster randomized controlled trial. PLoS One 2013;8:e77887. https://doi.org/10.1371/journal.pone.0077887

[31] Acharyaa D, Singhb JK, Adhikaric M, Gautamd S, Pandeye P, Dayalfa V. Association of water handling and child feeding practice with childhood diarrhoea in rural community of Southern Nepal. J Infect Public Health 2018;11:69-74. https://doi.org/10.1016/j.jiph.2017.04.007

[32] Kabinga FM, Mbewe A, Banda J. Environmental health factors associated with diarrhoea in the under five children in Mtendere, Lusaka, Zambia. Int J Infect Dis 2016;53:69. https://doi.org/10.1016/j.ijid.2016.11.173

[33] Kapwata T, Mathee A, le Roux WJ, Wright CY. Diarrhoeal disease in relation to possible household risk factors in South African villages. Int J Environ Res Public Health 2018;15:1665. https://doi.org/10.3390/ijerph15081665

[34] Siziya S, Muula AS, Rudatsikira E. Diarrhoea and acute respiratory infections prevalence and risk factors among under-five children in Iraq in 2000. Ital J Pediatr 2009;35:8. https://doi.org/10.1186/1824-7288-35-8

[35] Hadley C. Ethnic expansions and between-group differences in children's health: a case study from the Rukwa Valley, Tanzania. Am J Phys Anthropol 2005;128:682-92. https://doi.org/10.1002/ajpa.20056

[36] Hadley C, Mulder MB, Fitzherbert E. Seasonal food insecurity and perceived social support in rural Tanzania. Public Health Nutr 2007;10:544-51. https://doi.org/10.1017/S1368980007246725

[37] Islam MM, Sanin KI, Mahfuz M, Ahmed AMS, Mondal D, Haque R, Ahmed T. Risk factors of stunting among children living in an urban slum of Bangladesh: findings of a prospective cohort study. BMC Public Health 2018;18:197-. https://doi.org/10.1186/s12889-018-5101-x

[38] Angdembe MR, Dulal BP, Bhattarai K, Karn S. Trends and predictors of inequality in childhood stunting in Nepal from 1996 to 2016. Int J Equity Health 2019;18:42. https://doi.org/10.1186/s12939-019-0944-z

[39] Gatica-Domínguez G, Victora C, Barros AJD. Ethnic inequalities and trends in stunting prevalence among Guatemalan children: an analysis using national health surveys 1995-2014. Int J Equity Health 2019;18:110. https://doi.org/10.1186/s12939-019-1016-0

[40] Kabhele S, New-Aaron M, Kibusi SM, Gesase AP. Prevalence and factors associated with diarrhoea among children between 6 and 59 months of age in Mwanza City Tanzania. J Trop Pediatr 2018;64:523-30. https://doi.org/10.1093/tropej/fmx109

[41] Wasihun AG, Dejene TA, Teferi M, Marugán J, Negash L, Yemane D, McGuigan KG. Risk factors for diarrhoea and malnutrition among children under the age of 5 years in the Tigray Region of Northern Ethiopia. PLoS One 2018;13:e0207743. https://doi.org/10.1371/journal.pone.0207743

[42] Demirchyan A, Petrosyan V. Hand hygiene predicts stunting among rural children in Armenia: Anahit Demirchyan. Eur J Public Health 2017;27. https://doi.org/10.1093/eurpub/ckx186.287

[43] Mbuya MN, Humphrey JH. Preventing environmental enteric dysfunction through improved water, sanitation and hygiene: an opportunity for stunting reduction in developing countries. Matern Child Nutr 2016;12 Suppl 1:106-20. https://doi.org/10.1111/mcn.12220

[44] Maponga BA, Chirundu D, Gombe NT, Tshimanga M, Shambira G, Takundwa L. Risk factors for contracting watery diarrhoea in Kadoma City, Zimbabwe, 2011: a case control study. BMC Infect Dis 2013;13:567. https://doi.org/10.1186/1471-2334-13-567

[45] Hoque BA. Handwashing practices and challenges in Bangladesh. Int J Environ Health Res 2003;13:S81-S7. https://doi.org/10.1080/0960312031000102831

[46] Luby SP, Rahman M, Arnold BF, Unicomb L, Ashraf S, Winch PJ, Stewart CP, Begum F, Hussain F, Benjamin-Chung J, Leontsini E, Naser AM, Parvez SM, Hubbard AE, Lin A, Nizame FA, Jannat K, Ercumen A, Ram PK, Das KK, Abedin J, Clasen TF, Dewey KG, Fernald LC, Null C, diarrhoea and child growth in rural Bangladesh: a cluster randomised controlled trial. Lancet Glob Health 2018;6:e302-e15. https://doi.org/10.1016/S2214-109X(17)30490-4

[47] Pickering AJ, Djebbari H, Lopez C, Coulibaly M, Alzua ML. Effect of a community-led sanitation intervention on child diarrhoea and child growth in rural Mali: a cluster-randomised controlled trial. Lancet Glob Health 2015;3:e701-e11. https://doi.org/10.1016/S2214-109X(15)00144-8

[48] Gebreegziabher T, Regassa N. Ethiopia's high childhood undernutrition explained: analysis of the prevalence and key correlates based on recent nationally representative data. Public Health Nutr 2019;22:2099-109. https://doi.org/10.1017/s1368980019000569

[49] Chattopadhyay A, Sethi V, Nagargoje VP, Saraswat A, Surani N, Agarwal N, Bhatia V, Ruikar M, Bhattacharjee S, Parhi RN, Dar S, Daniel A, Sachdev HPS, Singh CM, Gope R, Nath V, Sareen N, De Wagt A, Unisa S. WASH practices and its association with nutritional status of adolescent girls in poverty pockets of eastern India. BMC Womens Health 2019;19:89. https://doi.org/10.1186/s12905-019-0787-1

[50] Behravesh CB, Brinson D, Hopkins BA, Gomez TM. Backyard poultry flocks and salmonellosis: A recurring, yet preventable public health challenge. Clin Infect Dis 2014;58:1432-8. https://doi.org/10.1093/cid/ciu067

[51] Gómara I, Miren, Kang G, Mammen A, Jana AK, Abraham M, Desselberger U, Brown D, Gray J. Characterization of G10P[11] Rotaviruses causing acute gastroenteritis in neonates and infants in Vellore, India. J Clin Microbiol 2004;42:2541. https://doi.org/10.1128/JCM.42.6.2541-2547.2004

[52] Luchs A, Timenetsky MTS. Unexpected detection of bovine G10 rotavirus in a Brazilian child with diarrhea. J Clin Virol 2014;59:74-6. https://doi.org/10.1016/j.jcv.2013.11.001

[53] Dione MM, Ikumapayi UN, Saha D, Mohammed NI, Geerts S, Ieven M, Adegbola RA, Antonio M. Clonal differences between non-typhoidal Salmonella (NTS) recovered from children and animals living in close contact in The Gambia. PLoS Negl Trop Dis 2011;5:e1148. https://doi.org/10.1371/journal.pntd.0001148

[54] Tun HM, Konya T, Takaro TK,. Brook JR, Chari R, Field CJ, Guttman DS, Becker AB, Mandhane PJ, Turvey SE, Subbarao P, Sears MR, Scott JA, Kozyrskyj AL, Sears MR, Subbarao P, Anand SS, Azad M, Becker AB, Befus AD, Brauer M, Brook JR, Chen E, Cyr M, Daley D, Dell S, Denburg JA, Duan Q, Eiwegger T, Grasemann H, HayGlass K, Hegele R, Holness DL, Hystad P, Kobor MS, Kollmann TR, Kozyrskyj AL, Laprise C, Lou WYW, Macri J, Mandhane PM,Miller G, Moraes T, Paré PD, Ramsey C, Ratjen F, Sandford A, Scott JA, Scott J, Silverman F, Simons E, Takaro T, Tebbutt S, To T. Turvey SE. Exposure to household furry pets influ-

ences the gut microbiota of infants at 3-4 months following various birth scenarios. Microbiome 2017;5:40. https://doi.org/10.1186/s40168-017-0254-x

[55] Passarelli s, Ambikapathi R, Gunaratna N, Madzorera I, Canavan C, Noor RA, Worku A, Berhane Y, Sibanda S, Sudfeld C, McConnell M, Fawzi W. Effects of chicken production on child nutrition and health outcomes Ethiopia. Annual Agriculture, Nutrition and Health (ANH) Academy Week. Hyderabad, India 2019.

[56] Contzen N, Meili IH, Mosler H-J. Changing handwashing behaviour in southern Ethiopia: a longitudinal study on infrastructural and commitment interventions. Soc Sci Med 2015;124:103-14. https://doi.org/10.1016/j.socscimed.2014.11.006

[57] Water Aid. wateraid Nepal's field experience in community-based resources management. field work paper. Kathmandu, Nepal: Water Aid 2008:28. https://www.wateraid.org/uk/search/wasearch

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Correspondence: Elpidius Rukambile, School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Australia - E-mail: erukambile@gmail.com

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ORIGINAL ARTICLE

Preferences of healthcare professionals regarding hexavalent pediatric vaccines in Italy: a survey of attitudes and expectations

GIANCARLO ICARDI[1,2], ANDREA ORSI[1,2], GIOVANNI VITALI ROSATI[3], ALESSIA TOGNETTO[4], GIOVANNI CHECCUCCI LISI[4], SALVATORE PARISI[4]

[1] Department of Health Sciences, University of Genoa, Italy; [2] Interuniversity Research Center on Influenza and Other Transmissible Infections (CIRI-IT), Genoa, Italy; [3] Italian Federation of Pediatricians FIMP, Family Pediatrician, Florence, Italy; [4] Sanofi Pasteur Medical Affairs, Italy

Keywords

Healthcare professionals • Hexavalent vaccine • Pediatric vaccination • Pre-filled syringe • Ready-to-use

Summary

Introduction. *In Italy, three hexavalent pediatric vaccines are available: two are ready-to-use (RTU) as pre-filled syringes, while the third must be reconstituted (need-for-reconstitution [NFR]). The formulation is related to the vaccination timing, safety of preparation and administration, and possible errors in immunization. We surveyed Italian healthcare professionals (HCPs) experienced with RTU and NFR vaccines in order to investigate their opinions on key aspects of the vaccines.*

Methods. *In Q1 2018, a qualitative study, ethnographic observations and in-depth interviews were performed in public vaccination settings of three Italian Regions. Data on how the vaccination process was managed and perceptions about the value of the RTU formulation were collected. In Q2 2018, face-to-face interviews were carried out to explore the attitude and preferences of Italian HCPs from nine Regions, assessing advantages and disadvantages of the two formulations from a quantitative point of view. In Q3-Q4 data analysis was carried out, using both qualitative and quantitative methodologies.*

Results. *The first phase demonstrated the following advantages of the RTU versus the NFR formulation: time-saving, lower probability of needle contamination and needle stick incidents, better handling, simpler procedure, easier disposal of waste. For the survey, 149 HCPs were interviewed; 80% and 40%, respectively, were very satisfied with the RTU and NFR vaccine.*

Conclusions. *Our study demonstrated that HCPs prefer the RTU formulation, as it simplifies vaccinations, reduces preparation time and minimizes the risk of errors. This formulation also saves time that can be spent on more in-depth counseling.*

Introduction

The development of combination vaccines can undoubtedly be considered an important innovation for the prevention of infectious disease that has led to enormous improvements on health, and has also brought economic benefits to healthcare systems [1]. Indeed, combined vaccines have played a central role in prophylaxis of the pediatric population from infectious diseases over the past decades. The availability of combination vaccines represents an important means of achieving successful protection against numerous pathogens simultaneously, and is associated with several advantages. By reducing the number of injections, a better compliance to the vaccination schedule and higher rates of coverage can be achieved, and a safer profile assured, since most adverse events reported after vaccination are related to the act of injection [2, 3].

Furthermore, in terms of healthcare service organizations, combination vaccines have been proven to improve the efficiency of the vaccination service, both for the healthcare professionals (HCPs) involved, namely physicians, nurses, and pediatricians, and for the organization itself. In fact, combination vaccines save HCPs time during vaccine preparation [4], reduce administration costs, minimize storage space needed and reduce waste [3, 5]. Depending on the practice of vaccination in terms of the number and role of HCPs involved, the impact of using combination vaccines can be very relevant, especially in situations of personnel constrains, which are common nowadays, as well as in crowded pediatric vaccination schedules, as already implemented in many high-income countries [6, 7].

Currently, several pediatric combination vaccines are available. Among these, hexavalent vaccines represent the most innovative formulation to protect babies against six diseases: diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis, and infection from *Haemophilus influenzae* type b. In the European Region, three hexavalent vaccines are authorized by the European Medicines Agency: Infanrix Hexa®, available since 2000 [8]; Hexyon®, available since 2013 [9]; and Vaxelis®, available since 2017 [10]. These three hexavalent vaccines have the same indication of use, including immunization against the six diseases and age of utilization, as described in their Summary of Product Characteristics (SmPC) [8-10]. Although a maximum age limit of use is not indicated for any of them, the fact that they contain a "pediatric" dose of antigens, make them recommended up to 7 years of age by health authorities and scientific

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societies in several countries [1]. Safety, immunogenicity and effectiveness of hexavalent vaccines is described in each SmPC and confirmed in several studies and clinical trials [1, 11-13]. Beyond indications, the main difference among the hexavalent vaccines is in regards to the preparation that is required for their administration: both Hexyon® and Vaxelis® are ready-to-use (RTU) in a pre-filled syringe, whereas for Infanrix Hexa® there is a need-for-reconstitution (NFR) of the Hib antigen with a syringe containing the five other components.
Preference for an RTU or NFR vaccine may be related to several factors, such as the preparation time required, the possibility to reduce mishandlings and dosage errors, cost, vaccination waste, the organization of the vaccination services in terms of time set for each vaccination, and to the characteristics of packaging that render the vaccine easier to integrate within existing databases. Moreover, individual experience and preferences of HCPs for a specific hexavalent vaccine may also dictate the selection of an RTU or NFR vaccine. Notably, it has been demonstrated that both physicians and nurses tend to prefer vaccines that require less time to prepare and manage [14]. As a consequence, the time saved may be spent on streamlining the vaccination session and providing parents with a more detailed vaccination counselling [15]. In addition, it has been reported that the higher acquisition costs of RTU vaccines are counterbalanced by lower administrative costs and increased safety compared with single-dose and multi-dose vial vaccines [16, 17].
In Italy, pediatric vaccinations are delivered by the public health sector, either in vaccination centers or in family pediatricians' medical offices. In vaccination centers, public health physicians (also defined as hygienists) are those medical doctor specialists who are in charge of vaccines in vaccination centers, from the organizational and practical point of view.
Within Italy, each Region runs independent tenders that are driven by price and/or scientific criteria, while product technical criteria are usually not taken into account in the assessment. To date, there remains limited data on the opinion of HCPs regarding technical aspects related to vaccination. To gain more insight into the opinions of HCPs on key aspects of the vaccination process, as well as on preferences for hexavalent vaccines, we carried out a survey of HCPs experienced in pediatric vaccinations, working in nine Italian Regions that differ by the organizational models of the vaccination services. Our survey investigated preferences and critical issues reported by the HCPs, in order to obtain information that may be useful for optimizing pediatric vaccinations in the public setting.

Methods

Qualitative phase

In Q1 2018 an experienced researcher performed ethnographic observations followed by in-depth interviews in public vaccination settings (vaccination centers and family pediatricians' offices) of three Italian Regions: in Liguria, with 6 HCPs (3 hygienists and 3 nurses) where the NFR hexavalent vaccine is used; in Apulia with 3 nurses and in Tuscany with 3 primary care pediatricians, where the RTU hexavalent vaccine is used. In general, all HCPs were experienced with both NFR and RTU formulations that are commonly available in Italy. The main purpose of the ethnographic observation was to understand how the vaccination process was managed in different Regions, in terms of HCPs involved and their role in the vaccination process.
The purpose of the subsequent interviews was to highlight and discuss critical issues emerging from the daily routine vaccination process, investigating the overall image of the hexavalent vaccine (safety and tolerability), and the value of the RTU formulation.

Quantitative phase: survey target

In Q2 2018, personal in-depth interviews were carried out by inviting 265 HCPs (hygienists, nurses, and family pediatricians) from nine Italian Regions covering the north, center, and south of the country (Liguria, Lombardy, Piemonte, Emilia Romagna, Tuscany, Calabria, Campania, Apulia and Sicily). In these Regions, three hexavalent vaccines are used, including both RTU and NFR vaccines.
Invited participants were selected through a purposive sampling methodology among those professionals that are in charge of the hexavalent pediatric vaccination at regional vaccination centers or as family pediatricians. The inclusion criteria for the HCPs to be interviewed were: a minimum of 10 years of experience in pediatric vaccinations and a minimum of 200 children under 2 years of age vaccinated monthly in vaccination centers or around 50 children under 2 years of age vaccinated monthly for family pediatricians.

Quantitative phase: survey characteristics

The survey consisted of 46 questions, requiring approximately 20 minutes for its completion (questionnaire in Annex 1). Computer-assisted interviews were conducted in person by an experienced interviewer and the anonymity of the results were assured before starting the interview. The overall objective was to identify the attributes of vaccination devices that may be valuable for HCPs and to evaluate advantages and disadvantages of the RTU formulation compared with the NFR formulation.
Firstly, demographic and professional data were collected including: region where HCPs work, gender, age, profession, years of experience in administering vaccination, number of children under 2 years of age vaccinated in a typical month (either in vaccination centers or with family pediatricians), number of children under 2 years of age vaccinated with hexavalent vaccines, and typology of the hexavalent vaccine used.
In order to investigate the daily practice of HCPs working in vaccination centers, where hygienists and nurses work together, the following data were collected: time and number of HCPs dedicated to vaccinations and ac-

tivities that each of the two professional categories mostly deal with.
With the aim of assessing perceptions and satisfaction towards hexavalent vaccines, participants were asked to describe: their individual experience while preparing and administering hexavalent vaccines to children, the attributes they consider more valuable for a hexavalent device, and the time dedicated to the various phases of the vaccination session (counselling, vaccine preparation, vaccine administration).
Lastly, the survey asked the participants to indicate which one of the two hexavalent formulations, RTU and NFR, had certain characteristics related to the ease and safety in the preparation, administration, and disposal of the vaccine.
The satisfaction and agreement of HCPs with the proposed statements were measured on a 1-10 scale (8-10 indicating high satisfaction/agreement).
Descriptive statistics were used to analyze and present results.

Results

Qualitative phase

In the Liguria region, the observed vaccination staff included 2 HCPs: one hygienist and one nurse (dedicated or working mainly in other specialties). It was observed that when the nurse was dedicated, the role of the hygienist and of the nurse were interchangeable, while when the nurse was "rented" temporarily from another unit, the nurse prepared the vaccine but vaccine administration and family counselling were managed by the hygienist.
In Apulia, the vaccination staff included 2 or 3 HCPs: one hygienist and one to two nurses (one in small towns, two in the cities). It was observed that in this setting the nurse played a major role in the vaccination process, being involved in all phases from ordering to administration to disposal of the vaccine. The hygienist was in charge of checking the child's record on the database, their vaccination history, their clinical history (filled in by the parents), and scheduling the following vaccination appointment.
Considering the time and the professional figures dedicated to vaccinations in vaccination centers, the respondents working in this setting declared that approximately 4 hours for 4 days were dedicated to the vaccination of children under 2 years of age, with 2 hygienists and 3 nurses dedicated to vaccination activities only.
In Tuscany, following a recent agreement with the Regional Health Authority, pediatric vaccinations have been shifted to family paediatricians, who also provide hexavalent vaccination in their practice.
As a result of the interviews, 6 HCPs (3 hygienists and 3 nurses) were interviewed in Liguria, 3 nurses in Apulia and 3 family pediatricians in Tuscany (Tab. I).
The hexavalent vaccine showed a positive image across the board: it was perceived as safe and with a good level of tolerability. Moreover, although on a practical point of view vaccination is considered easy and simple to manage for the HCP, on a more emotional level, vaccine administration often becomes a potentially anxious moment for the family. As a consequence, the need for family counselling when administering the first dose of hexavalent vaccination emerged strongly and was across all Regions. The value of the RTU formulation emerged clearly, across both target and geographic areas: its value was spontaneously recognized, by users of both RTU and NFR vaccines. The advantages of the RTU formulation that emerged compared with the NFR formulation can be ranked as follows (from more relevant to less relevant): time-saving, better safety profile, better handling, simpler procedure, easier disposal of waste, more convenient set of needles.
These results were considered as preliminary and were further tested during the survey phase.

Quantitative phase

In the quantitative phase, face-to-face computer-assisted personal interviews were carried out with 149 out of the 265 (56.2%) invited HCPs from the nine selected Italian Regions. Among the respondents, 60 were hygienists, 59 were nurses working in vaccination centers, and 30 were family pediatricians; 66% were female and the overall mean age was 55 years (58 years for hygienists, 51 years for nurses, and 63 years for pediatricians). The overall average number of years spent in vaccination activities was 15 years (18, 13, and 12 years, respectively, for hygienists, nurses and pediatricians). The sociodemographic and professional data of the survey participants are described in Table II.
Among the HCPs, 84 (56%) used the RTU hexavalent vaccine and 65 (44%) used the NFR one.
The activities in which HCPs reported being mostly involved varied amongst the professional category: talking to parents and collecting the medical history of the child were activities that hygienists mostly deal with, while nurses were in charge of preparing the vaccines and the room, taking inventory and orders, managing the stock, scheduling appointments and disposing of the waste materials. Pediatricians spent more time counselling (an average of 11 minutes) compared with hygienists (10 minutes) and nurses (8 minutes).

Tab. I. Qualitative phase: methodology used.

Region (hexavalent vaccine in use)	Ethnographic observation	Interviews
Liguria (NFR)	Vaccination center (2 days observation)	6 HCPs (3 Hygienists + 3 Nurses)
Apulia (RTU)	Vaccination center (2 days observation)	3 HCPs (3 Nurses)
Tuscany (RTU)	-	3 HCPs (3 Pediatricians)

Abbreviations: HCPs, healthcare professionals; NFR, need-for-reconstitution; RTU, ready-to-use.

Tab. II. Quantitative phase: demographic and professional characteristics of healthcare professionals.

	Hygienists in vaccination centers (n = 60)	Nurses (n = 59)	Pediatricians (n = 30)	Total (n = 149)
Female, n (%)	28	55	15	98 (66%)
Male, n (%)	32	4	15	51 (34%)
Age, mean (yrs)	58	51	56	55
Region				
Calabria	5	5	-	10
Campania	8	8	-	16
Emilia Romagna	6	6	-	12
Liguria	5	5	-	10
Lombardia	8	8	-	16
Piemonte	7	7	-	14
Apulia	11	10	-	21
Sicily	10	10	-	20
Tuscany	-	-	30	30
Experience with vaccinations, mean (yrs)	18	13	12	15
Approximate number of children < 2 yrs vaccinated monthly, n	229	210	48	185
Approximate number of children < 2 yrs vaccinated monthly with the hexavalent vaccine, n (% of total vaccinations)	165 (72%)	126 (60%)	28 (58%)	123 (66%)

N: number; yrs: years.

Assessment of hexavalent vaccines

As for the time spent during vaccination, HCPs answered that out of an average of 17 minutes requested for each vaccination, more than half (approximately 10 minutes) was spent explaining the hexavalent vaccine and vaccination process to the parents. Vaccine preparation required an average of 3 minutes, 2 minutes were spent administering the vaccine, and 2 minutes for disposal of waste materials.

Regarding hexavalent vaccination sessions, most HCPs (83.2% of the target pediatricians, 90.2% of the hygienists, and 97.2% of the nurses) expressed an 8-10 rate of agreement (very or mostly) with the declaration that giving information regarding vaccination/vaccines to parents was very demanding and time-consuming. As for managing and administrating the vaccine, 27.4% of hygienists, 29.4% of nurses, and 47.4% of pediatricians expressed an 8-10 rate of agreement (very or mostly) with the possibility of making errors during the vaccine preparation; 20.5% of hygienists, 22.5% of nurses, and 40.5% of pediatricians expressed a high rate of agreement (very or mostly) with the possibility of making errors during the vaccine administration; 18.6% of hygienists, 20.6% of nurses, and 33.6% of pediatricians very/mostly agreed that it could be possible to forget the reconstitution of the vaccine.

Key aspects of the hexavalent vaccines rated as "very important" were: minimizing the risk of needle contamination (80% of all respondent HCPs) and of needle stick injuries (79% of HCPs), being stable in case of problems of the cold chain (78% of HCPs), having low risk of errors in the reconstitution (78% of HCPs), being easy to prepare and to manage (74% of HCPs), and being ready to use (66% of HCPs). These last two aspects were particularly important for pediatricians.

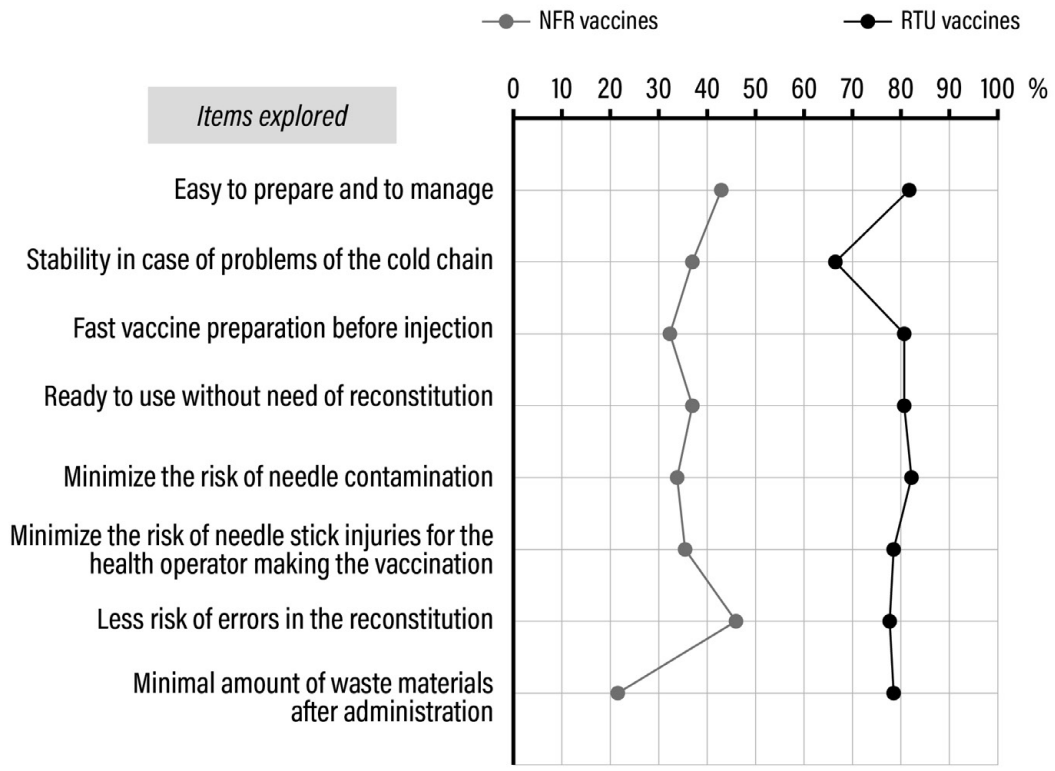
RTU vs NFR vaccines

As for the overall comparison between RTU and NFR hexavalent formulations, 80% of HCPs declared their satisfaction with the advantages of RTU hexavalent vaccines was "very good": easy preparation and administration, no risk to reconstitute, low risk of needle contamination and stick injuries. On the other hand, only 40% of HCPs declared they were satisfied by the NFR formulation to a level of "very good", due to more manipulations, higher risk of needle contamination and stick injuries (Fig. 1). Figures 2 and 3 describe in detail the assessment of the two formulations, as rated by HCPs.

As for safety issues related to the different syringe formulations, HCPs declared to be overall satisfied with the safety of hexavalent vaccines (49% very satisfied and 40% mostly satisfied), but a difference appeared between the two formulations with 61% of HCPs very satisfied with RTU overall syringe safety compared with only 34% of HCPs being very satisfied with NFR overall syringe safety (Fig. 4).

Lastly, when asked how much the use of an RTU vaccine could facilitate when vaccinating children under the age of 2 years, 92% (from 90% of hygienists to 93% of both nurses and pediatricians) expressed a score of 8-10 (indicating high satisfaction/agreement). Moreover, HCPs declared that the time saved in preparation of RTU vaccines can be more effectively spent on vaccination counselling during the visit.

Fig. 1. Perceived advantages of ready-to-use (RTU) vaccines over need-for-reconstitution (NFR) vaccines.



Question: *However, how do you consider your satisfaction ...for each of the following items?*
Answer reported: percentage of HCPs declaring that their satisfaction with the specific item was "very good"

Fig. 2. Assessment of the ready-to-use (RTU) formulation as rated by healthcare professionals.

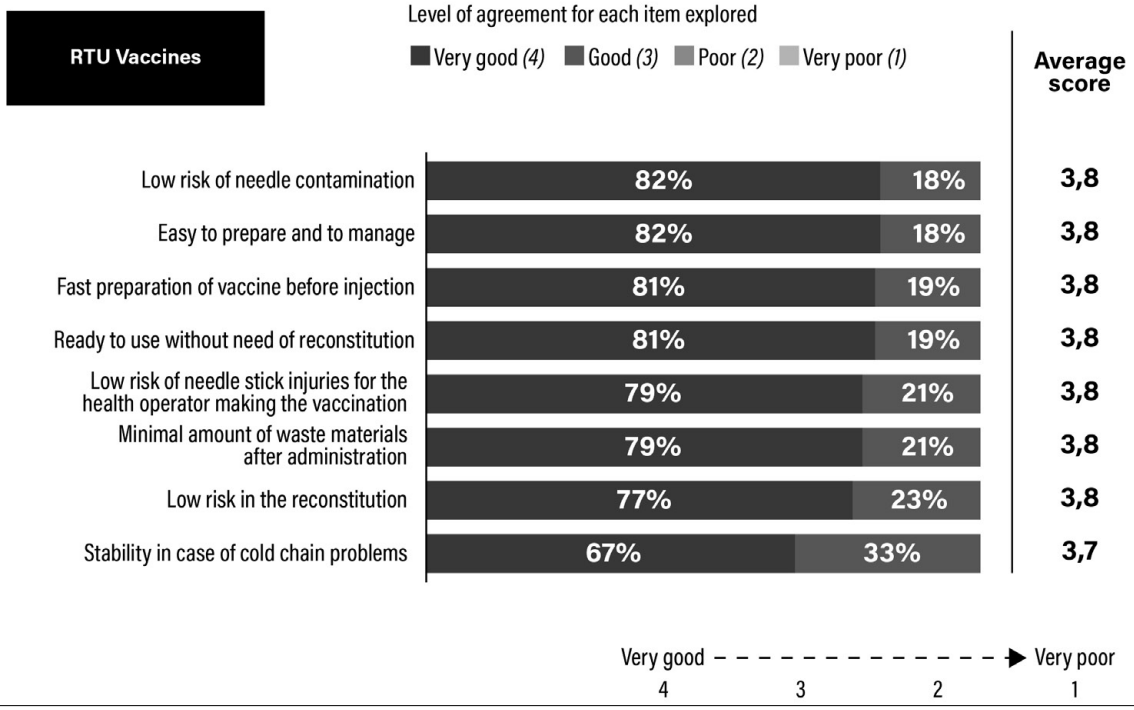


Fig. 3. Assessment of the need-for-reconstitution (NFR) formulation as rated by healthcare professionals.

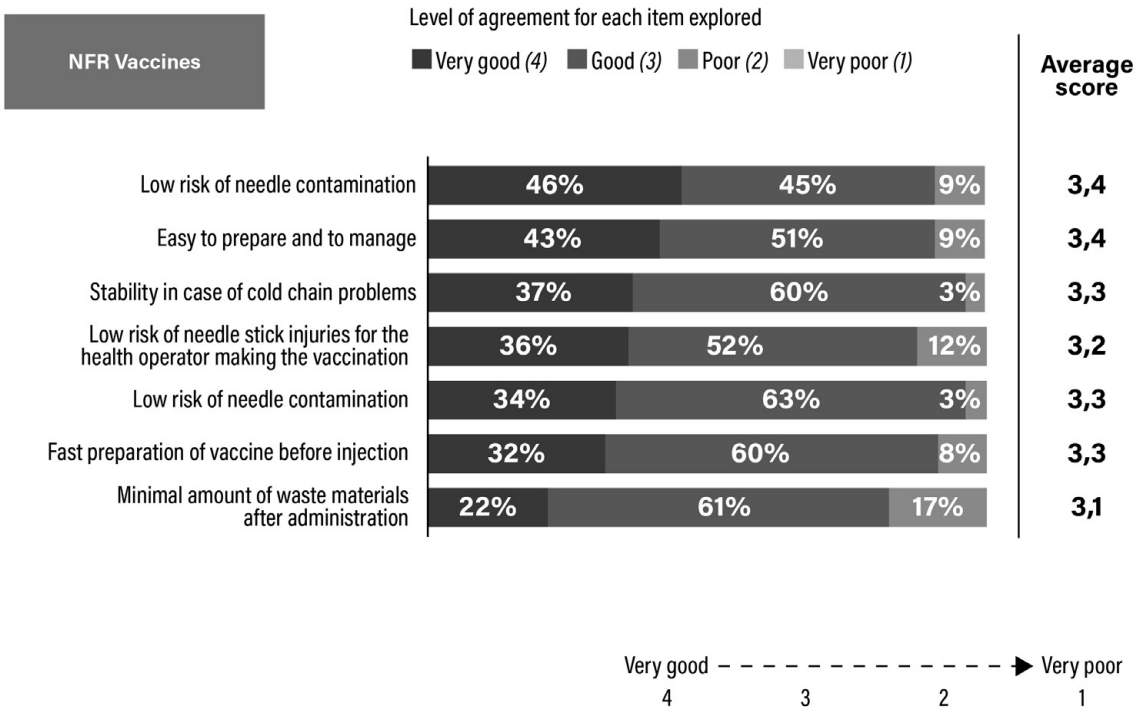
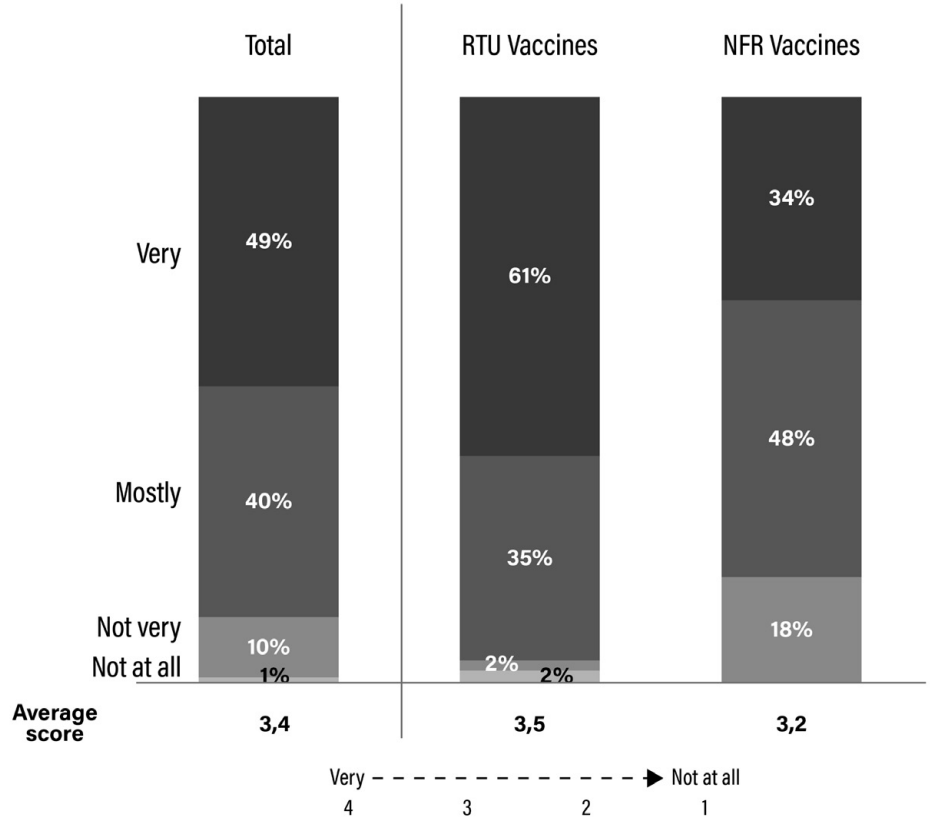


Fig. 4. Ready-to-use (RTU) versus need-for-reconstitution (NFR) vaccines: assessment of safety.



Discussion

This survey focused on relevant aspects of the hexavalent vaccines, such as handling, time needed for the different phases of vaccination sessions, errors and safety related to the formulation, with a comparison between RTU and NFR vaccines. Issues related to the safety or immunogenicity of hexavalent vaccines were not our objective because these aspects are already well documented and considered similar [18].

According to the inclusion criteria, vaccination centers and family pediatricians, respectively, had to vaccinate a minimum of 200 children and around 50 children under the age of 2 years each month. Of these, more than two-thirds were administered a hexavalent vaccine. Thus, the surveys respondents' long-standing knowledge of the issues involved in vaccinations constitutes a reasonable guarantee of validity in the assessment of hexavalent vaccines.

For Italian family pediatricians, vaccination is not a routine activity in their daily practice, but we chose to include this category as the Tuscany region has recently stated that family pediatricians should administer hexavalent vaccines in their medical offices, and this practice could be soon adopted by the other Italian Regions as a measure to increase coverage rates. In this regard, it has been demonstrated that physicians' recommendation is an important predictor of vaccine acceptance, constituting a major factor in receiving or intending to receive any vaccine [19]. For this reason, the involvement of all HCPs in our survey resulted essential to identify critical issues and thus highlight potential areas for additional intervention targeted at specific professional categories. Family pediatricians work autonomously in their office, thus being in charge of all the different phases of vaccine administration. As a consequence, as emerged in our study, they are able to perform only a limited number of vaccinations per month (i.e., 48 vaccinations to children < 2 years of age) and appeared more concerned about making errors during preparation, administration and reconstitution of the hexavalent vaccine compared with other HCPs. As is known in the literature, pediatricians can have a key role in increasing awareness about the benefits of pediatric vaccinations and educating parents [20]: in our study, pediatricians spent more time counselling than hygienists and nurses.

For all these reasons, an RTU formulation may be preferable, for all HCPs, and in particular for pediatricians, as it was demonstrated to render all processes not only easier and safer, but also more rapid. Similarly, our research demonstrated that RTU formulation of hexavalent vaccines was widely preferred to NFR vaccines among all HCPs because it simplified the preparation, minimized the number of manipulations and error risks: in fact, 80% of HCPs declared they were very satisfied with RTU vaccines compared with only 40% of HCPs who were very satisfied with NFR. The perceived benefits of an RTU vaccine included easier and quicker preparation with less risk of errors such as the risk of forgetting to reconstitute the Hib or not taking all the Hib antigen from the vial. It was also seen to minimize the risk of needle contamination and needle stick injury and to produce less waste material.

Although we should consider that previous published studies used different definitions in vaccine preparation time, as well as different methodologies for data collection and analysis, we can say that our results are in line with the existing literature. In fact, handling, dosage errors, and reduced preparation time were all highlighted as being important attributes of a fully-liquid RTU vaccine versus one that requires reconstitution in a previous survey of physicians and nurses conducted in Germany on hexavalent pediatric vaccines [14]. In particular, both the present and previous studies highlighted that HCPs are concerned about minimizing the risk of errors during vaccination, which may thus be reduced by using a fully-liquid hexavalent vaccine [4, 14, 21]. In fact, in a time and motion study, comparing RTU versus non-fully liquid vaccines showed that mishandlings were five times more common with a NFR hexavalent vaccine compared with the RTU vaccine [4]. In our study, 77% of HCPs rated as "very good" the low risk of errors in the reconstitution for RTU vaccines versus 46% for the NFR formulation. In addition to the reduced risk of error, it was reported that an RTU hexavalent vaccine can be prepared in less than half the time needed to prepare a NFR vaccine [4, 15]. Using the time difference of 35 seconds that was observed in the study of De Coster and colleagues for a HCP to prepare an RTU hexavalent vaccine versus a NFR vaccine, we can estimate the number of hours per year that are saved due to the simpler and quicker process of the RTU formulation. We applied these data to the Italian context, using hexavalent vaccination coverage (95%) of the birth cohort (440,000 newborns in 2018) and number of doses of hexavalent to be administered in the pediatric recommended schedule (3 doses, 2+1 schedule). We estimated approximately 12,000 hours saved/year, that correspond approximately to the workload of 7 HCPs working in public settings, that could therefore be re-allocated to other tasks or units, if a broader healthcare service perspective is used, with a potential saving for the public organization. Time saved is a significant aspect considering that the HCPs involved in our study devoted a substantial amount of time to vaccinations (approximately 17 minutes per vaccination), a large part of which was dedicated to informing and educating parents (around 10 minutes). Therefore, time saved in the act of preparing and administering the vaccine could be used in a more productive way with parents and the baby.

Our study is limited by the generalizability of our results. In fact, the purposive sampling methodology adopted to select the HCPs and the Regions involved in the two phases of the study may reduce the representativeness of our results. Moreover, our results may not generalize appropriately to other countries, due to potential differences in the organization of vaccination programs and cultural preferences for specific pharmaceutical forms. On the other hand, this study represents one of the very few evidences that support the switch

from NFR to RTU vaccines, taking in consideration HCPs preferences, as well as time saved, simplification of vaccine preparation and management, as is already known in the literature. The extension of this work to a larger sample and to other contexts could confirm our findings.

Conclusions

The present study has highlighted aspects that are important for HCPs when considering a hexavalent vaccine. We observed that a vaccine that can reduce the time needed for preparation, while reducing the risk of errors as much as possible, is preferred by HCPs. Accordingly, easy-to-use, fully liquid vaccines are desirable, and fully liquid, hexavalent vaccines in pre-filled syringes have many characteristics that HCPs value as important. An RTU vaccine minimizes the risk of errors, and especially the risk of forgetting to reconstitute the powder in the main syringe or reconstituting all the powder. RTU vaccines also reduce the risk of needle contamination and needle stick injuries as only one needle is used. The advantages in terms of time saving are clear as less time is needed for vaccine preparation and administration, which allows more time for counselling by the single HCP or can allow re-allocation to other tasks or units if a broader healthcare service perspective is used. Therefore, in comparable contexts of immunogenicity, tolerability and safety, it would thus seem likely that RTU vaccines present satisfactory characteristics over NFR vaccines. We also envisage that these technical aspects will be taken into account by regional decision makers in deciding to adopt one or another typology of vaccine.

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Conflict of interest statement

All Authors have participated in advisory boards or expert meetings or were speakers or organizers of congresses/conferences on hexavalent vaccines sponsored by GlaxoSmithKline Biologicals SA, Sanofi Pasteur-MSD, MSD or Sanofi Pasteur.

Authors' contributions

All Authors made a substantial contribution to the interpretation of data, read and approved the final manuscript.

References

[1] Orsi A, Azzari C, Bozzola E, Chiamenti G, Chirico G, Esposito S, Francia F, Lopalco P, Prato R, Russo R, Villani A, Franco E. Hexavalent vaccines: characteristics of available products and practical considerations from a panel of Italian experts. J Prev Med Hyg 2018;59:E107-E19.

[2] Decker MD. Principles of pediatric combination vaccines and practical issues related to use in clinical practice. Pediatr Infect Dis J 2001;20:S10-8. https://doi.org/10.1097/00006454-200111001-00002

[3] Skibinski DA, Baudner BC, Singh M, O'Hagan DT. Combination vaccines. J Glob Infect Dis 2011;3:63-72. https://doi.org/10.4103/0974-777X.77298

[4] De Coster I, Fournie X, Faure C, Ziani E, Nicolas L, Soubeyrand B, Van Damme P. Assessment of preparation time with fully-liquid versus non-fully liquid paediatric hexavalent vaccines. A time and motion study. Vaccine 2015;33:3976-82. https://doi.org/10.1016/j.vaccine.2015.06.030

[5] Maman K, Zollner Y, Greco D, Duru G, Sendyona S, Remy V. The value of childhood combination vaccines: From beliefs to evidence. Hum Vaccin Immunother 2015;11:2132-41. https://doi.org/10.1080/21645515.2015.1044180

[6] Bozzola E, Spina G, Russo R, Bozzola M, Corsello G, Villani A. Mandatory vaccinations in European countries, undocumented information, false news and the impact on vaccination uptake: the position of the Italian pediatric society. Ital J Pediatr 2018;44:67. https://doi.org/10.1186/s13052-018-0504-y

[7] World Health Organization (WHO). WHO vaccine-preventable diseases: monitoring system. 2019 global summary. Available at: http://apps.who.int/immunization_monitoring/globalsummary/schedules. Accessed on 23/03/2020.

[8] European Medicines Agency. Infanrix Hexa. Summary of Product Characteristics. Available at: https://www.ema.europa.eu/en/medicines/human/EPAR/infanrix-hexa. Accessed on 23/03/2020.

[9] European Medicines Agency. Hexyon. Summary of Product Characteristics. Available at: https://www.ema.europa.eu/en/medicines/human/EPAR/hexyon. Accessed on 23/03/2020.

[10] European Medicines Agency. Vaxelis. Summary of Product Characteristics. Available at: https://www.ema.europa.eu/en/medicines/human/EPAR/vaxelis. Accessed on 23/03/2020.

[11] Petrolini C, Chiappini E, Caffarelli C, Calvani M, Cardinale F, Duse M, Licari A, Manti S, Martelli A, Minasi D, Del Giudice MM, Pajno GB, Pietrasanta C, Pugni L, Tosca M, Mosca F, Marseglia GL. Vaccinazione esavalente nei nati pretermine. Rivista di Immunologia e Allergologia Pediatrica 2019;3-2019:7-23.

[12] Silfverdal SA, Icardi G, Vesikari T, Flores SA, Pagnoni MF, Xu J, Liu GF, Stek JE, Boisnard F, Thomas S, Ziani E, Lee AW. A Phase III randomized, double-blind, clinical trial of an investigational hexavalent vaccine given at 2, 4, and 11-12 months. Vaccine 2016;34:3810-6. https://doi.org/10.1016/j.vaccine.2016.05.054

[13] Vesikari T, Silfverdal SA, Jordanov E, Feroldi E. A Randomized, Controlled Study of DTaP-IPV-HB-PRP-T, a Fully Liquid Hexavalent Vaccine, Administered in a 3-, 5- and 11- to 12-month Schedule. Pediatr Infect Dis J 2017;36:87-93. https://doi.org/10.1097/INF.0000000000001358

[14] Lloyd AJ, Nafees B, Ziani E, Nicolas L, Fordham BA, Soubeyrand B, Bornhoft C. What are the preferences of health care professionals in Germany regarding fully liquid, ready-to-use hexavalent pedatric vaccine versus hexavalent pediatric vaccine that needs reconstitution? Patient Prefer Adherence 2015;9:1517-24. https://doi.org/10.2147/PPA.S87229

[15] Wiedenmayer KA, Weiss S, Chattopadhyay C, Mukherjee A, Kundu R, Aye R, Tediosi F, Hetzel MW, Tanner M. Simplifying paediatric immunization with a fully liquid DTP-HepB-Hib combination vaccine: evidence from a comparative time-motion

study in India. Vaccine 2009;27:655-9. https://doi.org/10.1016/j.vaccine.2008.11.045

[16] Nogier C, Hanlon P, Wiedenmayer K, Maire N. Can a Compact Pre-Filled Auto-Disable Injection System (cPAD) Save Costs for DTP-HepB-Hib Vaccine as Compared with Single-Dose (SDV) and Multi-Dose Vials (MDV)? Evidence from Cambodia, Ghana, and Peru. Drugs Real World Outcomes 2015;2:43-52. https://doi.org/10.1007/s40801-015-0010-0

[17] Pereira CC, Bishai D. Vaccine presentation in the USA: economics of prefilled syringes versus multidose vials for influenza vaccination. Expert Rev Vaccines 2010;9:1343-9. https://doi.org/10.1586/erv.10.129

[18] McCormack PL. DTaP-IPV-Hep B-Hib vaccine (Hexaxim(R)): a review of its use in primary and booster vaccination. Paediatr Drugs 2013;15:59-70. https://doi.org/10.1007/s40272-013-0007-7

[19] Gargano LM, Herbert NL, Painter JE, Sales JM, Morfaw C, Rask K, Murray D, DiClemente RJ, Hughes JM. Impact of a physician recommendation and parental immunization attitudes on receipt or intention to receive adolescent vaccines. Hum Vaccin Immunother 2013;9:2627-33. https://doi.org/10.4161/hv.25823

[20] Ferrara P, Zenzeri L, Fabrizio GC, Gatto A, Pio L, Gargiullo L, Ianniello F, Valentini P, Ranno O. Second-generation immigrant children: health prevention for a new population in terms of vaccination coverage and health assessment. Minerva Pediatr 2016;68:121-6.

[21] Sharp B, Whyte P. New directions in the development of prefilled syringes. Innov Pharm Technol 2010:64-8. Available at: http://iptonline.com/articles/public/p-8%20non-print.pdf. Accessed on 3/03/2020

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Correspondence: Salvatore Parisi, Sanofi Pasteur Medical Affairs, Rome, Italy, viale Bodio 37/b, 20158 Milan, Italy - E-mail: salvatore.parisi@sanofi.com

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Annex 1

Questionnaire
Paediatric Vaccination
36598

Length of interview: 20 minutes
Start fieldwork:
End fieldwork:

I. SAMPLE VARIABLES

- Region (label S01) [S]
- Target (label TARGET) [S]

II. QUOTA CHECK BASED ON SAMPLE VARIABLES

Total net n=150	Doctors of the vaccination centers	Nurses	Pediatricians
Toscana			***30***
Puglia	***10***	***10***	*-*
Lombardia	***5***	***5***	*-*
Campania	***5***	***5***	*-*
Liguria	***5***	***5***	*-*
Calabria	***5***	***5***	*-*
Sicilia	***10***	***10***	*-*
Piemonte	***10***	***10***	*-*
Emilia Romagna	***10***	***10***	*-*

III. INTRODUCTION

Good morning, the Healthcare Department of Gfk Italy Company is conducting a survey on pediatric vaccinations.
We would like to ask your willingness to cooperate with this survey. The interview will take about 20 minutes. Everything you say will be treated anonymously, with the utmost confidentiality and for statistical purposes only. Thanks for collaboration. (Privacy Law)

PHARMACOVIGILANCE

Adverse events/exposure to the drug during pregnancy/complaints about the product.
We are now being asked, as a company operating on marketing research, to pass on to PV services details on any adverse events, including exposure to drug during pregnancy or breast-feeding, suspected transmission of infectious agents, technical/qualitative issues,drug interactions and particular situations such as overdose, abuse, improper use, administration errors, drug prescription errors, occupational exposure and lack of effectiveness that are mentioned during the discussion in relation to a product of the Company who commissioned the survey.
Although what you say will, of course, be treated in confidence,should you mention during the discussion any adverse event (or any of the situations above described) happened in a specific patients, we will need to report even in the case you just reported it directly to the company or to the Italian bodies in charge of this (we remind you that you can report using the AIFA web site http://www.agenziafarmaco.gov.it/it/content/modalit%C3%A0-di-segnalazione-delle-sospette-reazioni-avverse-ai-medicinali).
In this situation you will be asked if you will be willing to waive the confidentiality given to you under the Codes of conduct specifically in relation to that adverse event/drug exposure during pregnancy or brest feeding/complaint about the product . All the other information that you will give during the interview will stay confidential .

PV_1 [S]
Are you willing to make this interview on the base of these premises?

1. Yes
2. No

PV_2 [O]
Name and full address:

If you are not willing to provide your name and full address, the communication to the PV service will be done anonymously, without indicating your name and personal data .

IV. SCREENER

Base: all respondents

S01 [S]
In which region do you work?
1. Calabria
2. Campania
3. Emilia Romagna
4. Liguria
5. Lombardia
6. Piemonte
7. Puglia
8. Sicilia
9. Toscana
98. Other region

SCRIPTER: all respondents with answer S01=98 go to the end of the questionnaire (SCREENOUT)

And in which province?

And in which municipality?

SCRIPTER: insert istat2016 folder

Base: S01=1-8

All regions except Tuscany

S02 [S]
In your clinical practice, do you work as …?

1. Nurse/health worker
2. Hygienist physician
3. Occupational physician
4. Pediatrician
98. Other medical specialization (specify) [O]

Base: S01=9

If Tuscany

S03 [S]
In your clinical practice, do you work as pediatrician?

1. Yes
2. No

If no close
SCRIPTER: all respondents with answer S03=2 go to the end of the questionnaire (SCREENOUT)

Base: all respondents

Create HIDDEN VARIABLE

TARGET

1. Vaccination centers phisicians = S02= 2-4 or 98
2. Vaccintionl centers nurses/health workers = S02=1
3. Pediatricians = S03=1

Base: TARGET=1-2

If physicians/nurses/health workers of the vaccination centers

S04 [Q]
Let's talk about the vaccinations administered to children under 2 years.
In a typical month, how many children under the age of 2 years are vaccinated in your center?

No. of children under 2 years vaccinated in a month

SCRIPTER: min.=0, max.=999

If none, close
SCRIPTER: all respondents with answer S04=0 go to the end of the questionnaire (SCREENOUT)

Base: TARGET=1-2

If physicians/nurses/health workers of the vaccination centers

S05 [Q]
Among the … (*SCRIPTER: show the answer at S04*) children under 2 years vaccinated in a month, how many of them have been administered with the hexavalent vaccine(diphtheria, tetanus, pertussis, polio, hepatitis B haemofilus influenzae type b)?

No. of children who are given a hexavalent vaccine/month

SCRIPTER: min.=0, max.=999
S04<=S03

If none, close
SCRIPTER: all respondents with answer S05=0 go to the end of the questionnaire (SCREENOUT)

Base: TARGET=1-2

If physicians/nurses/health workers of the vaccination centers

S06 [Q]
In your centers, in a typical week, how many days of the week (even if not whole) are dedicated to vaccinations of children under 2 years?

No. of days of the week dedicated to vaccinations of children under 2 years

SCRIPTER: min.=1, max.=6

Base: TARGET=1-2

If physicians/nurses/health workers of the vaccination centers

S07 [Q]
In a week, in total, how many hours are spent on vaccinations for children under 2 years?

No. of hours/week dedicated to vaccinations of children under 2 years

SCRIPTER: min.=1, max.=99

Base: TARGET=1-2

If physicians/nurses/health workers of the vaccination centers

S08 [Q]
In your Center, how many and which types of professional figures deal with / are dedicated to the vaccinations of these children?

1. No. of physicians
2. No. of nurses/health workers

SCRIPTER: min.=1, max.=9

Base: TARGET=1

If physicians of the vaccination centers

S09 [S]
In your center are there one or more nurses/health workers dedicated to vaccination activity or are you supported by nurses/health workers from other clinics / departments?

1. Nurses/health workers dedicated to vaccination activity
2. Nurses/health workers from other clinics / departments

Base: TARGET=2

If nurses/health workers of the vaccination centers

S10 [S]
Are you a nurse/health worker dedicated to this vaccination center or do you come from other departments / clinics?

1. Nurses/health workers dedicated to vaccination activity
2. Nurses/health workers from other clinics / departments

Base: TARGET=1-2

If physicians/nurses/health workers of the vaccination centers

S11 [M]
In particular, in your center, are you dealing with …? *Please indicate all activities in the center.*

1. Making inventory and/or managing the stock
2. Scheduling appointments
3. Making orders
4. Preparing the room where vaccinations are carrying out
5. Talking with the parents of the children / giving information about vaccination/the vaccine
6. Collecting the medical history of the child in view of the vaccination
7. Preparing the vaccine
8. Administering the vaccine
9. Registering the vaccination
10. Disposing of the waste materials
98. Other (specify) [O]

Base: TARGET=3

If pediatricians of Tuscany

S12 [Q]
In particular let's talk about the vaccinations administred to children under 2 years.
In a typical month, roughly, how many children under the age of 2 years do you vaccine?

No. of children under 2 years vaccinated in a month

SCRIPTER: min.=0, max.=999

If none, close
SCRIPTER: all respondents with answer S12=0 go to the end of the questionnaire (SCREENOUT)

Base: TARGET=3

If pediatricians of Tuscany

S13 [Q]
Among the … (*SCRIPTER: show the answer at S10*) children under 2 years vaccinated in a month, how many of them have been administered with the hexavalent vaccine (diphtheria, tetanus, pertussis, polio, hepatitis B haemofilus influenzae type b)?

No. of children/month

SCRIPTER: min.=0, max.=999
S13<=S12

If none, close
SCRIPTER: all respondents with answer S13=0 go to the end of the questionnaire (SCREENOUT)

V. MAIN QUESTIONNAIRE

A THE RTU VACCINES AND THE VACCINES TO RECONSTITUTE

A01 [S]
In general let's talk about ready-to-use and reconstituted vaccines that are administered to children under 2 years of age.
Which of the following characteristics are attributable to each of the two types of vaccine?

SCRIPTER: script as a grid

Items in row- randomize the items

1. Less time to prepare the vaccine
2. Less risk of error in preparing the vaccine
3. Easier preparation of the vaccine
4. Lower risk in the complete dissolution
5. Less risk of needling stick injury for physician /nurse
6. Less risk of error in the administration of the vaccine
7. Less risk of contamination of the needle
8. Lower production of disposal materials

Items in row- randomize the items

1. Ready-to-use vaccine
2. Reconstituted vaccine

Base: all respondents

A02 [S]
Thinking to the vaccination session performance, how much does the use of a ready-to-use vaccine facilitate when vaccinating children under the age of 2 years,? Answer by using a scale from 1 to 10 where 1 corresponds to “not at all” and 10 to “very “

1. 1
2. 2
3. 3
4. 4
5. 5
6. 6
7. 7
8. 8
9. 9
10. 10

B THE HEXAVALENT VACCINE IN GENERAL

Base: all respondents

B01 [S]
In particular let’s talk about the hexavalent vaccine.

In any case, based on your experience, how much the hexavalent vaccine is a vaccine …?

SCRIPTER: script as a grid

Items in row – randomize the items

1. Easy to manage
2. Vaccin safety
3. Requires a lot of family counseling at the time of first administration

Answers in column

1. Very
2. Mostly
3. Not very
4. Not at all

Base: all respondents

B02 [Q]
Again with reference to the hexavalent vaccine, on average, how much time is spent on *…? Please answer in minutes*

SCRIPTER: script as a grid

Items in row

1. Talking / giving explanations about vaccination/ the vaccine to parents
2. Preparing the vaccine
3. Administering the vaccine
4. Disposing of waste materials

Answer in column – 1 answer for each item

Minutes

SCRIPTER: min.=1, max.=99

Base: all respondents

B03 [S]
Usually the hexavalent vaccine is prepared …?

1. Before the interview with parents
2. During the interview with parents
3. After the interview with parents

Base: all respondents

B04 [S]
Concerning the hexavalent vaccine,based on your experience, how important is each of the following aspects?

SCRIPTER: script as a grid
Items in row – randomize the items

1. Easy to prepare and to manage
2. Stability in case of problems of the cold chain
3. Fast vaccine preparation before injection
4. Ready to use without need of reconstitution
5. Minimize the risk of needle contamination
6. Minimize the risk of needle stick injuries for the health operator making the vaccination
7. Less risk of errors in the reconstitution
8. Minimal amount of waste materials after administration

Answers in column

1. Very
2. Mostly
3. Not very
4. Not at all

Base: all respondents

B05 [S]
Below, find some statements about hexavalent vaccine managing and administaration. How much do you personally agree with each of the following statement?

SCRIPTER: script as a grid

Items in row- randomize the items

1. Talking/giving information about vaccination/vaccine to parents is very demanding. We have to reassure them.
2. Talking/giving information about vaccination/vaccine to parents takes a lot of time
3. It could be possible making errors during the vaccine preparation

4. It could be possible making errors during the vaccine administration
5. It could be possible to forget the reconstitution of the vaccine

Answers in column

1. Strongly disagree
2. Somewhat disagree
3. Somewhat agree
4. Strongly agree

C THE HEXAVALENT VACCINE USED: ASSESSMENT

Base: all respondents

C01 [S]
Let's talk about the hexavalent vaccinethat you use
Which hexavalent vaccine do you personally administer *(if TARGET=3)* /**is administered in your center** (*if TARGET=1-2*)?

1. Hexyon
2. Infranrix Hexa
3. Vaxelis
4. None of thesei

SCRIPTER: all respondents with answer C01=4 go to the end of the questionnaire (SCREENOUT)

Base: all respondents

C02 [O]
Based on your experience, which are the…….. (*SCRIPTER: show the answer at C01)* advantages/strengths?

Base: all respondents

C03 [O]
And which are the…… (*SCRIPTER: show the answer at C01)* disadvantages/weakenesses?

Base: all respondents

C04 [S]
However, how do you consider …… (*SCRIPTER: show the answer at C01)* for each of the following items?

SCRIPTER: script as a grid

Items in row- randomize the items

1. Easy to prepare and to manage
2. Stabiliy in case of cold chain problems
3. Fast preparation of vaccine before injection
4. Ready to use without need of reconstitution
5. Risk of needle contamination
6. Risk of needle stick injuries for the health operator making the vaccination
7. Risk in the reconstitution
8. Minimal amount of waste materials after administration

Answers in column

1. Very poor
2. Poor
3. Good
4. Very good

Base: all respondents

C05 [S]

Has you always used … (*SCRIPTER: show the answer at C01) or did you use in the past a different hexavalent vaccine* ?

1. Always this one
2. Another hexavalent vaccine

Base: C05=2

If another vaccine

C06 [S]
Which hexavalent vaccine did you use in the past?

1. Hexyon
2. Infranrix Hexa
3. Vaxelis

Base: C05=2

If another vaccine

C07 [O]
Thinking about … (show the answer at C06), which are the advantages/strengths of this vaccine?

Base: C05=2

If another vaccine

C08 [O]
And which are the…… (*SCRIPTER: show the answer at C06)* disadvantages/weakenesses?

Base: C05=2

If another vaccine

C09 [S]
And how do you consider …… (*SCRIPTER: show the answer at C06)* for each of the following items?

SCRIPTER: script as a grid

Items in row- randomize the items

1. Easy to prepare and to manage
2. Stabiliy in case of cold chain problems
3. Fast preparation of vaccine before injection
4. Ready to use without need of reconstitution
5. Risk of needle contamination
6. Risk of needle stick injuries for the health operator making the vaccination
7. Risk in the reconstitution
8. Minimal amount of waste materials after administration

Answers in column

1. Very poor
2. Poor
3. Good
4. Very good

Base: C01=2 and C05=1

If only Infranrix Hexa

C10 [O]
Following your experience, what are the advantages in the preparation and administration of Infranrix Hexa compared to a ready to use vaccine that is administered to children under 2 years (ad for example the PCV13)?

Base: C01=2 and C05=1

If only Infranrix Hexa

C11 [O]
And what are the disadvantages in the preparation and administration of Infranrix Hexa compared to a ready to use vaccine (as for example the PCV13) to be administered to children under 2 year?

D FOCUS ON PACKAGING AND SYRINGE

Base: all respondents

D01 [S]
Thinking about the packaging of……. (*SCRIPTER: show the answer at C01)*.
(*SCRIPTER: show the answer at C01)* are you provided …..... *(if TARGET=3)* /does your centre is provided (*if TARGET=1-2*) in pack of …?

1. Single pack
2. 10 syringes
3. 20 syringes
4. 50 syringes

Base: all respondents

D02 [S]
Overall, how do you evaluate the pack of … (*SCRIPTER: show the answer at D01)*?

1. Very good
2. Good
3. poor
4. Very poor

Base: D02=3-5

D03 [O]
And in particular, how do you evaluate the pack of….(*SCRIPTER: show the answer at D01) concerning …..*?
SCRIPTER: script as a grid

Items in row- randomize the items

1. The opening of the packaging
2. The opening of the blister
3. The recognition of the box

Answers in column

1. Very poor
2. Poor
3. Good
4. Very good

Base: all respondents

D04 [S]
And how much is the pack……(*SCRIPTER: show the answer at D01*) easy to store?

1. Extremely easy
2. Very easy
3. Not very easy
4. Not at all easy

Base: all respondents

D05 [S]
And which of the following packs would you prefer to have available?

Single pack

1. Single pack
2. 10 syringes
3. 20 syringes
4. 50 syringes

Base: all respondents

D06 [O]
Why do you prefer the pack you mentioned above?

Base: all respondents

D07 [S]
Let's briefly talk about the syringe of …. (*SCRIPTER: show the answer at C01)*.
Do you remember which kind of syringe is used for ……(*SCRIPTER: show the answer at C01)vaccine*?

SCRIPTER: show the item with its image

1. Luer slip (image 1)
2. Luer lock (image 2)
99. DK/ Don't remember

Image 1

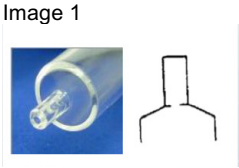
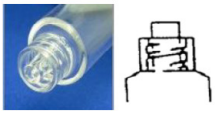


Image 2



Base: all respondents

D08 [S]
How much is safe/low risk of needle stick injury using ……(*SCRIPTER: show the answer at C01*) syringe?

1. Very
2. Mostly
3. Not very
4. Not at all

Base: all respondents

D09 [S]
In particular, speaking about the needles available in the packaging of (*SCRIPTER: show the answer at C01*)?
How much are you satisfied concerning this about…

SCRIPTER: script as a grid

Items in row- randomize the items

1. The number of needles present
2. The length of the needles
3. The diameters of the needles

Answers in column

1. Very
2. Mostly
3. Not very
4. Not at all

Base: all respondents

D10 [Q]
Following your experience, how many needles would you like to have available for administering the hexavalent vaccine …?

Items in row- randomize the items

1. Ready to use
2. Reconstituted

Answers in colum

N° needles

Base: all respondents

D11 [S]
And what is the length and the diameter of needles that you would prefer to have available for a ready to use exavalent vaccine?
And for a reconstituted exavalent vaccine? Interviewer: do not suggest,

SCRIPTER: script as a grid

Items in row

1. 16 mm – 25 G
2. 25 mm – 23 G
3. 25 mm – 25 G
98. Other (specify) [O]

Answers in column

1. Ready to use
2. Reconstituted

Base: all respondents

E NEEDS

E01 [S]
Finally,find listed below some improvement expectations/needs related to the hexavalent vaccine. How important is each of the following item for you?

SCRIPTER: script as a grid

Items in row- randomize the items

1. Having the ready to use vaccine available without the need of reconstitution
2. Having the vaccine available in pre-filled syringes
3. Making the inventory in digital way (bar codes, QR code.....)
4. Having packages /syringes easier to identify
5. Having packages easier to store
6. Having syringes with passive safety needle
7. Getting vaccine directly from the Pharmaceutical company
8. Availability of vaccine delivery service (*show only if TARGET=3*) (**only pediatrician)**
9. Availability of nurse to support the vaccinations (*show only if TARGET=3*) (**only pediatrician)**
10. Labels easier to detach
11. More time for family counseling
12. More information materials about vaccination/vaccine

Answers in column

1. Not at all
2. Not very
3. Mostly
4. Very

VI. DEMOGRAPHICS

Base: all respondents

Z01 [S]
Insert gender

1. Male
2. Female

Base: all respondents

Z02 [Q]
How old are you?

Years

SCRIPTER: min.=20, max.=80

Base: all respondents

Z03 [Q]
How long have you been dealing with vaccinations of children under two years?

Years

SCRIPTER: min.=1, max.=60

VII. STANDARD SCREENED OUT

Thank you very much for your willingness, but unfortunately it's not possible to continue with the interview since your characteristics don't satisfy the criteria requested for this survey.

END OF QUESTIONNAIRE

ORIGINAL ARTICLE

Screening approach among newly arrived asylum seekers: experience in a primary health care setting in Piacenza, Emilia Romagna, Northern Italy

ALESSANDRA DONISI[1], LAURA GERNA[2], TOMMASO FIETTA[1], CECILIA GRECCHI[1]
[1] Migration Health Unit, Department of Primary Health Care, AUSL Piacenza, Italy; [2] Infectious Diseases Unit, General Hospital of Piacenza, Italy

Keywords

Screening • Refugees • Infectious diseases • Migrants

Summary

In the last ten years the number of asylum seekers has increased in all of Europe. Our Migrants Health Unit in Piacenza, Emilia Romagna, Italy, is designated to provide primary health care for migrants without a regular permit of stay and, since 2015, is the reference center for asylum-seekers in our Province. Aim of this study is to describe the results of the screening for infectious diseases performed in asylum seekers from January 2015 to December 2015. For any asylum seekers referred to our Centre, we recorded demographical data and we offered screening for HIV, HBV, HCV, syphilis and active tuberculosis (TB). Descriptive statistics were used to analyze the characteristics of the cohort. In 2015, 316 asylum seekers accessed to our Centre. Of them, the majority were men (N= 275; 87,03%). Africa was the most represented geographical area (221, 69,94%), followed from Asia (95, 30.06%). The median age was 25,4 years. 301 patients underwent chest X-Ray, that resulted negative in 262 cases (87%). HBsAg testing proved to be positive in 17 (5,3%) cases. The screening test for HCV, HIV and syphilis resulted respectively positive in 1.9%, 0.3% and 1.6%.

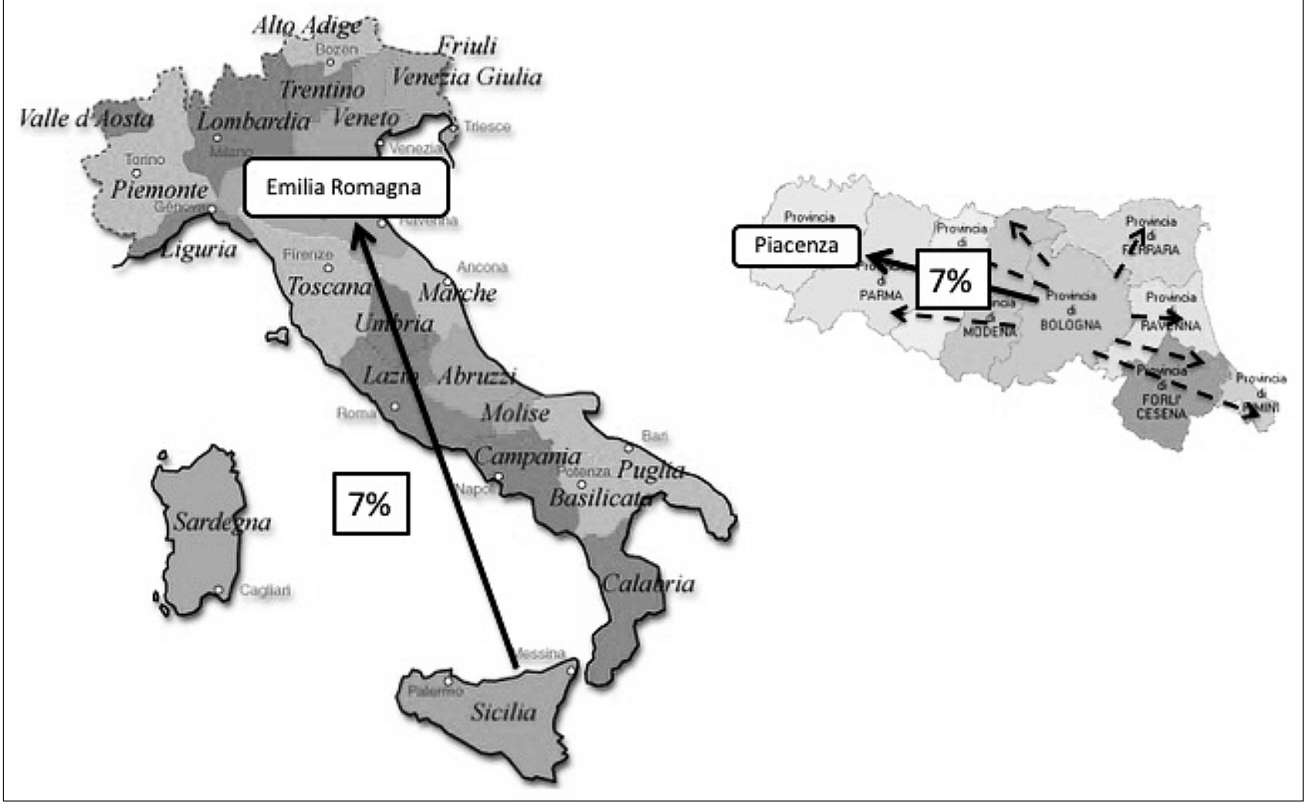
Introduction

In the last decade, migratory movements towards Europe bursted due to an increasing instability caused by conflicts, violence, natural disasters and human rights abuse in the nearby African and Asiatic region [1]. In 2015, the United Nations High Commissioner for Refugees (UNHCR) registered a dramatic growth in global forced displacement that involved approximately 65.3 million individuals [2]. In the same year, more than 1 million people entered Europe by boat. Of them, 153472 subjects arrived in Italy through the Mediterranean sea [3], that is the most deadly route to reach Europe [2]. Due to European resettlement policy, only 103972 individuals were admitted to Italian primary reception center [3]. According to Italian regulation, once international protection is requested, asylum-seekers are hosted in different locations placed along our country, till the application is resolved [4]. Consequently, a proportion of 7% of the total number of asylum seekers disembarked to Italy was assigned to Emilia Romagna and 7% of it was destinated to the province of Piacenza (Fig. 1).
At the moment of the disembarkation, migrants undergo a first aid procedure, aiming to individuate critical clinical situations, skin infections and signs or symptoms suspicious for tuberculosis. Later, at the arrival in the destination site, a second deeper medical visit with a screening for infectious diseases (HIV/HBV/HCV/Syphilis/Tuberculosis) and other clinical conditions (psychological trauma, diabetes, hypertension, anemia) is organized. These procedures are free of charge, and asylum seekers are regularly admitted to all national healthcare facilities and vaccination services till their legal status is cleared [5].
The Migrants Health Unit in Piacenza is designated to provide primary health care for all undocumented people and, since 2015, due to the presence of a skilled physician and cultural mediators, is the health referring center for all asylum-seekers, thanks to a joint venture with the Local Office of Interior Minister of Piacenza.
The aim of this study is to describe the results of infectious diseases screening in asylum seekers that accessed to our Unit from the 1st of January 2015 to the 31st December 2015.

Material and methods

This is an observational retrospective study that considers any asylum-seekers older than 14 years that referred to our Migration Health Unit from the 1st of January 2015 to the 31st December 2015.
For each individual, we reviewed paper records. Demographical data (name, age, sex, geographical origin, date of arrival) and screening results for HIV, HBV, HCV, syphilis and active tuberculosis (TB) were anonymously entered into a dedicated database. Descriptive statistics were used to analyze the characteristics of the cohort (χ square and T-student test); the geographical origin was

Fig. 1. Asylum seekers allocation proportion after their arrival.



divided into African and Asiatic (Not African) for statistical analysis.

At first visit, for each subject, we recorded in a paper file demographical data (name, age, sex, geographical origin, date of arrival, level of education, religion, marital status, children), behavioral data (smoke, alcohol consumption and drug abuse) and previous significant health problems and medicines taken. Blood pressure, heart frequency rate and temperature were obtained.

The main focuses of the visit were:

- to investigate the presence of symptoms suspicious for active tuberculosis (cough for more than 2 weeks, fever, night sweats, loss of weight);
- to detect skin signs of torture or ectoparasitic infection;
- to individuate signs or symptoms of genital, urogenital and gastrointestinal infections;
- to recognize signs or symptoms of diabetes, anemia, hypertension;
- to offer specialized psychological support for people who had suffered for violence or torture.

The screening for infectious disease was performed through a blood sample as follow:

- HIV: HIV ELISA test and Western Blot test to confirm a positive ELISA TEST;
- HBV: HBV surface antigen (HBsAg) search;
- HCV: HCV antibodies and, if positive, quantitative viremia;
- Syphilis infection: *Treponema pallidum* IgG ELISA and, in case of positivity, Treponema Pallidum Hemagglutination Assay.

In case of a diagnosis of viral chronic infection, patients were referred to the Infectious Diseases Department of our hospital for follow-up and treatment.

Syphilis was treated in our Unit through injections of penicillin according to international guidelines.

Active tuberculosis was investigated through chest X-Ray and, where necessary, a CT scan. We considered chest X-rays suspicious for tuberculosis in case of infiltrate or consolidation in the pulmonary apexes, cavitary lesions and pleural effusion. If TB was suspected, patients were sent to the Pneumology Department of our Hospital for further investigation, treatment and follow up. No screening for latent tuberculosis infection was performed till the end of 2015 due to our regional directive.

In case of a diagnosis of a communicable disease, patients were informed on how to avoid the spread of infection even through cultural mediators. Moreover, we provided informative materials in different languages and condoms in case of sexually transmitted infection.

Thanks to a partnership with the Public Health and Hygiene Department, we gave to each subject an appointment for vaccinations, according to a schedule approved by the Italian Ministry.

In case of pregnancy, patients were addressed to the Antenatal Care of our Hospital.

Results

Three hundred sixteen asylum seekers were referred to our Centre in 2015, of them the majority were men (M 275, 87.03%; F 41, 12.97%). All the females were coming from Africa; 4 of them were diagnosed pregnant, and 1 having a miscarriage. Subjects came from 16 different countries. Africa was the most represented geographical area (69.94%, 221), while the remaining were coming from Asia (30.06%, 95). Nigeria was the country with the largest share (32.91%, N = 104), followed by Pakistan (20.25%; N = 64) and Gambia (11.07%, N = 35). The median age was 25.4 years (confidence range 17-64). Age fell between 18 and 30 years in 82.9% of cases. The median age was 23 ± 5 years in African subjects, and slightly higher in those from Asia (28 ± 7 years), although this difference was not statistically significant ($p > 0.05$). Sierra Leone had the youngest population (18.5 years). Two subjects were older than 50 years old, a man from Eritrea of 64 years and one from Pakistan of 59 years (Tab. I).

Three hundred and fifteen people underwent HBV, HCV, HIV and syphilis screening (Tab. II).

HBsAg was detected in 17 (5.3%) subjects (16 M; 1 F); 16 cases were from Africa and only 1 from Asia (Pakistan) ($p = 0.032$). All of them were addressed to the infectious diseases unit for second level tests.

HCV antibodies were identified in 6 persons (1.9%), all men, coming from Africa in 4 cases and Asia (Pakistan) in 2 cases ($p > 0.05$). A dual infection HBV/HCV was diagnosed in 1 subject.

HIV infection was diagnosed in 2 males (0.3%) from Nigeria.

No statistically significant difference resulted for HBV/HCV/HIV screening positivity according to the geographical area of origin (22/243 from Africa; 3/98 Non-African; $\chi = 3.69$; $p = 0.054$).

Six patients resulted positive at the syphilis screening, 1 woman with VDRL positivity (0,3%) and 5 men (1,58%)

Tab. I. Demographic characteristics.

Origin	Men	Women	Mean	Median	Max	Min
Afghanistan	9	0	23,55556	19	46	18
Bangladesh	22	0	29,45455	29	45	20
Costa d'Avorio	12	8	25,3	25	36	19
Eritrea	1	0	64	64	64	64
Gambia	32	3	21,45714	21	29	17
Ghana	9	0	26,33333	27	39	18
Guinea	13	0	22,84615	22	30	19
Liberia	2	0	28,5	28,5	29	28
Mali	15	0	22,73333	22	31	18
Nigeria	74	30	24,70192	24	40	17
Pakistan	64	0	29,48438	28	59	19
RCA	1	0	24	24	24	24
Senegal	15	0	21,6	20	26	18
Sierra Leone	2	0	18,5	18,5	19	18
Togo	4	0	22,25	21,5	27	19

Tab. II. Screening serological data by country of origin.

Origin	N. Total	HBsAg +	HCVAb + (%)	VDRL + (%)	TPHA + (%)	HIVAb + (%)
Afghanistan	9	0	0	0	0	0
Bangladesh	22	0	0	0	0	0
Cote Ivoire	20	0	0	1	3	0
Eritrea	1	0	1	0	0	0
Gambia	35	4	0	0	0	0
Ghana	9	2	1	0	0	0
Guinea	13	0	0	0	0	0
Liberia	2	1	0	0	0	0
Mali	15	2	0	0	1	0
Nigeria	103	4	0	0	0	2
Pakistan	64	1	2	0	0	0
RCA	1	0	0	0	0	0
Senegal	15	2	1	0	1	0
Sierra Leone	2	0	0	0	0	0
Togo	4	1	1	0	0	0
Total	315	17 (5.3%)	6 (1.9%)	1 (0.3%)	5 (1.58%)	2 (0.63%)

with TPHA positivity: all of them came from Africa (1 woman and 3 men from Ivory Coast; 1 man from Senegal and 1 man from Mali).
A chest X-ray was proposed to any patients, except pregnant women. Of the 312 patients, only 301 individuals underwent the procedure, that revealed normal findings in 262 (87%) cases. Eleven patients produced documents proving that they already did the screening before their arrival in our Province.
Among the 39 positive X-Ray, 4 showed signs of pneumonia (1,3%), 8 showed signs compatible with tuberculosis (2,7%), 29 showed signs compatible with other pathologies (9.6 %).
Of the 8 cases of X-Ray scans suggesting Tb, only a single TC scan confirmed the suspicion of tuberculosis. In the remaining population, during follow up, 2 other pulmonary tuberculosis cases have been diagnosed, both of them with a negative X-Ray scan at the screening.

Discussion

We presented the results of the screening procedure conducted in our Unit, that was in line with our regional guidelines.
Various studies have been carried out in Italy over the prevalence of infectious diseases among migrants [6-8], but only few consider hepatitis, HIV, tuberculosis and syphilis at the same time [9, 10].
Our data are consistent with national data for subject's origin, as Nigeria, Pakistan and Gambia were the more represented countries. Contrarily, we had a higher percentage of female (12.97 % vs 9.69%), especially coming from Nigeria [11].
Due to the numerosity and the variableness of the sample, no consideration can be formulated on different prevalence of infectious diseases based on the origin, especially that our study doesn't include economic migrants [12, 13], but the overall prevalence of hepatitis, syphilis and HIV was low.
Active tuberculosis was diagnosed in 3 subjects, although more complete data could have been provided by latent tuberculosis infection screening, that was not yet recommended in Emilia Romagna at the study time.
In our region, even nowadays, active tuberculosis screening consists not only in a syndromic surveillance but even in a chest X-ray that proved to be helpful in diagnosing early and asymptomatic cases[14].
Even if migrants do not generally pose a health threat to the host population, some subgroups of migrants, as refugees, asylum seekers and irregular migrants, are particularly vulnerable to infectious diseases not only for higher prevalence in their countries of origin, but also for poor living conditions and deprivations experienced during the journey to reach the host country and in the host country itself [12, 15].
Expert consultations pointed out the importance of conducting migrant screening for communicable diseases according to their native land, since prevalence rates differ considerably by each country [16-21], but a national and European health policy on this target population was not yet formulated in 2015 [15, 22].
Alongside this, the importance of verify how to deliver effective and cost-effective screening, vaccination, and health services to this group is becoming crucial in a resource-constrained system [12]. Asylum seekers, by the way, are a particular population that has special pathways of integration in the welcoming country either at a social either at a sanitary and legal level. Many studies were conducted to assess the compliance to screening program in this population allover Europe, finding a very high acceptability [12, 23-25]. Data on treatment adherence however are lacking, due to the extreme mobility of this population and the difficulty to rebuild their healthcare pathway beyond a local level [12, 23-25]. Our study unfortunately was not conceived to assess the cost effectiveness of the intervention. Nevertheless, considering some of the parameters of a cost-effective analysis, such as the coverage and the uptake, we can affirm that we reached a coverage of almost 100% as any asylum seekers allocated to our Province had to be visited by our equip within 48-72 hours from the arrival thanks to the notification of the Local Office of Interior Minister of Piacenza. The uptake, defined as the percentage of persons who agreed to be screened after being offered screening [12], was also very high, thanks to the presence of cultural mediators and the completion of all the screening procedures in a single day. Concerning treatment compliance, we personally provided only syphilis therapy, that was completed by all patients, but our sample is low. Since we addressed people to other Units for tuberculosis, viral hepatitis and HIV cure, we have no data on their treatment compliance.
In general, however, we can affirm that our intervention was cost effective for coverage and uptake, mainly thanks to the close interaction among the stakeholders (Local Office of Interior Minister, Health system, hosting community), and contributed to diagnose diseases at early stage with damage limitation at personal and health care system level.
Alongside this, we believe it's important to stress that an infectious diseases screening should not be averted from a parallel screening to psychological needs as migrants arriving on Italian coasts experience mental and psychosocial diseases in a considerable number. As we noticed even in our Unit, depression and anxiety disorders, indeed, are related to traumas, either physical, mental or sexual, roused from violence that these people experience in their country of origin and throughout their journey [26-28].
We believe that this study enlightens the utility of a screening procedure in newly arrived asylum seekers. This is not only an overall public health strategy, but it's even the first chance for this population to access to the National Health Service and have accurate and faster specific treatment when and where needed.

Key points

- No systematic association between migration and communicable diseases.
- Migrant screening procedures for communicable diseases accordingly to their country of origin.
- Screening procedures as a part of a public health strategy
- screening procedures offer the opportunity to access to previously undiagnosed diseases.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

Conceptualization; AD, TF, CG; methodology: AD and CG; formal statistical and epidemiological analysis: AD and CG; investigation: AD, LG; data curation: AD, LG; writing - original draft preparation: AD and TF; writing - review and editing: AD and CG; supervision: AD.

References

[1] World Health Organization Regional Officer for Europe 2018. No public health without refugee and migrant health - Report on the health of refugees and migrants in the WHO European Region. Available form: https://apps.who.int/iris/bitstream/handle/10665/311347/9789289053846-eng.pdf?sequence=1&isAllowed=y

[2] UNHCR 2015 Global trends forced displacement in 2015. Available from: https://www.unhcr.org/statistics/unhcrstats/576408cd7/unhcr-globaltrends-2015.html

[3] Anci, Caritas Italiana, Cittalia, Fondazione Migrantes, Servizio Centrale dello Sprar 2016. Rapporto sulla protezione internazionale in Italia. Available from: https://www.sprar.it/pubblicazioni/rapporto-sulla-protezione-internazionale-in-italia-2016

[4] Attuazione della direttiva 2013/33/UE recante norme relative all'accoglienza dei richiedenti protezione internazionale, nonchè della direttiva 2013/32/UE, recante procedure comuni ai fini del riconoscimento e della revoca dello status di protezione internazionale, D. L. 142/2015, GU 214 (Sep 15, 2015) (Implementation of Directive 2013/33 / EU containing rules relating to the reception of applicants for international protection, as well as Directive 2013/32 / EU, containing common procedures for the recognition and withdrawal of international protection status)

[5] Indicazioni operative alle Aziende Sanitarie per l'organizzazione di modalità omogenee ed adeguate di assistenza sanitaria alle persone straniere – programma nazionale "Mare Nostrum". DPG Regione Emilia-Romagna 291902/2014. (Operational indications for Healthcare Organizations for homogeneous and adequate methods of health care for foreign people - national program "Mare Nostrum)

[6] Germinario C, Serena Gallone M, Tafuri S. Migrant health: the Apulian model. Epidemiol Prev 2015;39:76-80.

[7] Del Pinto R, Pietropaoli D, Russomando U, Evangelista P, Ferri C. Health status of Afro-Asian refugees in an Italian urban area: a cross-sectional monocentric study. Public Health [Internet] 2018;158:176-82. https://doi.org/10.1016/j.puhe.2018.03.011

[8] Coppola N, Alessio L, Gualdieri L, Pisaturo M, Sagnelli C, Minichini C, Di Caprio G, Starace M, Onorato L, Signoriello G, Macera M, Angelillo IF, Pasquale G, Sagnelli E. Hepatitis B virus infection in undocumented immigrants and refugees in Southern Italy: demographic, virological, and clinical features. Infect Dis Poverty 2017;6:33. https://doi.org/10.1186/s40249-016-0228-4

[9] Cuomo G, Franconi I, Riva N, Bianchi A, Digaetano M, Santoro A, Codeluppi M, Bedini A, Guaraldi G, Mussini C. Migration and health: A retrospective study about the prevalence of HBV, HIV, HCV, tuberculosis and syphilis infections amongst newly arrived migrants screened at the Infectious Diseases Unit of Modena, Italy. J Infect Public Health [Internet] 2019;12:200-4. https://doi.org/10.1016/j.jiph.2018.10.004

[10] Papadakis G, Okoba NA, Nicolaou C, Boufidou F, Ioannidis A, Bersimis S, Chatzipanagiotou S. Serologic markers for HBV, HCV and HIV in immigrants visiting the Athens' polyclinic of 'Doctors of the World – Greece'. Public Health 2013;127: 1045-7. https://doi.org/10.1016/j.puhe.2013.08.001

[11] Dipartimento per le Libertà civili e l'Immigrazione Ministero dell'Interno. I numeri dell'asilo. Riepilogo anni 2015-2016. Available from: http://www.libertaciviliimmigrazione.dlci.interno.gov.it/sites/default/files/allegati/riepilogo_dati_2015_2016_0.pdf

[12] Seedat F, Hargreaves S, Nellums LB, Ouyang J, Brown M, Friedland JS. How effective are approaches to migrant screening for infectious diseases in Europe? A systematic review. Lancet Infect Dis 2018;18:e259-e271 https://doi.org/10.1016/S1473-3099(18)30117-8

[13] Kentikelenis A, Karanikolos M, Williams G, Mladovsky P, King L, Pharris A, Suk JE, Hatzakis A, McKee M, Noori T, Stuckler D. How do economic crises affect migrants' risk of infectious disease? A systematic-narrative review Eur J Public Health 2015;25:937-44 https://doi.org/10.1093/eurpub/ckv151

[14] Vanino E, Tadolini M, Attard L, Po C, Francia F, Giannini A, Viale P. Systematic tuberculosis screening in Asylum Seekers in Italy. Clin Infect Dis 2017;65:1407-9. https://doi.org/10.1093/cid/cix503

[15] ECDC. Public health guidance on screening and vaccination for infectious diseases in newly arrived migrants within the EU/EEA. 2018. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/Public%20health%20guidance%20on%20screening%20and%20vaccination%20of%20migrants%20in%20the%20EU%20EEA.pdf

[16] Hirsch-Moverman Y, Daftary A, Franks J, Colson PW. Adherence to treatment for latent tuberculosis infection: systematic review of studies in the US and Canada. Int J Tuberc Lung Dis 2008;12:1235-54.

[17] Burnett A. Asylum seekers and refugees in Britain: Health needs of asylum seekers and refugees. BMJ 2001;322:544-7.

[18] Kärki T, Napoli C, Riccardo F, Fabiani M, Dente M, Carballo M, Noori T, Declich S. Screening for infectious diseases among newly arrived migrants in EU/EEA Countries - varying practices but consensus on the utility of screening. Int. J Environ Res Public Health 2014;11:11004-14 https://doi.org/10.3390/ijerph11101100419

[19] Treviño-Maruri B, Collazos-Sanchez F, Pou-Ciruelo D, Serre-Delcor N, Soriano-Arandes A, Sulleiro E, Molina-Romero I, Ascaso C, Bocanegra-Garcia C. Health status of asylum seekers, Spain. Am J Trop Med Hyg 2018;98:300-7. https://doi.org/10.4269%2Fajtmh.17-0438

[20] Pérez-Molina JA, Álvarez-Martínez MJ, Molina I. Medical care for refugees: a question of ethics and public health. Enferm In-

fecc Microbiol Clin 2016;34:79-82 https://doi.org/10.1016/j.eimc.2015.12.00722

[21] Greenaway C, Thu Ma A, Kloda LA, Klein M, Cnossen S, Schwarzer G, Shrier I. The seroprevalence of hepatitis C Antibodies in immigrants and refugees from intermediate and high endemic countries: a systematic review and meta-analysis. PLoS One 2015;10:e0141715. https://doi.org/10.1371/journal.pone.0141715

[22] Istituto Nazionale per la promozione della salute delle popolazioni Migranti ed il contrasto delle malattie della Povertà, Istituto Superiore di Sanità, Società Italiana di Medicina delle Migrazioni. I controlli alla frontiera - La frontiera dei controlli. 2017 Available from: https://www.inmp.it/lg/LG_Migranti-integrata.pdf

[23] Suijkerbuijk AWM, van Hoek AJ, Koopsen J, de Man RA, Mangen M-JJ, de Melker HE, Polder JJ, de Wit GA, Veldhuijzen IK. Cost-effectiveness of screening for chronic hepatitis B and C among migrant populations in a low endemic country. PLoS One 2018;13:e0207037. https://doi.org/10.1371/journal.pone.0207037

[24] Myran D, Morton R, Biggs B-A, Veldhuijzen I, Castelli F, Tran A, Staub L, Agbata E, Rahman P, Pareek M, Noori T, Pottie K. The effectiveness and cost-effectiveness of screening for and vaccination against hepatitis B virus among migrants in the EU/EEA: a systematic review. Int J Environ Res Public Health 2018;15:1898. https://doi.org/10.3390/ijerph15091898

[25] Pottie K, Lotfi T, Kilzar L, Howeiss P, Rizk N, Akl E, Dias S, Biggs B-A, Christensen R, Rahman P, Magwood O, Tran A, Rowbotham N, Pharris A, Noori T, Pareek M, Morton R. The effectiveness and cost-effectiveness of screening for HIV in migrants in the EU/EEA: a systematic review. Int J Environ Res Public Health 2018;15:1700. https://doi.org/10.3390/ijerph15081700 .

[26] Ministero della Salute. Linee guida per la programmazione degli interventi di assistenza e riabilitazione nonché per il trattamento dei disturbi psichici dei titolari dello status di rifugiato e dello status di protezione sussidiaria che hanno subito torture, stupri o altre forme. 2017.

[27] Available from: http://www.salute.gov.it/imgs/C_17_pubblicazioni_2599_allegato.pdf

[28] Pavli A, Maltezou H. Health problems of newly arrived migrants and refugees in Europe. J Travel Med 2017. https://doi.org/10.1093/jtm/tax016

[29] Bettini M, Donisi A, Gerna L. Sportello psicologico per migranti richiedenti asilo: l'esperienza di Piacenza. Proceeding of the XIV SIMM (Italian Migration Health Society) Congress; 2016 May 11-14; Turin, IT. Available from www.simmweb.it

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Correspondence: Cecilia Grecchi, Migration Health Unit, Department of Primary Health Care, AUSL Piacenza, Italy - E-mail: c.grecchi@ausl.pc.it

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ORIGINAL ARTICLE

The impact of PrEP: results from a multicenter Health Technology Assessment into the Italian setting

LUCREZIA FERRARIO[1], EMANUELA FOGLIA[1], ELISABETTA GARAGIOLA[1], VALERIA PACELLI[1], GIOVANNI CENDERELLO[2], ANTONIO DI BIAGIO[3], GIULIANO RIZZARDINI[4], MARGHERITA ERRICO[5], ROSARIA IARDINO[6], DAVIDE CROCE[7]
[1] MEcon, Centre for Health Economics, Social and Health Care Management, LIUC-Università Cattaneo, Castellanza, Italy; [2] Galliera Hospital, Department of Infectious Diseases, Genova, Italy - ASL-1 Imperiese Hospital, Department of Infectious Diseases, Sanremo, Italy; [3] Policlinico San Martino Hospital, Unit of Infectious Diseases, Genova, Italy; [4] Fatebenefratelli Sacco Hospital, Department of Infectious Diseases, Milan, Italy - School of Clinical Medicine, Faculty of Health Science, University of the Witwatersrand, Johannesburg, South Africa; [5] President of the NPS (Network Persone Sieropositive), Italy; [6] President of The Bridge Foundation, Milan, Italy; [7] MEng, Centre for Health Economics, Social and Health Care Management, LIUC-Università Cattaneo, Castellanza, Italy - School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Keywords

Pre-exposure prophylaxis • HTA • Economic sustainability • Organizational impact • PrEP

Summary

Introduction. *The use of oral tenofovir/emtricitabine (FTC/TDF) for pre-exposure prophylaxis (PrEP) among high-risk people without Human Immunodeficiency Virus (HIV), is emerging as an innovative strategy to decrease HIV epidemic. The study aims at evaluating the implications related to PrEP introduction, from a multidimensional point of view, as required by Health Technology Assessment (HTA) approach, with a particular attention on sustainability and social factors, influencing PrEP implementation.*
Methods. *An analysis was conducted involving 35 Italian Infectious Disease Departments. The introduction of PrEP (applied both as "add-on" and "substitute" prevention strategy) into the clinical practice was compared with a baseline scenario, consisting of condoms among men who have sex with men, and serodiscordant couples, and the use of Needle Syringe Programme among injection drugs users The above scenarios were analysed by means of a Health Technology Assessment (HTA) approach. The 9 EUnetHTA Core Model domains were assessed through comparative information, retrieved from literature evidence, and collection of qualitative and quantitative information, derived from real-world evidence, in particular from 35 Infectious Disease Departments and potential PrEP' users involved. A final multi-criteria decision analysis approach (MCDA) was implemented to simulate the appraisal phase and providing evidence-based information with regard to the preferable technology.*
Results. *Despite the improvement in patients' quality of life, PrEP would generate the development of other sexually transmitted and blood-borne diseases, with a consequent decrease of patients' safety in case of PrEP applied as a "substitute" prevention strategy. In addition, PrEP would generate an increase in staff workflow, with investment in medical supplies and training courses. PrEP would lead to significant economic investments both for the NHS (+40%), and for citizens (+2,377%) if used as an add-on strategy, assuming FTC/TDF patent cost. With the off-patent drug, the NHS would benefit from an advantage (37%), and a shrink of the patients' expenditure emerged (+682%). More economic resources are required if PrEP is applied as a substitute strategy, considering both the patent (NHS: 212%; citizens: 3,423%) and the off-patent drug (NHS: 73%; citizens: 1,077%). Conclusions. The most cost-containing strategy would be the use of PrEP, as an add-on strategy, with a consequent improvement in patients' safety, even if drug-related adverse events would be considered. The implementation of the off-patent drug would decrease the economic burden of the innovative prevention strategy. Hence, the organizational aspects related to its adoption would be deeply investigated, with the potential opportunity to create specific ambulatories devoted to PrEP users' especially for medium and big size hospitals.*

Introduction

The success achieved in the past few years in the HIV infection treatment has not been paralleled by remarkable improvements in the effectiveness of HIV prevention strategies [1, 2]. More than 1.8 million new HIV-1 infections were reported in 2017 worldwide, and an incidence rate equal to 5.7 new cases per 100,000 residents emerged in Italy [2].
As result, innovative and potent prevention strategies were required to reduce the risk of viral transmission from infected persons to healthy individuals, given the poor adherence to the traditional prevention strategy, as condoms and needle-syringe programs (NSPs). In the last few years, the use of oral tenofovir/emtricitabine (FTC/TDF), for pre-exposure prophylaxis (PrEP) among high-risk persons without HIV, has emerged as an innovative strategy to decrease HIV epidemic.
PrEP was approved by the Food and Drug Administration in 2012, and the US Centers for Disease Control and Prevention (CDC) released clinical guidelines on its use in 2014, on the basis of the drugs' clinical effectiveness [3-6], thus recommending the use of PrEP, in addition to condoms and NSPs, for HIV negative individuals, with the following characteristics [7]: 1) serodiscordant sexual relationship; 2) anyone who is not in a monogamous relationship with an HIV negative person; 3) men who have sex with other men; 4) sexual risk in general,

including individuals who have had sex without using a condom; and 5) injection drug users. In 2016, also the European Medicines Agency (EMA) approved the use of the oral TDF/FTC for PrEP in adults at high-risk for contracting HIV infection, and guidelines were issued recommending that oral PrEP should be offered as an additional prevention choice, for people at substantial risk of HIV infection, as part of combination prevention approaches. EDCD's annual survey [8] revealed that by 2019, 14 of 53 reporting countries declared that their national healthcare service provided reimbursed PrEP (Belgium, Bosnia and Herzegovina, Croatia, Denmark, France, Germany, Iceland, Luxembourg, Moldova, the Netherlands, Norway, Portugal, Sweden, and Northern Ireland and Scotland within the United Kingdom - UK), either through insurance or from the public sector. The results show that progress has been made since 2016, when only France reported that PrEP was nationally available and reimbursed [9]. Despite the different behaviors of European Countries, the Italian provisions of PrEP remains limited, since, in the Italian setting, PrEP (as preventive strategy for high-risk people) is available only if totally paid by citizens.

Based on the above suggestions, while there have been a significant number of studies reporting the high potential efficacy of PrEP [3-6], its implementation is strictly related to significant economic and organizational concerns, as well as to the different behaviours and adherence of high-risk populations. Besides the high PrEP cost, there emerged an organizational difficulty to guarantee an adequate hospital pathway to the HIV negative individuals assuming PrEP [10], since specialist visits, cultural blood tests and treatment of other sexually transmitted diseases [11], could be ensured to the PrEP treated population, in particular, for whom not using condoms or NSPs. Furthermore, the ethical aspects related to the medicalization of a healthy person become an urgent pattern, in Countries characterized by limited economic resources, since the use of Highly Active Antiretroviral Therapy (HAART) is related to several monitoring activities for drugs toxicities [12].

Despite the relevance of the topic, no evidence has emerged with regard to the potential impacts associated to the introduction of PrEP in the Italian clinical practice. Thus, the aim of the present study was a multi-dimensional evaluation of PrEP adoption in Italy (as an "add-on" or a "substitute" prevention strategy), compared with the traditional HIV prevention strategies, in order to protect high-risk HIV-negative individuals, useful to support evidence-based decision-making processes, taking into consideration the individual's and the National Healthcare Service (NHS) perspectives.

Methods

An analysis was conducted involving 35 Italian Infectious Disease Departments in Italy, including 15 Italian Regions, in order to achieve a complete national landscape of the centers devoted to the enrollment and treatment of these patients, in terms of regional distribution, centers dimensions and private/public ownerships.

A letter for participation to the study was sent via-mail to the Italian Infectious Disease clinicians, in order to gather information with regard to the number of potentially PrEP users, as well as to retrieve their perceptions with regard to the introduction of such preventive strategy.

Thus, the introduction of PrEP (prescribed as an "add-on" or a "substitute" prevention strategy) into the clinical practice, was compared with a baseline scenario consisting of the use of condoms among men who have sex with other men (MSM), and serodiscordant couples (SCs), and the use of NSPs among injection drugs users (IDUs), thus being consistent with the guidelines available on the topic. The above scenarios were analyses by means of a Health Technology Assessment (HTA) approach, in order to cover all the domains required by the EUnetHTA Core Model according to real-life qualitative and quantitative information.

Since no sensitive and human data were collected, both the ethics approval and the compliance with the STROBE guidelines for reporting observational studies, were not applicable.

Because of the multi-dimensional nature of HTA [13], the present analysis considered several aspects of the medical technologies under evaluation, in accordance with the EUnetHTA Core Model [14]. Thus, the assessment of the EUnetHTA Core Model domains, was completed by a prioritisation phase, and a final multi-criteria decision analysis – MCDA [15-17], simulating the appraisal phase.

Assessment of EUnetHTA Core Model domains

Due to the multi-dimensional and multi-disciplinary nature of HTA, several aspects of the preventive approaches taken into account (PrEP and other alternatives, in add-on or not), were analyzed as suggested by literature [14]: i) health problem and current use of technology; ii) description and technical use of technology; iii) safety; iv) clinical effectiveness; v) costs and economic evaluation; vi) ethical analysis, in terms of access to care; vii) organizational aspects; viii) social aspects and ix) legal aspects.

The above domains were deployed, considering scientific evidence, economic evaluations (quantitative information) and qualitative approaches (expert opinion and potential PrEP's users perceptions.

Literature review

With regard to the systematically searching medical literature, the PICO (Population, Intervention, Comparator and Outcome) was defined as follows: P – High-risk persons without HIV; I – FTC/TDF for pre-exposure prophylaxis; C – use of condoms among MSM and SCs, and use of NSPs among IDUs, to prevent HIV infection; O: HIV occurrence rate adverse events and sexually transmitted disease (STDs) incidence rate.

Literature evidence came from the systematic search of literature databases (Pubmed, Embase and Cochrane Library). The search terms were the followings: "pre-exposure prophylaxis", "high-risk individuals", "clinical effectiveness", "HIV occurrence rate", "drug-related adverse events", "STDs", "men who have sex with men - MSM", "serodiscordant couples - SCs", "injection drug users - IDU".
Peer-reviewed papers that explicitly described the clinical effectiveness of the different preventive strategies under assessment, were consequently included, and synthetized according to a PRISMA flow diagram, thus mapping out the number of records (in terms of papers) identified, included and/or excluded, and the reasons for exclusion motivated [18].
The validation of the scientific evidence available on the topic, was performed through the ROBINS II Cochrane risk of bias tool [19], and the AMSTAR-Assessing the Methodological Quality of Systematic Reviews check-list, on the basis of the nature of the study included. Literature was used for highlighting efficacy profile in terms of HIV occurrence rate with or without PrEP, and safety profile (measured as drug-related adverse events rate). Since only primary evidence have been considered, the literature review proposed in the present paper, collected high-quality efficacy and safety information.

Economic evaluation

For the economic evaluation, both a process mapping technique [21] and a budget impact analysis [22] were conducted, comparing the clinical pathway costs of a PrEP user *versus* the ones related to an individual not using PrEP. Information was gathered according to the standard clinical pathway performed in the 35 Italian Infectious Diseases Departments involved in the study, by means of a Delphi approach [23], consistent with International and National HIV Guidelines, and Regional Clinical Pathways.
The following determinants of costs were deeply considered: i) cost of the prevention strategies; ii) cost of the drug-related adverse events; iii) cost of the other sexually transmitted infections; and iv) cost of the medical monitoring of PrEP users.
The pathways were valorized considering the reimbursement tariffs of the Italian NHS valid for the year 2018/2019 and assumed a 12-month time horizon. On the one hand, the cost supported by the NHS for the proper cure and follow-up of PrEP users, considered the following cost drivers, representing all the healthcare direct costs: specialist visits, diagnostic procedures, hematological exams, hospitalizations and drugs, all in terms of typology and quantity of healthcare services administered to the potential PrEP user. On the other hand, the cost supported by the PrEP users for the disease management, was calculated in terms of "out of pocket" healthcare expenditure, productivity loss (time spent in hospital valorized on the basis of the PrEP users average monthly gross salary), as well as the average transport costs (estimated according to the Italian Automotive Club - ACI tables price list), to reach the Infectious Disease Department for outpatient procedures. It should be noted here that on the one hand, the average monthly gross salary derived from the most recent JP Salary Outlook for Italy [24], approaching with the Human Capital Method [25, 26] on the other hand, the average transport cost was calculated according to the average distance spent by the patient to reach the hospital of reference, that in the Italian setting is equal to 55.8 km [27].
After evaluating the management cost of the high-risk individuals, stratified by prevention technology, and including both the costs for the management of sexually transmitted infection and adverse events, a Budget Impact Analysis was developed considering a scenario in which PrEP is totally reimbursed by the NHS, and a scenario in which the drug, is directly purchased by the citizens (as happened in the Italian setting).
As mentioned before, all the economic analyses assumed both the NHS and the patient's perspectives (in terms of "out-of-pocket expenditure" supported for the management and care of their clinical conditions), considering both branded and off-patent drugs costs (considering a 70% reduction in drug costs).

Domains investigated through qualitative approaches

Qualitative questionnaires were administered to 35 clinicians referring to the 35 Infectious Diseases Departments involved in the study, who completed the questionnaire according to their own experience and perceptions.
The qualitative questionnaires were used for examining ethical, legal and organizational aspects, considering a comparative approach (use of PrEP as add on strategy *vs* not-use of PrEP in prevention activities), in accordance with a validated 7-item Likert scale ranging from –3 to +3 [28]. According to the above, the scenario consisting of the PrEP absence is always represented as a neutral situation, corresponding to zero value, and the perceptions related to the different domains, in case of PrEP adoption, were studied, with a comparative and incremental or decremental approach (the items studied are presented in Tab. I) It should be noted that all the items used for the deployment of each qualitative domain derived from the EUnetHTA Core Model issues [14], and were, then characterized and integrated, considering the specific topic of investigation.
The analysis of the social domain was conducted considering both the 35 clinicians and the potential PrEP users' perceptions. For this last part of the sample, an online questionnaire, administered, using a Survey Monkey tool, was completed by a sexually high-risk population, representing potential PrEP users. In particular, the on-line questionnaire was sent to two different citizens' association, where people voluntarily decided to complete the questionnaire. Data were collected anonymously, in accordance with the EU Regulation n. 679 of 04.05.2016, guaranteeing privacy and legal issues. In addition to information with regard their risk factor for HIV, their PrEP knowledge, attitudes and willingness-to-pay were deeply investigated

considering a 5-item evaluation scale, ranging from a minimum of 1 (completely disagree) to a maximum of 5 (completely agree).
Detailed information regarding the specific items related to each domain is shown in Table I.

Statistical methods

Focusing on the statistical methods, qualitative data were first analyzed, considering descriptive statistics. The existence of statistically significant differences (according to a significance level lower than 0.05 p-value) was assessed in the comparison between i) baseline scenario and innovative scenario, with regard to the qualitative assessment of the domains; ii) MSM and heterosexuals, with regard to the online survey conducted with the involvement of potentially PrEP users in the social domain deployment. In particular, independents sample t-test for either parametric or not-parametric variables were conducted.

Multi-criteria decision analysis

After the assessment of all the EUnetHTA Core Model domains, a Multi-Criteria Decision Analysis (MCDA)

Tab. I. Methods used for the deployment of the EUnetHTA dimensions.

Domains	Description of the domains	Quantitative and qualitative metrics for the evaluation	Scores and their related descriptions for the application of the MCDA
Health problem and current use of the technology	*Target population eligible to PrEP administration*	Definition of the high-risk population, potentially eligible for PrEP, in accordance with the Italian epidemiological data available and with real-life information retrieved by the 35 Infectious Disease Departments involved. Thus, information with regard to the overall number of adult MSM, SCs and IDUs, approaching the above 35 Departments were collected	1 – Small number of potentially eligible PrEP users 2 – Moderate number of potentially eligible PrEP users 3 – Significant number of potentially eligible PrEP users
Description and technical characteristics	*Definition of evidence-based information and assessment of their quality*	After the definition of the PICO guiding the literature review, the paper included in the HTA were synthetized according to a PRISMA flow diagram, thus mapping out the number of records (in terms of papers) identified, included and/or excluded, and the reasons for exclusion motivated. Furthermore, the validation of the scientific evidence available on the topic, was performed through the ROBINS II Cochrane risk of bias tool, and the AMSTAR-Assessing the Methodological Quality of Systematic Reviews check-list , on the basis of the nature of the study included	1 – Poor quality of evidence-based information 2 – Medium quality of evidence-based information 3 – High quality of evidence-based information
Safety	*Rate of mild, moderate and severe adverse events*	Identification of the possible adverse events for PrEP users, in terms of evidence-based incidence-rate data, with regard to drug-related adverse events, and sexually transmitted/blood borne infections, derived from literature evidence available on the topic. These events were also economically evaluated, in order to analyze their economic impact, considering both NHS and patients' perspective, in accordance with the standard clinical pathways, declared by the 35 hospitals involved in the study – according to the Delphi methods, consistent with International and National HIV Guidelines, and Regional Clinical Pathways	1 – The prevention strategy presents a significant decrease in PrEP user's safety 2 – The prevention strategy presents no impact in PrEP user's safety 3 – The prevention strategy presents a significant increase in PrEP user's safety
Clinical effectiveness	*Efficacy indicators*	Identification of the HIV occurrence rate related to the use of the three technologies (PrEP, condoms and syringes, as single prevention strategies or as add-on strategies), based on the evidence available, and validated in the Prisma Flow Chart	1 – The prevention strategy presents a significant increase in HIV occurrence rate 2 – The prevention strategy presents no impact in HIV occurrence rate 3 – The prevention strategy presents a significant decrease in HIV occurrence rate
Costs and economic evaluation	*Activity based costing analysis*	Clinical pathway economic evaluation, considering individual assuming or not assuming PrEP	1 – The prevention strategy presents a substantial economic impact on the clinical pathway 2 – The prevention strategy presents an insignificant and sustainable economic impact on the clinical pathway 3 – The prevention strategy presents a favorable and low economic impact on the clinical pathway
	Budget impact analysis	The above-mentioned clinical pathway cost per PrEP user was multiplied by the total number of patients potentially eligible to PrEP thus comparing a baseline with an innovative scenario and assuming different hypotheses: 1) PrEP used as an add-on or substitute strategy; 2) PrEP totally reimbursed by the Italian NHS or paid by the citizens; 3) administration of the branded or the off-patent drug	1 – The prevention strategy presents a substantial economic impact on both the NHS and individuals healthcare budget 2 – The prevention strategy presents an insignificant and sustainable impact on both the NHS and individuals healthcare budget 1 – The prevention strategy presents a favorable economic impact on both the NHS and individuals healthcare budget
Ethical aspects	Perceived aspects related to the access to care	The 35 clinicians involved in the analysis, completed a comparative qualitative questionnaire, based on a 7-item Likert scale (ranging from-3 to +3), considering the following items: 1) Access to care on local level; 2) Access to care for persons on a legally protected status; 3) Impact of the preventive strategy on the accessibility to care related to the management of adverse events; 4) Generation of healthcare migration phenomena; 5) Impact of the preventive strategy on the patients' willingness to pay; 6) General equity; 7) Accessibility to the prevention strategy, in case of full payment by the potential PrEP's users; 8) accessibility to the prevention strategy, in case of co-payment	1 – The prevention strategy presents a decrease in the access to care for PrEP users at local level 2 – The prevention strategy presents no impact in the access to care for PrEP users at local level 3 – The prevention strategy presents an increase in the access to care for PrEP users at local level
Social aspects	*Social and ethical perceived aspects: the clinicians' point of view*	The 35 clinicians involved in the analysis, completed a comparative qualitative questionnaire, based on a 7-item Likert scale (ranging from -3 to +3), considering the following items: 1) Ability of the drug to protect the patients' autonomy; 2) Ability of the drug to protect the human rights; 3) Ability of the drug to protect the PrEP users' integrity; 4) Ability of the drug to protect the PrEP users' dignity; 5) Impact of the drug on the PrEP users' willingness to pay; 6) Impact of the drug on PrEP users'religion; 7) Impact of the drug on social costs; 8) Impact of the drug on the citizens' medicalization; 9) Impact of the drug on the PrEP users' satisfaction; 10) Impact of the drug on the PrEP users' perceived quality of life; 11) Impact of the drug on the PrEP users' lifestyle; 12) Impact of the drug on sexual behaviours disinhibition	1 – The prevention strategy presents a decrease in individuals reported outcomes, considering the clinicians' perspective 2 – The prevention strategy presents no impact in individuals reported outcomes, considering the clinicians' perspective 3 – The prevention strategy presents an increase in individuals reported outcomes, considering the clinicians' perspective

continues

follows

Tab. I. Methods used for the deployment of the EUnetHTA dimensions.

	Social and ethical perceived aspects: the PrEP users' point of view	Definition of the PrEP users' awareness and knowledge with regard its adoption into the clinical practice, by means of an online questionnaire administration, filled in by potentially PrEP users	1 – The prevention strategy presents a decrease in individuals reported outcomes, considering the potential PrEP users' perspectives 2 – The prevention strategy presents no impact in individuals reported outcomes, considering the potential PrEP users' perspectives 3 – The prevention strategy presents an increase in individuals reported outcomes, considering the potential PrEP users' perspectives
Legal aspects	*Legal perceived aspects*	The 35 clinicians involved in the analysis, completed a comparative qualitative questionnaire, based on a 7-item Likert scale (ranging from -3 to +3), considering the following items: 1) Authorization level (national/European/international); 2) Legal impact on safety issues; 3) Infringement of intellectual property rights; 4) Impact on the production warranties; 5) Need to regulate the drug acquisition and costs; 6) The legislation covers the regulation of technology, for all categories of users; 7) Impact on the not-availability of PrEP in hospitals	1 – The prevention strategy presents the need to regulate its acquisition 2 – The prevention strategy presents no need to regulate its acquisition 3 – The prevention strategy presents an improvement of the related legal concerns
Organizational aspects	*Organizational perceived aspects*	The 35 clinicians involved in the analysis completed a comparative qualitative questionnaire, using a 7-item Likert scale (ranging from -3 to +3), in accordance with the following aspects: 1) Additional staff; 2) Training courses devoted to Infectious Disease clinicians; 3) Training courses devoted to healthcare professionals; 4) Training courses devoted to potentially PrEP users; 5) Internal hospital meetings; 6) Additional rooms and services; 7) Additional furniture; 8) Impact on the internal processes; 9) Impact on the processes between the Pharmaceutical Department and the Infectious Diseases Department; 10) Impact on the number of access; 11) Impact on the number of hematological exams, specialist visits related to the administration of the drug; 12) Impact on the management of other infectious diseases, different from HIV and HBV; 13) Organisational impact on the development of complications and adverse events; 14) Organisational impact on the development of drug toxicities and resistances; 15) Impact on the taking in charge of a higher number of users	1 – The prevention strategy presents a qualitative negative impact, since it requires important organizational efforts without any advantage for the hospital 2 – The prevention strategy presents no qualitative organizational impact 3 – The prevention strategy presents a qualitative positive impact, since it requires small organizational efforts with some advantages for the hospital
	Organizational quantitative aspects	Definition of the organizational ceasing or incremental costs related to the prevention strategies under assessment, considering additional persons, training courses, additional equipment, spaces or rooms	1 – The prevention strategy requires important and significant organizational investments 2 – The prevention strategy requires no or small organizational investment 3 – The prevention strategy presents the possibility to free-up organizational resources

approach [17] was implemented, thus simulating the appraisal phase.

At first, the domains were prioritized by the 35 clinicians involved, using a rating scale ranging from 1 (more important dimension), to 9 (less important dimension).

Furthermore, the quality of the information retrieved for the deployment of each EUnetHTA domain was evaluated by three Medical Directors, that assigned to each sub-dimension (listed in Tab. I), a three-level mark (ranging from a minimum of 1 – less performant – to a maximum of 3 – more performant –), after having carefully read a first draft of the evidence proposed in the present manuscript, in order to fully understand the potential impacts of the PrEP introduction or not introduction. Detailed information with regard the specific score assigned to each domain are reported in Table I.

This experiment was carried out to lead to a final concise result, useful in the choice of the "preferable" technology, supporting the appraisal phase and the policy-making process.

The final score was obtained by multiplying the normalized score, calculated for each domain, with the normalized value of priority, as suggested by scientific evidence [17]; the higher the score acquired, the more preferable is the technology.

Results

Assessment of the domains

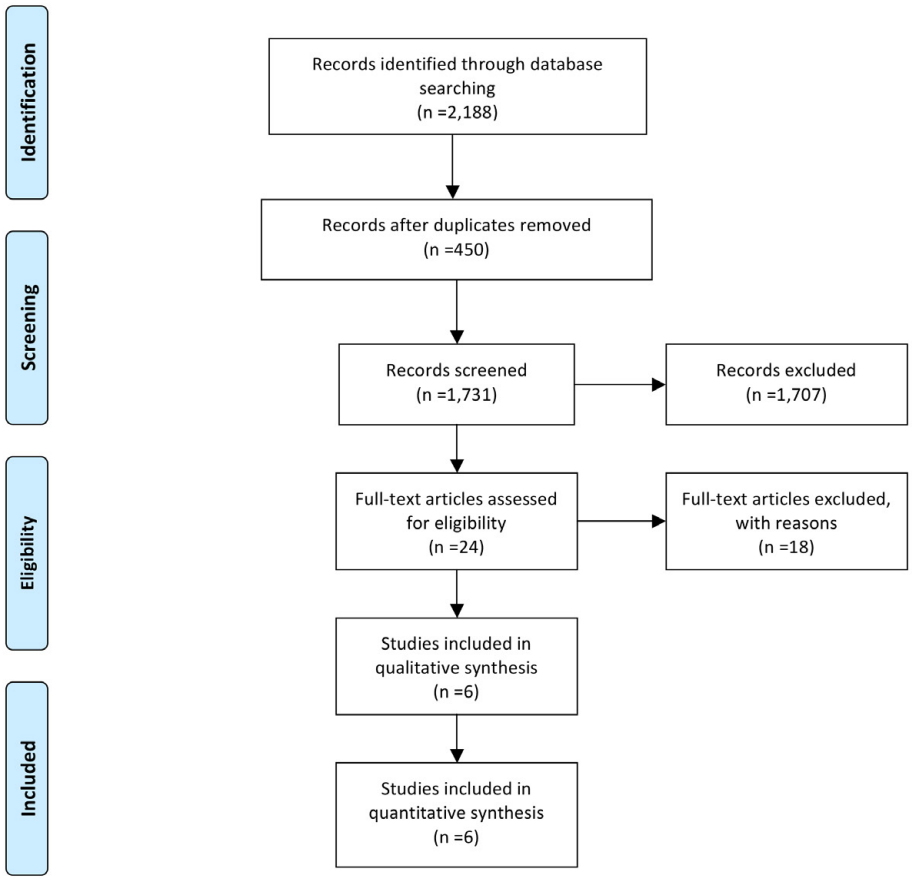
Results from literature review

Out of 2,118 papers identified through databases searching, according to the proposed PICO of the study, only six of them [3-6, 29-30] met the inclusion criteria, in accordance with the above-mentioned search strategy, focusing on the administration of PrEP for high-risk individuals as detailed in the Prisma Flow Chart for literature synthesis (Fig. 1). In particular, four of them [3-6] were RCTs investigating the effectiveness and the safety profiles within the target populations, and two of them [29, 30] were meta-analysis with regard to the effectiveness of the traditional preventive strategies. The rejected articles had different aims, without focusing the attention on efficacy/safety data, nor focusing on different populations than MSM, IDUs or SCs.

The literature review revealed the lack of scientific evidence concerning the head-to-head comparison of PrEP, as preventive strategy, used in add-on with the traditional preventive strategies (condoms or NSPs), or used alone, in particular observing the safety, and the efficacy profile of the alternatives.

Despite the above missing information, the articles included in the analysis presented quality and reliable data

Fig. 1. PRISMA 2009 flow diagram.



assessed, in accordance with the ROBINS II Cochrane risk of bias tool, the CASP checklist and the AMSTAR tool. Focusing on the RCTs included, useful for the retrieval of PrEP efficacy and safety profile, ROBINS II tool revealed that the risk of bias was not high.

All the studies were at low risk of bias since the classification of PrEP *vs* control was made clearly. The outcomes measurement proved to be relevant in most cases, and both positive and negative outcomes were determined and explained. According to scientific evidence [3-6], the innovative technology would lead to an increase in drug-related adverse events, whose incidence rates and economic evaluation are presented in Table II.

Furthermore, since PrEP presents a protective effect only with regard to HIV infection, even if used as an add-on strategy, and considering the real-life adherence to condoms and NSPs strategies, individuals could acquire other sexually transmitted/blood borne infections [3-5], resulting in a final worst safety profile. The general population presents an HIV occurrence rate equal to 33% [2], with a consequent NHS resource absorption per patient of € 11,694.86 and an individual's "out-of-pocket" expenditure of € 751.94.

Focusing on the efficacy profile, the parameter used in the present HTA for this specific domain, was the ability of each strategy to prevent the individual from HIV infection and derived from literature evidence available on the topic. With regard to the baseline scenario, condoms and the NSPs present an efficacy rate equal to 99% [29] and 17% [30], respectively. On the contrary, literature declares an efficacy rate of 86% for MSM [3, 4], 75% for SCs [6], and 48.9% for IDUs [5], strictly dependent form treatment adherence [31].

Results from the economic evaluation

Before conducting the economic evaluation, the number of HIV negative high-risk individuals, and, thus, individuals potentially eligible for PrEP treatment, was defined. Data derived from the number of individuals approaching the 35 Italian Infectious Disease Departments involved, for the conduction of the HIV test or for counselling were projected. Forecasting collected hospital data declared, considering the entire Italian potential population, it emerged that at least 16,577 individuals could be eligible for PrEP: 6,653 SCs, 5,943 MSM and 3,981 IDUs were considered and projected in the present economic analysis.

At first, as reported in Table II, the management cost of drug-related AEs and STDs developed by the PrEP population were considered.

Furthermore, the annual cost of each prevention strategy was accordingly investigated, assuming a 12-month time horizon and valorizing all the items of cost according to the reimbursement tariffs of the Italian NHS valid

Tab. II. Safety profile.

Drug-related adverse events: incidence and costs					
Drug-related adverse events	**Men who have sex with other men [3, 4]**	**Serodiscordant couples [3, 4]**	**Injection drug users [3-5]**	**Cost for the NHS**	**Cost for citizen**
Nausea	8%	8%	8%	€ 52.74	€ 22.15
Vomiting	2%	2%	5%	€ 52.74	€ 22.15
Diarrhea	4%	4%	11%	€ 1,822.21	€ 306.26
Abdominal pain	7%	7%	9%	€ 50.00	€ 21.00
Bone disease	2%	2%	2%	€ 661.44	€ 527.40
Creatinine Increase	18%	10%	7%	€ 1,474.61	€ 549.73
Headache	8%	8%	8%	€ 20.07	€ 8.43
Rash	8%	8%	8%	€ 760.13	€ 264.72
Other sexually transmitted/ blood borne infections	**% PREP [3, 4]**	**% NO PREP [2]**	**Cost for the NHS**	**Cost for citizen**	
HCV	1%	2%	€ 8,545.97	€ 950.89	
Syphilis	10%	9%	€ 117.75	€ 49.55	
Chlamydia	27%	22%	€ 76.00	€ 31.98	
Gonorrhea	38%	37%	€ 88.67	€ 37.31	
Rectal or vaginal Infection	36%	32%	€ 267.72	€ 112.66	

for the years 2018/2019. The annual average cost (per person) of condoms was hypothesized equal to € 192.00; whereas the annual average cost (per person) of NSPs was equal to € 75.25, being consistent with literature evidence [31]. On the one hand, given an average value for each condom equal to € 1.00, the model assumed a use of this strategy four times a week [6]. On the other hand, the average cost for a clean and sterile syringe was equal to € 0.056, with on average 4 doses per day [32].

With regard to the branded drug, the model assumed a drug cost equal to € 5,339.95 and € 9,011.28 per year (per person), in case of NHS reimbursement and citizens purchased respectively. If the off-patent drug was used and introduced into the clinical practice, a cost equal to € 1,601.99 and to € 2,703.38 was hypothesized, considering the NHS and the citizen's perspective respectively.

In addition to the annual economic value of PrEP drug, its monitoring cost was evaluated. Patients should attend at least 2 specialist visits, and 2 follow-up medical controls, as well as conduct full blood test panels, with the inclusion of creatinine, phosphorus, urine, proteinuria, and tests for sexually transmitted diseases, such as HIV, HCV, HBV and syphilis. These procedures required on average € 306.40 and € 68.62 considering the NHS and the patient point of view respectively.

The following tables reported the budget impact analysis derived from the introduction of PrEP, both as an add-on, and as a substitute strategy to the traditional prevention technologies, considering branded (Tab. III) and off-patent (Tab. IV) drugs.

If PrEP is used as an "add-on" strategy, distributed and paid by the NHS, considering branded drugs, NHS investments would increase significantly (+40%),

Tab. III. Budget impact analysis, considering PrEP as an add-on strategy to condoms and NSPs.

	100% PrEP reimbursed by the Italian NHS, considering branded drugs				**100% PrEP purchased by citizens, considering branded drugs**			
	Italian NHS point of view				**Italian NHS point of view**			
	Baseline scenario	Innovative scenario	Difference	Difference %	Baseline scenario	Innovative scenario	Difference	Difference %
MSM and sexual risk	€ 47,049,798	€ 114,119,651	€ 67,069,852	143%	€ 47,049,798	€ 20,491,208	-€ 26,558,590	-56%
SCs	€ 52,670,757	€ 131,873,657	€ 79,202,900	150%	€ 52,670,757	€ 29,012,864	-€ 23,657,893	-45%
IDUs	€ 99,007,920	€ 31,559,987	-€ 67,447,933	-68%	€ 99,007,920	€ 24,948,467	-€ 74,059,453	-75%
Total high-risk population	**€ 198,728,475**	**€ 277,553,294**	**€ 78,824,819**	**40%**	**€ 198,728,475**	**€ 74,452,539**	**-€ 124,275,936**	**-63%**
	PrEP users' point of view				**PrEP users' point of view**			
	Baseline scenario	Innovative scenario	Difference	Difference %	Baseline scenario	Innovative scenario	Difference	Difference %
MSM and sexual risk	€ 5,183,416	€ 6,720,833	€ 1,537,416	30%	€ 5,183,416	€ 164,720,834	€ 159,537,418	3078%
SCs	€ 5,802,670	€ 7,120,791	€ 1,318,121	23%	€ 5,802,670	€ 180,700,582	€ 174,897,912	3014%
IDUs	€ 6,498,759	€ 2,778,990	-€ 3,719,769	-57%	€ 6,498,759	€ 87,616,622	€ 81,117,862	1248%
Total high-risk population	**€ 17,484,846**	**€ 16,620,614**	**-€ 864,232**	**-5%**	**€ 17,484,846**	**€ 433,038,038**	**€ 415,553,192**	**2377%**
	100% PrEP reimbursed by the Italian NHS, considering off-patent drugs				**100% PrEP purchased by citizens, considering of patent drugs**			
	Italian NHS point of view				**Italian NHS point of view**			
	Baseline scenario	Innovative scenario	Difference	Difference %	Baseline scenario	Innovative scenario	Difference	Difference %
MSM and sexual risk	€ 47,049,798	€ 48,579,741	€ 1,529,943	3%	€ 47,049,798	€ 20,491,208	-€ 26,558,590	-56%
SCs	€ 52,670,757	€ 59,871,102	€ 7,200,345	14%	€ 52,670,757	€ 29,012,864	-€ 23,657,893	-45%
IDUs	€ 99,007,920	€ 16,772,989	-€ 82,234,931	-83%	€ 99,007,920	€ 24,948,467	-€ 74,059,453	-75%
Total high-risk population	**€ 198,728,475**	**€ 125,223,832**	**-€ 73,504,643**	**-37%**	**€ 198,728,475**	**€ 74,452,539**	**-€ 124,275,936**	**-63%**
	PrEP users'point of view				**PrEP users'point of view**			
	Baseline scenario	Innovative scenario	Difference	Difference %	Baseline scenario	Innovative scenario	Difference	Difference %
MSM and sexual risk	€ 5,183,416	€ 6,720,833	€ 1,537,416	30%	€ 5,183,416	€ 54,120,833	€ 48,937,417	944%
SCs	€ 5,802,670	€ 7,120,791	€ 1,318,121	23%	€ 5,802,670	€ 59,194,728	€ 53,392,058	920%
IDUs	€ 6,498,759	€ 2,778,990	-€ 3,719,769	-57%	€ 6,498,759	€ 30,040,415	€ 23,541,656	362%
Total high-risk population	**€ 17,484,846**	**€ 16,620,614**	**-€ 864,232**	**-5%**	**€ 17,484,846**	**€ 143,355,976**	**€ 125,871,130**	**720%**

Tab. IV. Budget impact analysis, considering PrEP as a substitute strategy to condoms and NSPs.

	100% PrEP reimbursed by the Italian NHS, considering branded drugs				100% PrEP purchased by citizens, considering branded drugs			
	Italian NHS point of view				Italian NHS point of view			
	Baseline scenario	Innovative scenario	Difference	Difference %	Baseline scenario	Innovative scenario	Difference	Difference %
MSM and sexual risk	€ 44,524,325	€ 126,062,875	€ 81,538,550	183%	€ 44,524,325	€ 35,401,844	-€ 9,122,482	-20%
SCs	€ 31,786,125	€ 150,349,598	€ 118,563,473	373%	€ 31,786,125	€ 52,557,382	€ 20,771,257	65%
IDUs	€ 45,636,657	€ 104,088,731	€ 58,452,074	128%	€ 45,636,657	€ 50,601,093	€ 4,964,435	11%
Total high-risk population	€ 121,947,108	€ 380,501,204	€ 258,554,096	212%	€ 121,947,108	€ 138,560,318	€ 16,613,210	14%
	PrEP users'point of view				PrEP users'point of view			
	Baseline scenario	Innovative scenario	Difference	Difference %	Baseline scenario	Innovative scenario	Difference	Difference %
MSM and sexual risk	€ 5,360,454	€ 6,149,260	€ 788,806	15%	€ 5,360,454	€ 159,141,693	€ 153,781,239	2869%
SCs	€ 3,826,853	€ 6,895,607	€ 3,068,754	80%	€ 3,826,853	€ 171,922,065	€ 168,095,212	4393%
IDUs	€ 2,995,535	€ 4,658,200	€ 1,662,665	56%	€ 2,995,535	€ 94,919,735	€ 91,924,200	3069%
Total high-risk population	€ 12,182,842	€ 17,703,066	€ 5,520,224	45%	€ 12,182,842	€ 425,983,493	€ 413,800,651	3397%
	100% PrEP reimbursed by the Italian NHS, considering off-patent drugs				100% PrEP purchased by citizens, considering off-patent drugs			
	Italian NHS point of view				Italian NHS point of view			
	Baseline scenario	Innovative scenario	Difference	Difference %	Baseline scenario	Innovative scenario	Difference	Difference %
MSM and sexual risk	€ 44,524,325	€ 62,600,238	€ 18,075,913	41%	€ 44,524,325	€ 35,401,844	-€ 9,122,482	-20%
SCs	€ 31,786,125	€ 81,895,138	€ 50,109,013	158%	€ 31,786,125	€ 52,557,382	€ 20,771,257	65%
IDUs	€ 45,636,657	€ 66,647,434	€ 21,010,777	46%	€ 45,636,657	€ 50,601,093	€ 4,964,435	11%
Total high-risk population	€ 121,947,108	€ 211,142,810	€ 89,195,703	73%	€ 121,947,108	€ 138,560,318	€ 16,613,210	14%
	PrEP users'point of view				PrEP users'point of view			
	Baseline scenario	Innovative scenario	Difference	Difference %	Baseline scenario	Innovative scenario	Difference	Difference %
MSM and sexual risk	€ 5,360,454	€ 6,149,260	€ 788,806	15%	€ 5,360,454	€ 52,046,922	€ 46,686,468	871%
SCs	€ 3,826,853	€ 6,895,607	€ 3,068,754	80%	€ 3,826,853	€ 56,403,471	€ 52,576,618	1374%
IDUs	€ 2,995,535	€ 4,658,200	€ 1,662,665	56%	€ 2,995,535	€ 31,736,620	€ 28,741,086	959%
Total high-risk population	€ 12,182,842	€ 17,703,066	€ 5,520,224	45%	€ 12,182,842	€ 140,187,013	€ 128,004,171	1051%

while NHS economic benefits (-63%) are found if PrEP is purchased by citizens (individuals' investment: +2,377%). As for off-patent drugs, the NHS would benefit from an advantage (-37%) and a shrinkage of the patients' "out-of-pocket" expenditure (+720%).

If PrEP is introduced as a "substitute" strategy, the economic burden would be higher, both for the NHS (+212%) and the citizens' (+3,397%). Even considering the off-patent drug, the NHS and patients face a relevant economic challenge, equal to +73% and +1,051% respectively.

Results from the qualitative approaches

As stated in the Method section, the qualitative assessment of the ethical, social and organisational dimensions was conducted through the involvement of 35 clinicians referring to different Italian Regions, giving a representativeness of the Italian landscape. With respect to the geographical origin, 49%, 31% and 20% of clinicians referred to north, south and islands, and centre of Italy respectively.

A synthesis of the clinicians' perceptions is reported in Table V, in terms of incremental or decremental value of PrEP (from -3 to + 3), in comparison with the baseline scenario, without PrEP (always neural, and equal to 0).

While the use of traditional prevention strategies did not have an impact on the NHS accessibility, a critical impact on ethical aspects emerged in case of PrEP introduction (-0.50 *vs* 0.00, $p < 0.05$), in particular in case of full payment of PrEP by the potential users (-1.84 *vs* 0.00, $p < 0.05$), since this drug is expensive, thus limiting its accessibility to the different population categories.

However, the clinicians declared an improvement both in the PrEP users' quality of life (0.58 *vs* 0.00, $p < 0.05$) and in their satisfaction (1.52 *vs* 0.00, $p < 0.05$), even if professionals have the perception of disinhibition of the sexual behavior of the individuals who assume PrEP (-2.20 *vs* 0.00, $p < 0.05$), thus being consistent with literature evidence [33-37].

From a legal point of view, investments are required to regulate the use of PrEP in hospitals (-1.21 *vs* 0.00, $p < 0.05$). Despite the EMA approval, in Italy the administration of this preventive strategy should be regulated and inserted in the clinical protocols.

With regard to the organizational impact, clinicians declared an increase in staff workflow (-1.62 *vs* 0.00, $p < 0.05$) due to the high number of HIV negative patients attending regular doctor appointments, and follow-up procedures, thus requiring additional clinicians (-1.44 *vs* 0.00, $p < 0.05$). The assessment of the quantitative organizational impact, assuming a 12-month time horizon, confirmed their perceptions, considering an Infectious Disease Department (taking in charge on average 745 HIV+ treatment-experienced and 32 HIV+ treatment-naïve individuals, 777 in total). There emerged the need to invest in additional working professionals, as well as to organize specific training courses devoted to individuals directly involved in the provision of PrEP to the citizens. At least 2 clinicians, 2 nurses, and 1 psychologist could be involved in the training activities, with an economic resources' absorption equal to € 1,750.00 only for the first year.

Ninety percent of the Infectious Disease Department involved in the analysis, required the creation of a new ambulatory devoted to the high-risk population potentially assuming PrEP, with a consequent additional investment in both medical supplies and equipment, for an average amount equal to € 666.26 and to € 1,158.7 respectively. At the 12-month time point of the base-case scenario for market penetration, a medium size hospital would invest on average a total amount of € 3,574.96, for organizational arrangements.

Tab. V. Clinicians' perceptions.

The clinicians' perceptions		
Ethical aspects	**PREP**	**No PREP**
Access to care on local level	0.25	0.00
Access to care for persons on a legally protected status	0.69	0.00
Impact of the preventive strategy on the accessibility to care related to the management of adverse events	-0.81	0.00
Generation of health migration phenomena	0.43	0.00
Impact of the preventive strategy on the patients' willingness to pay	-1.19	0.00
General equity	-0.44	0.00
Accessibility to the prevention strategy, in case of full payment by the potential PrEP users	-1.84	0.00
Accessibility to the prevention strategy, in case of co-payment.	-1.09	0.00
Average value	*-0.50*	*0.00*
Social aspects	**PREP**	**No PREP**
Ability of the drug to protect the potential PrEP users' autonomy	0.00	0.00
Ability of the drug to protect the human rights	0.00	0.00
Ability of the drug to protect the potential PrEP users' integrity	0.00	0.00
Ability of the drug to protect the potential PrEP users' dignity	0.00	0.00
Impact of the drug on potential PrEP users' religion	0.00	0.00
Impact of the drug on social costs	-1.76	0.00
Impact of the drug on the citizens' medicalization	-0.91	0.00
Impact of the drug on the potential PrEP users' satisfaction	1.52	0.00
Impact of the drug on the potential PrEP users' perceived quality of life	0.98	0.00
Impact of the drug on the potential PrEP users' lifestyle	0.19	0.00
Impact of the drug on sexual behaviors disinhibition	-2.21	0.00
Average value	*-0.20*	*0.00*
Legal aspects	**PREP**	**No PREP**
Authorization level (national/European/international)	-1.94	0.00
Legal impact on safety issues	-1.18	0.00
Infringement of intellectual property rights	-0.48	0.00
Impact on the production warranties	-0.79	0.00
Need to regulate the drug acquisition and costs	-1.48	0.00
The legislation covers the regulation of technology for all categories of patients	-1.00	0.00
Impact on the not-availability of PrEP in hospitals	-1.58	0.00
Average value	*-1.21*	*0.00*
Organisational aspects	**PREP**	**No PREP**
Additional staff	-1.44	0.00
Training courses devoted to Infectious Disease clinicians	-1.35	0.00
Training courses devoted to healthcare professionals	-1.41	0.00
Training course devoted to potentially PrEP users	-1.38	0.00
Internal hospital meetings	-1.35	0.00
Additional rooms	-1.00	0.00
Additional furniture	-0.59	0.00
Impact on the internal processes	-1.26	0.00
Impact on the processes between the Pharmaceutical Department and the Infectious Diseases Department	-0.76	0.00
Impact of the number of access for conducting HIV tests	-0.68	0.00
Impact on the number of hematological exams, specialist visits related to the administration of the drug	-1.68	0.00
Impact on the management of other infectious diseases, different from HIV and HBV	-2.06	0.00
Organisational impact on the development of complications and adverse events	-0.47	0.00
Organisational impact on the development of drug toxicities and resistance	-1.21	0.00
Impact on the taking in charge of a higher number of individuals	-1.62	0.00
Average value	*-1.22*	*0.00*

Focusing on the potentially PrEP users' perceptions, the on-line survey involved 129 individuals referring to two different citizens' associations. Individuals were well-matched in terms of homosexuals (MSM) and heterosexuals (54% *vs* 46%) risks factors. The sample presented scarce knowledge of PrEP (2.78 ± 1.19), even if MSM reported a better awareness in comparison with heterosexuals (2.99 *vs* 2.54, p = 0.035). Individuals also declared poor information sharing, both from healthcare agencies and medical providers (1.77±0.72), and from

media information (1.51 ± 0.06). In this view, statistically significant differences emerged between MSM and heterosexuals, with regard to the perception concerning the quality of information from healthcare agencies and medical providers (1.60 *vs* 1.97, p = 0.010).
A total of 69 individuals (only 53.48% of the entire sample), revealed their intention to pay for the administration of PrEP, by introducing the drug into their personal healthcare budget, showing no difference between MSM and heterosexuals (50.7% *vs* 49.3%, p>0.05). Despite 70% (n = 49) of the individuals having the intention to pay for PrEP had a job, no relations emerged between the job category and the willingness to pay for PrEP (p > 0.05), thus leading to the fact that having a job is not a determinant of the individuals' willingness-to-pay.
The sample agreed that PrEP is not responsible of any modification of daily activities (1.82 ± 0.20), sexual behaviors (2.33 ± 0.11), as well as a possible accentuation of sexual disinhibition (2.18 ± 0.10), with no statistically significant differences between the two groups. In addition, they do not think that the development of drug-related adverse events could be a reason for PrEP discontinuation (2.78 ± 0.09), adhering to their treatment, in order to achieve clinical effectiveness. However, they felt slightly uncomfortable in going to the hospital to obtain PrEP (3.09 ± 0.09), and in conducting diagnostic and blood tests for PrEP (3.29 ± 0.09), as follow-up procedures.

Appraisal phase

The experimental appraisal phase required both a prioritisation of the domains (performed by the 35 Infectious Disease Clinicians), and the implementation of a multi-criteria decision approach (based on the above results and the marks proposed by three Medical Directors). The prioritization reported that the most important aspects were both safety and efficacy profiles, as well as the social domain. The MCDA revealed that the adoption of PrEP resulted in a score of 0.484, underlying a disadvantage for acquiring the new HIV prevention strategy, with respect to the baseline scenario (0.516), even if the alternative technology could present a positive impact from a safety and social perspective (Tab. VI).

Discussion

PrEP is now established as a biomedical HIV prevention approach, with the potential to contribute significantly to global HIV prevention efforts and decreased HIV incidence rates, in several different populations considered at high-risk of acquiring HIV.
However, the population health benefits and costs of adopting PrEP remain unclear, since there are multiple barriers to worldwide provision of PreP to all eligible high-risk populations [38]. Concerns around safety and potential side effects, effectiveness, cost, and adherence challenges become key issues to be considered. Thus, the present study paved the way to the determination of potential strengths and weaknesses of PrEP adoption into the clinical practice.
Accordingly, advantages include a reduction of HIV infection, if associated with the traditional prevention strategies (only if PrEP is used as an "add-on" strategy), as demonstrated in recent clinical trials with regard to the administration of PrEP to IDUs [5], MSM [3,4] and SCs [6]. This consideration would suggest that PrEP could substantially reduce the lifetime risk of HIV infection for individuals at high-risk also in Italy, thus protecting the community from HIV infection.
Results showed that PrEP would increase satisfaction and quality of life for its users, justifying the social acceptance and implications of the drug. Focusing the attention on the potential population eligible to PrEP, the study reported a limited knowledge of PrEP in Italy, suggesting the setting up and implementation of PrEP training programs, targeted at the eligible population through public health campaigns, in order to raise awareness and disseminate correct information. In this regard, efforts should be taken to challenge the stigma and marginalization of minority groups, such as IDUs and MSM, both within the community and at governmental level.
However, PrEP would also provoke safety and economic concerns that should be taken into consideration from the policy-makers point of view, and that significantly impact both on Italian healthcare expenditure and on the citizens' healthcare budget. The deployment of

Tab. VI. Appraisal phase.

	Normalised score of the domains		Prioritisation	Final result	
Domains	Baseline scenario	Introduction of PrEP		Baseline scenario	Introduction of PrEP
Health problem and current use of technology	0.471	0.529	0.044	0.021	0.024
Description and technical characteristics	0.500	0.500	0.022	0.011	0.011
Safety	0.438	0.563	0.178	0.078	0.100
Clinical effectiveness	0.545	0.455	0.200	0.109	0.091
Cost and economic evaluation	0.611	0.389	0.133	0.081	0.052
Ethical analysis	0.625	0.375	0.089	0.056	0.033
Social aspects	0.485	0.515	0.156	0.075	0.080
Legal aspects	0.375	0.625	0.067	0.025	0.042
Organisational aspects	0.538	0.462	0.111	0.060	0.051
Total	**4.588**	**4.412**		**0.516**	**0.484**

the safety profile, derived from the HTA exercise, is consistent with scientific evidence available. Despite PrEP remaining significantly protective against HIV infection, its general safety is strictly related to the high-risk population behaviors, in terms of development of other sexually transmitted diseases if used as a substitute prevention strategy. In addition, some studies reported small, subclinical decreases in liver function [6, 39] and bone mineral density [40, 41].

Focusing on the economic impact, results revealed the impossibility for the Italian NHS to cover the cost of drugs, despite nowadays the off-patent drug is available. In particular, the acquisition of PrEP especially depends on its reimbursement and consumption, with the generation of financial problems, both in term of NHS investment and in terms of citizens' "out-of pocket" expenditure.

However, except for the cost of the preventive strategy itself, the management of the potential PrEP users', thus considering also the so-called risk compensation, require additional healthcare investments, since the need to intensively monitor the PrEP users, with a consequent negative organizational impact, due to the taking in charge of more patients.

At least, in the investigated setting, the best cost-containing strategy would be the use of PrEP with off-patent molecules, thus decreasing the economic burden of the innovative prevention strategy. Hence, in order to limit the need of the above-mentioned organizational investment, the potential opportunity to create specific ambulatories devoted to PrEP users' especially for medium and big size hospitals should be considered. Moving on from these elements, and considering the results derived from the MCDA approach, the decision to not implement PrEP into the clinical practice, with a public expenditure, could be the preferable option, at least, within the Italian healthcare setting. However, the quantitative difference between the baseline and the innovative scenario is not significant (0.516 *vs* 0.484, $p > 0.05$), thus leading to the consideration that the use of PrEP could be a positive solution devoted to high-risk patients given its high clinical effectiveness if applied as an "add-on" strategy considering the off-patent drug cost. The social, economic and organizational conditions of the Italian NHS could not be ready yet for the introduction of the prophylaxis, requiring the definition of proper clinical pathways to become sustainable. Given this fact, the preferable solution could be the adoption of PrEP as an "add-on" prevention strategy, but supposing also a co-payment, in order to guarantee the sustainability and affordability of the strategy. Considering a 50% co-payment, with the branded drugs, an economic burden emerged both for the NHS (+26%) and the patients (1,193%), whereas, with the off-patent drug the NHS would benefit from an economic advantage equal to -53% and patients would invest for 218%, in comparison with a baseline scenario consisted of the purchase of condoms and NSPs.

A future step of further research could be the integration of the present results, considering the innovative molecules emtricitabine/tenofovir alafenamide (FTC/TAF), that has been recently approved for PrEP use, by the American Food and Drugs Administration (FDA). The introduction of TAF, instead of TDF, could thus improve the economic resources absorption of the innovative preventive strategy, but also the safety profile, in terms of management cost for drug-related side effect. TAF regimen has a reduced potential for causing kidney injury and thinning bones than TDF, with a consequent positive impact both on the economic pathway, and on the organisational aspects, with a lower impact of controls, laboratory exams and specialistic visits.

In conclusions, since the study was not design for the collection of PrEP users' reported outcome, in terms of quality of life, it could be interesting to investigate this topic thus examining the head to head differences between PrEP users and high-risk individuals, not assuming PrEP.

Conclusions

To the best of the authors' knowledge, this study could be considered the first attempt to fully evaluate the implications derived from the PrEP introduction into the clinical practice, with an holistic vision of all the impacts that could play an important role in the PrEP introduction' choice, offering new insights to advance the ongoing debate regarding the relevance and feasibility of its adoption, in contexts characterized by the paucity of economic and human resources.

On the whole, a proper stratification of the potential population eligible to PrEP could optimize the clinicians' choice and the correct use of PrEP, with a lower and more sustainable economic impact, and a maximization of both safety and efficacy profile. In particular, the individuals who are at risk, should identify themselves to their doctors, and every effort should be made, to ensure that a safe, stigma-free environment is created for them.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

EF, GC, ADB, GR, ME, RI, and DC made substantial contributions to conception and design. LF, EF, EG and VP acquire and analyzed data, and wrote the paper. GC, ADB, GR, ME, RI, and DC critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

References

[1] Puro V, Palummieri A, De Carli G, Piselli P, Ippolito G. Attitude towards antiretroviral pre-exposure prophylaxis (PrEP) prescription among HIV specialists. BMC Infect Dis 2013;13:217. https://doi.org/10.1186/1471-2334-13-217

[2] Joint United Nations Programme on HIV/AIDS (UNAIDS). Geneva: UNAIDS Data 2017. Available at: http://www.unaids.org/en/resources/documents/2017/2017_data_book

[3] Molina JM, Capitant C, Spire B, Pialoux G, Cotte L, Charreau I, Tremblay C, Le Gall JM, Cua E, Pasquet A, Raffi F, Pintado C, Chidiac C, Chas J, Charbonneau P, Delaugerre C, Suzan-Monti M, Loze Benedicte, Fonsart J, Peytavin G, Cheret A, Timsit J, Girard G, Lorente N, Préau M, Rooney JF, Wainberg MA, Thompson D, Rozenbaum W, Doré V, Marchand L, Simon MC, Etien N, Aboulker JP, Meyer L, Delfraissy JF, ANRS IPERGAY Study Group. On-demand preexposure prophylaxis in men at high risk for HIV-1 infection. N Engl J Med 2015;373:2237-46. https://doi.org/10.1056/NEJMoa1506273

[4] McCormack S, Dunn DT, Desai M, Dolling DI, Gafos M, Gilson R, Sullivan AK, Clarke A, Reeves I, Schembri G, Mackie N, Bowman C, Lacey CJ, Apea V, Brady M, Fox J, Taylor S, Antonucci S, Khoo SH, Rooney J, Nardone A, Fisher M, McOwen A, Phillips AN, Johnson AM, Gazzard B, Gill ON. Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection (PROUD): effectiveness results from the pilot phase of a pragmatic open-label randomised trial. Lancet 2016;387:53-60. https://doi.org/10.1016/S0140-6736(15)00056-2

[5] Choopanya K, Martin M, Suntharasamai P, Sangkum U, Mock PA, Leethochawalit M, Chiamwongpaet S, Kitisin P, Natrujirote P, Kittimunkong S, Chuachoowong R, Gvetadze RJ, McNicholl JM, Paxton LA, Curlin ME, Hendrix CW, Vanichseni S; Bangkok Tenofovir Study Group. Antiretroviral prophylaxis for HIV infection in injecting drug users in Bangkok, Thailand (the Bangkok Tenofovir Study): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet (London, England) 2013;381:2083-90. https://doi.org/10.1016/S0140-6736(13)61127-7

[6] Baeten JM, Donnell D, Ndase P, Mugo NR, Campbell JD, Wangisi J, Tappero JW, Bukusi EA, Cohen CR, Katabira E, Ronald A, Tumwesigye E, Were E, Fife KH, Kiarie J, Farquhar C, John-Stewart G, Kakia A, Odoyo J, Mucunguzi A, Nakku-Joloba E, Twesigye R, Ngure K, Apaka C, Tamooh H, Gabona F, Mujugira A, Panteleeff D, Thomas KK, Kidoguchi L, Krows M, Revall J, Morrison S, Haugen H, Emmanuel-Ogier M, Ondrejcek L, Coombs R, Frenkel L, Hendrix C, Bumpus NN, Bangsberg D, Haberer JE, Steven WS, Lingappa JR, Celum C, for the Partners PrEP Study Team. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. N Engl J Med 2012;367:399-410. https://doi.org/10.1056/NEJMoa1108524

[7] Centres for Disease Control and Prevention. Preexposure prophylaxis for the prevention of HIV infection in the United States. A clinical practice guidelines. 2014. Available at: https://www.cdc.gov/hiv/pdf/guidelines/PrEPguidelines2014.pdf

[8] Hayes R, Schmidt AJ, Pharris A, Azad Y, Brown AE, Weatherburn P, Hickson F, Delpech V, Noori T and the ECDC Dublin Declaration Monitoring Network. Estimating the 'PrEP Gap': how implementation and access to PrEP differ between countries in Europe and Central Asia in 2019. Euro Surveill 2019;24(41). https://doi.org/10.2807/1560-7917.ES.2019.24.41.1900598

[9] European Centre for Disease Prevention and Control (ECDC). Evidence brief: Pre-exposure prophylaxis for HIV prevention in Europe. Stockholm: ECDC 2016. Available from: https://www.ecdc.europa.eu/en/publications-data/ecdc-evidence-brief-pre-exposure-prophylaxis-hiv-prevention-europe

[10] Di Biagio A, Riccardi N, Signori A, Maserati R, Nozza S, Gori A, Bonora S, Borderi M, Ripamonti D, Rossi MC, Orofino G, Quirino T, Nunnari G, Celesia BM, Martini S, Sagnelli C, Mazzola G, Colletti P, Bartolozzi D, Bini T, Ladisa N, Castelnuovo F, Saracino A, Lo Caputo S. PrEP in Italy: the time may be ripe but who's paying the bill? A nationwide survey on physicians' attitudes towards using antiretrovirals to prevent HIV infection. PLoS One 2017;12:e0181433. https://doi.org/10.1371/journal.pone.0181433

[11] Rossotti R, Borghi V, Callegaro AP, Micheli V , Bruzzone B, Colao G, Gismondo MR, Vicenti I, Penco G, Zazzi M, Rusconi S. Impact of circulating resistance-associated mutations on HIV pre-exposure prophylaxis (PrEP) efficacy: modeling from antiretroviral resistance cohort analysis (ARCA) national database. J Clin Virol 2016;83:48-53. https://doi.org/10.1016/j.jcv.2016.08.293

[11] Restelli U, Croce D, Rizzardini G. Antiretroviral treatments' durability and costs: important elements in the choice of first-line therapy. AIDS 2016;30:2247-9. https://doi.org/10.1097/QAD.0000000000001210

[12] Drummond M.F, Schwartz JS, Jönsson B, Luce BR, Neumann PJ, Siebert U, Sullivan SD. Key principles for the improved conduct of health technology assessments for resource allocation decisions. Int J Technol Assess Health Care 2008;24:244-58. https://doi.org/10.1017/S0266462308080343

[13] EUNetHTA, The HTA Core Model, Version3.0., April 2016. Available online at: http://eunethta.eu/sites/5026.fedimbo.belgium.be/files/HTACoreModel3.0.pdf

[14] Foglia E, Lettieri E, Ferrario L, Porazzi E, Garagiola E, Pagani R, Bonfanti M, Lazzarotti V, Manzini R, Masella C, Croce D. Technology assessment in hospitals: lessons learned from an empirical experiment. Int J Technol Assess Health Care 2017:1-9. https://doi.org/10.1017/S0266462317000356

[15] Radaelli G, Lettieri E, Masella C, Merlino L, Strada A, Tringali M. Implementation of Eunethta core model® in Lombardia: the VTS framework. Int J Technol Assess Health Care 2014;30:105-12. https://doi.org/10.1017/S0266462313000639

[16] Thokala P, Devlin N, Marsh K, Baltussen R, Boysen M, Kalo Z, Longrenn T, Mussen F, Peacock S, Watkins J, Ijzerman M. Multiple criteria decision analysis for health care decision making — an introduction: Report 1 of the ISPOR MCDA Emerging Good Practices Task Force. Value Health 2016;19:1-13. https://doi.org/10.1016/j.jval.2015.12.003

[17] Moher D, Liberati A, Tetzlaff J, Altman DG. The PRISMA Group Preferred Reporting Items for Systematic Reviews and Meta-Analyses: the PRISMA Statement. PLoS Med 2009;6(7). https://doi.org/10.1371/journal.pmed.1000097

[18] Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, Cates CJ, Cheng HY, Corbett MS, Eldridge SM,

Emberson JR, Hernán MA, Hopewell S, Hróbjartsson A, Junqueira DR, Jüni P, Kirkham JJ, Lasserson T, Li T, McAleenan A, Reeves BC, Shepperd S, Shrier I, Stewart LA, Tilling K, White IR, Whiting PF, Higgins JPT. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ 2019;366:l4898. https://doi.org/10.1136/bmj.l4898

[19] Shea BJ, Grimshaw JM, Wells GA, Boers M, Andersson N, Hamel C, Porter AC, Tugwell P, Moher D, Bouter LM. Development of AMSTAR: A measurement tool to assess the methodological quality of systematic reviews. BMC Med Res Methodol 2007;7:1-7. https://doi.org/10.1186/1471-2288-7-10

[20] Baker J. Activity-based costing and activity based management for healthcare. Gaithersburg: Aspen Publishers 1998.

[21] Mauskopf JA, Sullivans SD, Annemans L, Caro J, Mullins CD, Nuijten M, Orlewska E, Watkins J, Trueman P. Principles of good practice for budget impact analysis: report of the ISPOR task force on good research practices-budget impact analysis. Value Health 2007;10:336-47. https://doi.org/10.1111/j.1524-4733.2007.00187.x

[22] Quinn Patton M. Qualitative Research & Evaluation Methods, third edition. Sage publications 2002.

[23] https://www.jobpricing.it/blog/project/salary-outlook-seconda-edizione

[24] Weisbrod BA. The Valuation of Human Capital. Journal of Political Economy 1961;69:425-36. www.jstor.org/stable/1828532 (accessed June 9, 2020).

[25] Krol M, Brouwer W. How to estimate productivity costs in economic evaluations. Pharmacoeconomics 2014;32:335-44. https://doi.org/10.1007/s40273-014-0132-3

[26] http://www.sisreg.it/index.php?option=com_content&view=article&id=85&Itemid=112

[27] Mitton C, Dionne F, Damji R, Campbell D, Bryan S. Difficult decisions in times of constraint: criteria based Resource Allocation in the Vancouver Coastal Health Authority. BMC Health Serv Res 2011;11:169. https://doi.org/10.1186/1472-6963-11-169

[28] Weller SC. A meta-analysis of condom effectiveness in reducing sexually transmitted HIV. Soc Sci Med 1993;36:1635-44. https://doi.org/10.1016/0277-9536(93)90352-5

[29] Mathers BM, Degenhardt L, Ali H, Wiessing L, Hickman M, Mattick RP, Myers B, Ambekar A, Strathdee SA, 2009 Reference Group to the UN on HIV and Injecting Drug Use. HIV prevention, treatment, and care services for people who inject drugs: a systematic review of global, regional, and national coverage. Lancet 2010;375:1014-28. https://doi.org/10.1016/S0140-6736(10)60232-2

[30] Anderson PL, Glidden DV, Liu A, Buchbinder S, Lama JR, Vicente Guanira J, McMahan V, Bushman LR, Casapía M, Montoya-Herrera O, Veloso VG, Mayer KH, Chariyalertsak S, Schechter M, Bekker LG, Kallás EG, Grant RM, For the iPrEx Study Team. Emtricitabine-tenofovir exposure and pre-exposure prophylaxis efficacy in men who have sex with men. Sci Transl Med 2012;4(151). https://doi.org/10.1126/scitranslmed.3004006

[31] Wilson D, Fraser N. The economics and financing of harm reduction. The World Bank 2013.

[32] Cassell MM, Halperin DT, Shelton JD, Stanton D. Risk compensation: the Achilles' heel of innovations in HIV prevention? BMJ 2006;332:605-7. https://doi.org/10.1136/bmj.332.7541.605

[33] Cohen MS. HIV treatment as prevention and " The Swiss Statement": in for a dime, in for a dollar? Clin Infect Dis 2010;51:1323-24. https://doi.org/10.1086/656810

[34] Eaton LA, Kalichman S. Risk compensation in HIV prevention: Implications for vaccines, microbicides, and other biomedical HIV prevention technologies. Curr HIV/AIDS Rep 2007;4:165-72. https://doi.org/10.1007/s11904-007-0024-7

[35] Hogben M, Liddon N. Disinhibition and risk compensation: scope, definitions, and perspective. Sex Transm Dis 2008;35:1009-10. https://doi.org/10.1097/OLQ.0b013e31818eb752

[36] Underhill K. Study designs for identifying risk compensation behavior among users of biomedical HIV prevention technologies: balancing methodological rigor and research ethics. Soc Sci Med 2013;94:115-23. https://doi.org/10.1016/j.socscimed.2013.03.020

[37] Cáceres cF, O'reilly Kr, Mayer Kh, Baggaley r. PreP implementation: moving from trials to policy and practice. J Int AIDS Soc 2015;18 (Suppl 3). https://doi.org/10.7448/IAS.18.4.20222

[38] Van Damme L, Corneli A, Ahmed K, Ahmed K, Agot K, Lombaard J, Kapiga S, Malahleha M, Owino F, Manongi R, Onyango J, Temu L, Monedi MC, Mak'Oketch P, Makanda M, Reblin I, Makatu SE, Saylor L, Kiernan H, Kirkendale S, Wong C, Grant R, Kashuba A, Nanda K, Mandala J, Fransen K, Deese J, Crucitti T, Mastro TD, Taylor D, for the FEM-PrEP Study Group. Preexposure prophylaxis for HIV infection among African women. N Engl J Med 2012;367:411-22. https://doi.org/10.1056/NEJMoa1202614

[39] Kasonde M, Niska RW, Rose C, Henderson FL, Segolodi TM, Turner K, Smith DK, Thigpen MC, Paxton LA. Bone mineral density changes among HIV-uninfected young adults in a randomised trial of pre-exposure prophylaxis with tenofovir-emtricitabine or placebo in Botswana. PLoS One 2014;9:e90111. https://doi.org/10.1371/journal.pone.0090111

[40] Liu AY, Vittinghoff E, Sellmeyer DE, Irvin R, Mulligan K, Mayer K, Thompson M, Grant R, Pathak S, O'Hara B, Gvetadze R, Chillag K, Grohskopf L, Buchbinder SP. Bone mineral density in HIV-negative men participating in a tenofovir pre-exposure prophylaxis randomized clinical trial in San Francisco. PLoS One 011;6:e23688. https://doi.org/10.1371/journal.pone.0023688

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Correspondence: Lucrezia Ferrario, Centre for Health Economics, Social and Health Care Management, LIUC - Università Cattaneo, corso Matteotti 22, 21053 Castellanza (VA), Italy - Tel. +39 0331 572504 - E-mail: lferrario@liuc.it

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ORIGINAL ARTICLE

Validation of pediatric height estimation formulae in suburban communities in South-east Nigeria: a cross-sectional study

KENECHUKWU K. ILOH[1], OBIANUJU O. IGBOKWE[1], OGOCHUKWU N. ILOH[1], LINDA N. NWOKEJI-ONWE[2], UZOAMAKA C. AKUBUILO[1], OGECHUKWU H. NWACHUKWU[3], CHIDIEBERE D.I. OSUORAH[4]
[1] Department of Pediatrics, College of Medicine, University of Nigeria, Ituku/Ozalla, Enugu; [2] Department of Pediatrics, Alex Ekwueme Federal University Teaching Hospital Abakaliki Ebonyi State; [3] Department of Pediatrics, Enugu State University of Science and Technology, Enugu state Nigeria; [4] Child Survival Unit, Medical Research Council UK, The Gambia Unit

Keywords

Children • Height • Estimation • Accuracy

Summary

Background. *Height measurement is one of the common essential anthropometric measurements in clinical pediatrics. The most accurate method of determining a child's height is to measure the height. However, in emergency situations and some resource limited settings, obtaining the actual height of a child may not be feasible hence the need to estimate. The most common age-based formulae for height estimation in children is the Nelson-Wheech formula, 6n + 77 where n = age in years. The accuracy of this height estimation formulae has not been assessed in a developing setting like ours with high prevalence of malnutrition. This study therefore sought to evaluate the accuracy of the height estimation formula in children in communities across Enugu southeast Nigeria.*
Method. *Children 2-12 years old who met the inclusion criteria were enrolled over 12 months from three of the 17 Local Government Area of Enugu State. Height was measured using a standard stadiometer and estimated height was calculated 6n + 77. Data collected was analyzed using SPSS.*
Result. *Of the 4046 children enrolled, majority (86.1%) were of normal height. The formula underestimated height of children in the two, 3, 4, 5, 6, 7, 9 and 10 years old categories by a factor of 1.2%-10.0% while overestimating height in 8-year old children by 5.1%, 11-year old by 0.2% and 12-year by 2.9%. Overall, the estimated height using the formula was within ± 10% agreement of the actual height of surveyed children in 77.0% of children surveyed.*
Conclusion. *The 6n + 77 formula is a reasonable but not entirely accurate for height estimation for children in our setting.*

Introduction

Accurate measurement of height is important for assessment of growth, development and nutritional status. Not only is measurement of height necessary for tracking growth and development in children, it is as well important for determining body surface area for pharmacological dosing. Additionally, height is essential in the calculation of body mass index (BMI), one of the most widely used screening tools to monitor nutritional status and obesity. Knowledge of a patient's height is vital for daily practice in the intensive care unit, for either assessment of renal function [1], calculating cardiac function indices or tidal volume setting [2]. In some circumstances, actual measurements of weight or height may not be feasible and estimates becomes imperative. The most accurate method of determining a child's height is to measure the height with an appropriate height measuring tool. However, in some settings, measuring the actual height may not be possible as a result of the child's clinical status and/or unavailability of a height measurement tool. The most used formulae in our setting for height estimation in children aged 2-12 years is *6n + 77* where n = age in years [3]. This so called ''Nelson-Wheech formula'' has little literature as to how it was derived but has universal usage. The accuracy of height estimates derived from this formula has not been validated in a developing setting like ours where an estimated 2 million children suffer from severe acute malnutrition [4]. This study therefore sought to assess the accuracy of the height estimation formula in children in communities in Enugu southeast Nigeria.

Materials and methods

Study area

This study was conducted in Enugu state in south east Nigeria located on latitude 6° 27´N and longitude 7° 30´E [5]. The economy of Enugu state is dependent mainly on national oil revenue and commerce. Enugu state is made up of 17 local government areas and majority of the inhabitants are of Igbo ethnicity with Christianity being the dominant religion. The minimum monthly income like the national average is ₦18,000 (110 US$). Literacy rate is 66%, higher than the national literacy rate of 45%, and there are 955 males per 1,000 females [6]. The fertility rate and neonatal mortality rate is similar to the national mean of 4.5 births per woman and 40 per 1,000 live births respectively [6].

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Study design, subject and sampling technique

This is a community based cross-sectional descriptive and analytical study conducted over 12 months among children aged 2 to12 years recruited from pre-school and primary schools in three of the 17 Local Government Area of Enugu State. Prior to the commencement of the study, the study protocol was explained to the parents and caregivers during the parents-teachers association meeting.

Multi-stage sampling method was used to select study participants (Fig. 1). In the first stage, convenient sampling method was used to select 3 LGAs based on proximity to the researchers. In the second stage, one community was selected from each LGA using simple random sampling. In the third stage, two schools (i.e. one private and public school) were selected from each community using simple random selection process. Each selected school had children in pre-school (2-5 years) and primary school (6-12 years). The number of children enrolled from each school was based on pupil's population in each school. With proportionate allocation method, the sample size of each school was proportionate to the population of the school. Strata sample sizes are determined by the following equation: $_{Nh}$ = (Nh / N)* n; where Nh is the sample size of each school h, Nh is the population size for school h, N is the total population size of the schools to be studied and n is total sample size. The children enrolled were selected randomly using a computer-generated table of random numbers. Following this, the selected children were given a take-home questionnaire for their parents to complete as well as a consent form to be signed and/or thumb printed. They were instructed to return the questionnaires the next day which was the day for the study. Respondents who were seven years of age and above who returned a well completed questionnaire with an endorsed consent form from their caregivers were given the assent form before enrollment. The anthropometric data of all the selected participants were taken.

Based on malnutrition rate 39.4% [7], a three percent margin of error and an anticipated non-response rate of 10% the minimum number of children needed to make a valid assessment of height estimation was 1122 for each community giving a total minimum sample size of 3366. The choice of this prevalence of malnutrition was because it was the closest locally available published cross-sectional survey among primary school children that assessed both under- and overnutrition and reported the total prevalence of malnutrition.

Inclusion criteria

In this study were included both pre-school age and primary school age individuals:

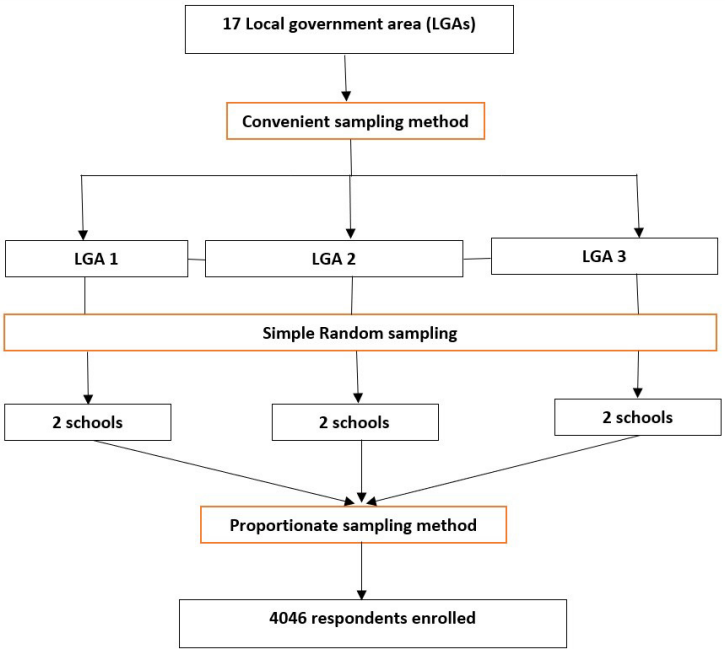
1. Children between the ages of 2 and 12 years.
2. Children who had lived in the study area for at least 12 months and completed the three terms of the preceding academic year in the present school.
3. Children whose parents and/or caregivers gave consent and completed the accompanying questionnaire and/or aged seven years and above who gave assent.

Measure

Height measurements

Height was measured to the nearest 0.1 cm using a stadiometer [SECA213, Hamburg August 2014] by trained research assistants. This stadiometer has a measuring range of 20-205 cm with a precision of up to 0.1 cm. The participants stood with the weight evenly distributed on both feet, heels together and the head positioned so that the line of vision was at right angles to the body. The correct position for the head is in the Frankfort plane [8] i.e. low-

Fig. 1. Summary of sampling technique in recruitment of study respondents.



er margins of the orbits and the upper margins of the ear canals lay in the same horizontal plane. The hands hung freely by the sides. The head, back, buttocks and heels were positioned vertically so that the buttocks and heels were in contact with the vertical board to obtain a consistent measure. The subjects were asked to inhale deeply and stretch to their fullest height. The measurements were taken to the nearest 0.1 cm. A repeat measurement was taken by a second reader after asking the subject to step off and step back onto the stadiometer while observing all the previous steps. Where the two measurements disagreed by equal to or more than 0.5 cm, a third measurement was taken. The subjects measured height was the mean of the two observations or the mean of the two closest measurements if a third is taken [9]. The estimated weight was also calculated using the Nelson-Wheech formula, *6n+77* where n is the age at the child's last birthday.

Socio-demographic characteristics

i) Age of respondent: in years at last birthday was assessed and grouped into eleven categories from 2-12 years *ii) Socioeconomic status:* defined as the wealth index of the household was derived using maternal and paternal highest educational attainment and occupation based on Oyedeji classification [10]. This was then categorized as lower, middle, and upper class; *iii) Stunting* was calculated using Height-for-age Z score using WHO Anthro-Plus software Values which were compared to the recommended 2007 WHO growth charts. Based on the z-score, respondents were re-categorized as severely stunted, stunted, normal and tall.

Data collection and statistical analysis

Data collected were inputted into the relevant sections of the questionnaire and subsequently transferred into a Microsoft Excel Sheet. Statistical analysis was performed using SPSS (version 21; SPSS Inc., Chicago, IL USA) software To compare the heights estimated using formula and actual height, the absolute error (estimated height minus measured height) were calculated and the mean percentage error [100 x (estimated height minus measured height)/measured height]. A Bland-Altman plot was displayed to graphically present the bias and 95% limits of agreement. The percentage differences (errors) between estimated and measured heights were plotted on the y-axis while the averages of the two were on the x-axis. The green lines represented the line of agreement (LOA) while the red lines represent the limits of agreement (confidence interval) showing the degree of reliability.

Results

Characteristics of children enrolled

Four thousand and forty-six children (4046) were enrolled for this study. The male to female ratio was 0.94 and almost half (46.0%) were from the families of low socio-economic status. Majority of the enrolled children (86.1%) had normal height for age while approximately 1-in-10 were tall for age. Ninety-two of the 4046 (2.3%) surveyed children were stunted with 6/92 (6.5%) of these being severely stunted (Tab. I).

Mean difference and Mean Percentage Error (MPE) of formula for height estimation

Tables II and III show the mean difference and MPE (or measurement bias) of the height estimation formula. It was noted that the formula underestimated height of children in the 2-7 and 9-10 years old categories by a factor of 3.0%, 4.4%, 7.2%, 5.6%, 10.0%, 7.8%, 4.8%

Tab. I. Summary statistics of children enrolled in study.

Study parameter	Variables	Frequency (n)	Percentage (%)
Age of respondents[†1]	2 years	104	2.6
(N = 4046)	3 years	86	2.1
	4 years	69	1.7
	5 years	57	1.6
	6 years	366	9.0
	7 years	508	12.6
	8 years	636	15.7
	9 years	681	16.8
	10 years	685	16.9
	11 years	482	11.9
	12 years	373	9.2
Gender	Male	1971	48.7
(N = 4046)	Female	2075	51.3
Socio-economic class	High	1053	26.0
(N = 4046)	Middle	1133	28.0
	Low	1860	46.0
HFA z-score category[†2]	Severe stunting	6	0.6
(N = 4035)	Stunted	86	2.1
	Normal	3923	86.1
	Tall	448	11.1

[†1] Age at last birthday, [†2] Height for age

Tab. II. Difference in height between the mean formulae estimation and actual measurement in each age category.

Variables	Formulae estimation		Actual height	Difference	Confidence Interval
Age category	N	Mean	Mean ± SD†	Mean ± SD†	(Lower, upper)
2 years	104	89.0	92.3 ± 8.3	-3.2 ± 8.3	-4.9, -1.9
3 years	86	95.0	99.7 ± 6.4	-4.7 ± 6.4	-6.2, -3.4
4 years	69	101.0	109.2 ± 6.6	-8.2 ± 6.6	-9.7, -6.7
5 years	57	107.0	113.8 ± 6.8	-6.8 ± 6.8	-8.5, -4.9
6 years	366	113.0	126.2 ± 9.4	-13.2 ± 9.4	-14.3, -12.2
7 years	508	119.0	131.7 ± 53.7	-12.7 ± 53.7	-18.2, -9.8
8 years	636	125.0	133.9 ± 10.9	-8.9 ± 10.9	-9.8, -8.2
9 years	681	131.0	137.7 ± 8.5	-6.7 ± 8.5	-7.4, -6.1
10 years	685	137.0	140.7 ± 9.8	-3.8 ± 10.8	-4.7, -3.0
11 years	482	143.0	143.9 ± 17.7	-0.9 ± 17.7	-2.8, 0.4
12 years	373	149.0	145.5 ± 9.7	3.4 ± 9.7	2.5, 4.4
Overall	4046	128.3	134.5 ± 24.4	-5.9 ± 29.1	-7.0, -5.6

† Standard deviation

Tab. III. Mean Percentage Error (MPE) or BIAS for formulae estimated heights.

Age variable	Mean Percentage	Confidence interval of MPE (%)		
	Error (%) †	Standard deviation	Lower	Upper
2 years	-3.0	7.2	- 20.9	-17.1
3 years	-4.4	6.1	- 16.4	7.6
4 years	-7.2	5.6	-18.2	3.8
5 years	-5.6	5.8	-17.0	5.8
6 years	-10.0	6.4	-22.5	2.5
7 years	-7.8	7.0	- 21.5	5.9
8 years	5.1	28.7	- 61.4	51.2
9 years	-4.8	5.9	-16.4	6.8
10 years	-1.2	33.5	- 66.9	64.5
11 years	0.2	8.0	- 15.9	15.5
12 years	2.9	7.1	- 16.8	11.0
Overall	-2.1	115.1	- 227.7	223.5

† Positive and negative values of MEP indicate over- and under-estimation of height respectively.

and 1.2% respectively while overestimating height in 8-year old children by 5.1%, 11-year old by 0.2% and 12-year by 2.9%. Figure 2 shows the Bland Altman plot of the estimated heights using the formula.

Accuracy of formula for height estimation

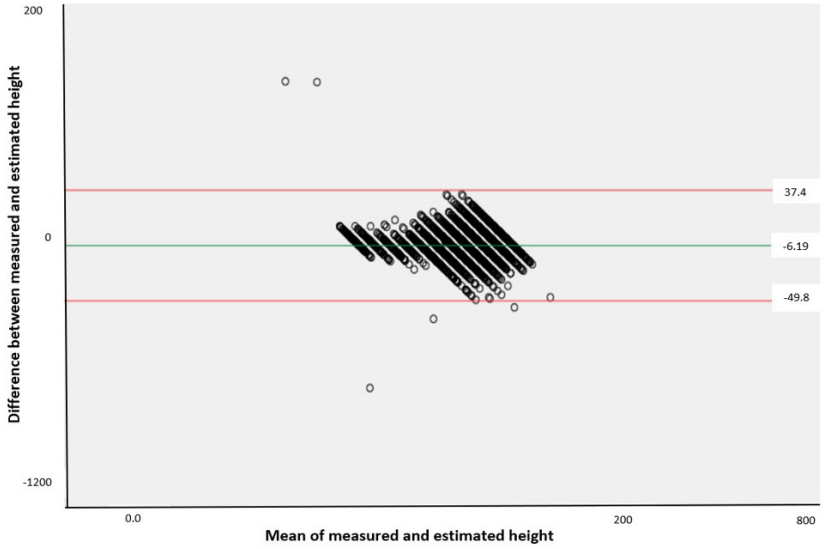
Overall, the estimated height using the formula was within ± 10% and ± 20% agreement of the actual height of surveyed children by 77.0% and 97.9% respectively. Table IV shows the degree of agreement stratified by age categories. The accuracy of the formula in estimating actual height within 10% interval of actual height was greatest in children that were two year old (85.6%), 9-years (83.0%), 10-years (87.3%) 11-years (86.3) and 12-years (83.9%) but worst in 6-years old children where its accuracy was slightly above 50%. The estimated height was fairly accurate and in agreement within 20% of the actual heights for all age categories (Tab. IV).

Discussion

Height measurement is one of the common essential anthropometric measurements for calculation of body mass index and body surface area [11]. These parameters are useful in adjusting drug dosage [12]. The knowledge of the accurate height of a child is an invaluable tool in pediatric practice hence the need to determine the accuracy of the common formula used in height estimation especially in emergency situations when the actual height of the child may be difficult to determine.

Our study showed that the formula estimated heights underestimated height in children surveyed. It was further noted that the underestimation of height progressively worsened as age increased from 2 to 6 years. Eke et al. [13], also documented similar results of an underestimation of the heights of children with the formula in 370 children in Enugu, Nigeria. Beyond 6 years, the formula calculated height followed no clear pattern of estimation error compared to the actual height. While the authors cannot give a concrete explanation for this finding, we believe that the effect of genetics and sex hormones in older children that results in non-linear growth curves may make prediction or estimation of height be more difficult using formula alone. This is unlike in younger age where growth curve is linear, and nutrition is the main determinant [14]. This reasoning is buttressed by a study conducted in 20 countries, including 180,520 paired measurements of heights in ages 1-19 years which showed that the relative genetic

Fig. 2. Bland-Altman plot of mean difference and average of measured and estimated height (Graph scaled to size for adequate formatting; Standard Deviation =22).



Tab. IV. Agreement within 10 and 20% actual height of Formulae Estimated Height.

Age	Estimated Height AGREEMENT with Actual Height		
(years)	N	Within ± 10%	Within ± 20%
2	104	89 (85.6)	103 (99.0)
3	86	66 (76.7)	86 (100.0)
4	69	43 (63.2)	68 (100.0)
5	57	43 (75.4)	57 (100.0)
6	366	194 (53.0)	335 (91.5)
7	508	351 (69.1)	494 (92.7)
8	636	437 (68.7)	621 (97.6)
9	681	565 (83.0)	679 (99.7)
10	685	598 (87.3)	683 (99.7)
11	482	416 (86.3)	472 (97.9)
12	373	313 (83.9)	363 (97.3)
Overall	4046	3115 (77.0)	3961 (97.9)

contribution increased with age and was greatest in adolescence [15].

Additionally, our study showed based on the Bland Altman plot that the estimated height was well clustered around the line of agreement and vast majority of the under and overestimated height were well within the limits of agreement. Overall, the estimated height using the formula was 77% of cases within ± 10% of actual height and in 97.9% measures within ± 20% agreement of the actual height of surveyed children. This suggests that the formula though not entirely accurate for children in our setting, is a reasonable clinical tool for height estimation especially in children less than 6 years in scenarios where conventional height measurement is either unavailable or impossible. We therefore recommend further study that would explore derivation of formula that is better suited for height estimation in children from developing setting like ours.

Limitations

Due to logistic and proximity to researchers, convenience sampling method was used in the selection of the study locations. This may have resulted in sampling and recruitment bias. However, the authors aimed to improve the validity and reliability of the data by employing multi-stage sampling technique and using the same research assistants and measurements tools across the 3 communities surveyed.

Conclusions

We conclude that the *6n + 77* formula is a reasonable but not totally accurate for height estimation for children in our setting. We recommend further study to assist in the devising of a height estimation formula that is better suited for children in developing settings like ours.

Ethics approval and consent to participate

Ethical approval was obtained from the Ethics and Research Committee of the University of Nigeria Teaching Hospital. Informed consent was obtained from the parents of the children. The retrieved information was transferred into a private computer and pass worded. Data was anonymized, and questionnaires had no names. Participation in the study was entirely voluntary, and no financial inducement whatsoever was involved. Participants were informed that voluntary withdrawal at any stage of interaction was guaranteed for them without any adverse effect.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

KKI, OOI and ONI conceptualized the study. IKK and ODIC developed and wrote the methodology. ODIC did the statistical data analysis of study and wrote the result section of the work. OOI, UCA and OHN wrote the discussion of the study. All authors contributed in writing and reviewed the final draft of the manuscript.

References

[1] Martin JH, Fay MF, Udy A, Roberts J, Kirkpatrick C, Ungerer J, Lipman J. Pitfalls of using estimations of glomerular filtration rate in an intensive care population. Intern Med J 2011;41:537-43. https://doi.org/10.1111/j.1445-5994.2009.02160

[2] Diacon AH, Koegelenberg CFN, Klüsmann KJC, Bolliger CT. Challenges in the estimation of tidal volume settings in critical care units. Intensive Care Med 2006;32:1670-1. https://doi.org/10.1186/s13613-016-0154-4

[3] Keane V. Assessment of growth. In: Nelson textbook of Paediatrics, 18th edition. Philadelphia, 2006.

[4] UNICEF. National Nutrition and Health Survey (NNHS) 2018. Report on the Nutrition and Health Situation of Nigeria. Available and assessed on 24/04/2020 from https://www.unicef.org/nigeria/nutrition.

[5] Wikipedia, the free encyclopedia. Retrieved from http://en.wikipedia.org/wiki/Nnewi.

[6] Nigerian Population Commission. State and local government demographic profile: 1991-2010, Abuja.

[7] Fetuga MB, Ogunlesi TA, Adekanmbi AF, Alabi AD. Nutritional status of semi-urban Nigerian school children using the 2007 WHO Reference population. West Afr J Med 2011;30:331-6. PMID: 22752820

[8] Definition of Frankfort horizontal plane. Available and assessed on June 25, 2017 from http://medical-dictionary.thefreedictionary.com/Frankfort+horizontal+plane

[9] WHO. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert committee. Technical report series No 854. Available and assessed on June 20, 2018 from www.who.int/childgrowth/publications/physical_status

[10] Oyedeji GA. Socioeconomic and cultural background of hospitalized children in Ilesa, Nigeria. Nig J Paediatr 1985;12:111-7.

[11] Katzmarzyk PT. Anthropometric indicators in relation to the gold standards In: Bray GA, Bouchard C. Handbook of Obesity Epidemiology, Etiology, and Physiopathology 2014. 3rd ed. Boca Raton: CRC Press Taylor & Francis Group 2017, pp. 37-44.

[12] Zind BJ. Determining drug dosages by height: applying a model made for coastal children in Papua New Guinea to children from the highlands P N G Med J 1992;35:194-6. PMID: 1296421

[13] Eke CB, Ubesie AC, Ibe BC. Comparison of actual (measured) weights and heights with the standard formula methods of estimation among children in Enugu. Niger J Paed 2014;41:307-11. https://doi.org/10.4314/njp.v41i4,3

[14] Zelalem TW. Magnitude and determinants of stunting among children in Africa: a systematic review. Current Research in Nutrition and Food Science 2014;2:88-93. https://doi.org/10.12944/CRNFSJ.2.2.05d

[15] Jelenkovic A, Sund R, Hur Y-M, Yoko Y. Genetic and environmental influences on height from infancy to early adulthood: an individual-based pooled analysis of 45 twin cohorts. Sci Rep 2016;6:284-96. https://doi.org/10.1038/srep28496

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Correspondence: Osuorah DI. Chidiebere, Child Survival Unit, Medical Research Council UK, The Gambia Unit - E-mail: chidi.osuorah@gmail.com

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ORIGINAL ARTICLE

Knowledge, attitude, and practice of breast cancer and breast self-examination among female detainees in Khartoum, Sudan 2018

AHMED OSMAN AHMED MOHAMED[1], MUNEER MAKKI MUSA NORI[1], ALMEGDAD SHARAFALDIN MOHAMED AHMED[1], RANDA AHMED ABDALRHEEM ALTAMIH[1], EZZAN SAEED MOHAMED KUNNA[2]
[1] Faculty of Medicine, University of Khartoum, Khartoum, Khartoum, Sudan; [2] Department of International Health, Institute of Tropical Medicine-Nagasaki University, Nagasaki, Japan

Keywords

Breast cancer • Prevention • Breast self-examination • Awareness • Detainees

Summary

Introduction. *Breast cancer is the common cancer among females and a leading cause of mortality among them globally, its rates are three times higher in developing countries. Breast self-examination (BSE) had an important role in early detection, increasing the survival rate of breast cancer patients, despite these benefits, the rates of practicing BSE are low, especially in developing countries.*
Objective. *To assess the levels of awareness about breast cancer, and BSE among the detainees of Dar-Altaebat facility, a female's detention facility in Khartoum, Sudan.*
Methods. *A cross-sectional study conducted at Dar-Altaebat a female imprisonment facility in Khartoum, Sudan. 354 participants were randomly selected from the total population of the facility. Data was collected using a self-administered structured questionnaire, which was composed of socio-demographic section, knowledge section, attitude section, and breast self-examination section. Data was analyzed using statistical package for social sciences(SPSS) software.*
Results. *330 participants responded to the questionnaire, their mean age was (31, SD: 11.2) years, 126 (38.2%) of them were illiterate, 196 (59.4%) were married, and the majority of them 177 (76.3%) were housewives. their overall knowledge score revealed that 185(56.2%) of them had poor knowledge about breast cancer. 218 (66.3%) of them had poor knowledge about breast self-examination, however 314 (95.3%) thought that it was important. 315 (95.5%) of the participants had poor practice towards breast self-examination.*
Conclusion. *Poor levels of knowledge about breast cancer were reported among the detainees. Additionally, their level of knowledge and practice of breast self-examination was also low, thus more health education campaigns are recommended in such facilities.*

Introduction

Breast cancer is the most prevalent cancer among females globally, encompassing nearly a quarter of diagnosed cases among females, since 1.15 million cases are diagnosed annually around the world [1-3]. Breast cancer is common in both developed and developing countries. Nevertheless, its rates are three times higher in developing countries [4]. Breast cancer is a leading cause of mortality among females in Africa [5]. According to studies 1:6 women worldwide undergo breast biopsy, most reported cases are benign changes yet cases with malignancy are many, breast diseases range from inflammatory changes, benign fibroblastic to fibrocystic changes and malignant diseases [6], risk factors include age, early menstruation, late menopause, family history of breast disease, usage of hormonal replacement therapy, breastfeeding state and lifestyle habits [6-9].
Early-onset of the disease and poor prognosis has been reported more among African populations compared to Caucasians [8]. It is estimated that 70-90% of breast cancer cases present with late-stages in African countries, which is a cause of decreasing survival probabilities among patients [10]. The lack of early detection in Africa has been linked to the scarcity of resources [11]. In Africa generally, breast cancer is higher in Sub Saharan region especially in Sudan where the number of new cases is increasing steadily [7]. In Sudan, breast cancer is the third most common cancer in the country, and the most common among females [12, 13].
Studies have shown that breast cancer screening programs like mammography, breast self-examination (BSE), and breast clinical examination (BCE) had an important role in early detection, increasing the survival rate, decreasing fatality, and preventing recurrence among breast cancer patients [14-16]. BSE is recommended by the American cancer society, it has been reported that women who practiced BSE regularly were more likely to present with early stages of the disease, this is due to the fact that regular BSE familiarizes the female with feel and appearance of the breast, and thus she will be more able to detect any change as early as possible [17]. Nonetheless, the implementation of such programs in Africa is challeng-

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ing due to the lack of adequate substructure, trained personnel with regard to financial barriers [18].
Evidence suggests that practicing BSE depends on different factors including females' knowledge, attitude, socio-demographic and sociocultural factors [19]. Other reasons for low rates of practice have been reported including lack of time, forgetfulness, and low level of education [20]. In Sudan, results from a previous study concluded that there are insufficient knowledge and practice of BSE among medical students [21].

Methods

Study settings

This was a facility-based, descriptive, cross-sectional study, conducted at *Dar-Altaebat* Prison which is a female's imprisonment facility located in Khartoum state and considered to be one of the largest female detention facilities in Sudan. This study was the first phase of a breast cancer prevention campaign conducted by SCOPH office. Detainees from all age groups and backgrounds were eligible to be included in this study.

Data collection tools

354 participants were randomly selected from the total population of the facility which is 820 persons, using a formula with prevalence of 0.5 and a confidence level of 95, by randomly picking 354 numbers out of 820 numbers list of the total population. Data was collected a self-administered structured questionnaire which was adopted from a previous study [26].
The questionnaire is divided into 4 sections; socio-demographic section, knowledge section, attitude section, and breast self-examination section. Nine items were used to assess the knowledge by asking about symptoms, signs, protective factors, diagnosis, risk factors and curability, and three items were used for the attitude section. In breast self-examination section two items were used to assess the knowledge about breast self-examination by asking about the reasons, place, steps of self-examination, two items were used for the attitude, and three items for the practice.
Copies were handed in person to participants. Codes were used instead of names to ensure confidentiality. The Scoring for knowledge, attitude and practice for breast cancer and self-examination items was performed by a consultant oncologist. Data was collected during July-September 2018.

Data analysis

Statistical Package for Social Science 24.0 (SPSS) software was used for data entry and analysis. Categorical variables were presented as frequencies, and continuous Variables as means and standard deviations. Additionally, Chi-square test was used to test the association between categorical variables. P.value of less than 0.05 was considered statistically significant.

Ethical considerations

An ethical approval was obtained from the institutional review committee at the radiation and isotopes center in Khartoum (RICK). In addition, a written consent was taken from the prison administration and verbal consent was obtained from each participant before conducting data collection.

Results

Out of 354 selected detainees, 330 participants responded to the questionnaire with a response rate of 93%, their mean age was (31, standard deviation (SD): 11.2) years, and the ages of more than half of them 185 (56.7%) are from 18-30 years. 126 (38.2%) of them were illiterate and 124 (37.6%) had primary education. Regarding their marital status, the majority of them 196 (59.4%) were married. Additionally, 177 (76.3%) were housewives, Table I demonstrates the demographic characteristics of the study participants. About the participants' knowledge about breast cancer, the majority of them 201 (60.9%) agreed that breast cancer is the most common cancer among females, and 202 (61.2%) said that breast cancer is a curable disease. Moreover, nipple discharge was the most commonly chosen symptom by the participants 137 (41.5%), and smoking was the most commonly selected risk factor for breast cancer 175 (53%). Fur-

Tab. I. Demographic characteristics of the study participants.

	Frequency	Percentage
Age		
Mean 31 Minimum 13 Maximum 80		
Age groups		
18-30	185	57
31-40	89	27
41-50	34	10
More than 50	13	6
Educational level		
Illiterate	126	38.2
Khalwa	11	3.3
Primary	124	37.6
Secondary	31	9.4
University	31	9.4
Post-graduate	7	2.1
Occupation		
House wife	177	76
Employee	55	24
Marital status		
Single	63	19
Married	196	59
Divorced	42	13
Widowed	29	9

thermore, 175 (53%) of the participants did not know the methods of breast cancer diagnosis. The overall score of the participants' revealed that more than half of them 185 (56.2%) had poor knowledge about breast cancer, poor knowledge about breast cancer was significantly associated with low educational status (p-value: < 0.001), however it was not associated with the occupation of the participants (p-value: 0.82), and wasn't associated with neither their marital status (p-value: 0.05), nor their age (p-value: 0.22) Table II shows the participants knowledge about breast cancer. Regarding the attitude of participants towards breast cancer, only 92 of the participants responded to the questions, 87 (95%) of them thought that hospitals are the best places to receive treatment for breast cancer and neither the *Sheikh* (local traditional healer), nor alternative medicine centers. Respondents were asked about the time appropriate to seek doctors when they sense the presence of a lump in the breast, and the majority 74 (80%) of them said that they would go to the doctor immediately. Overall assessment of the participants revealed that 69 (75%) of them had a good attitude regarding breast cancer. Attitude of the participants was not associated with their Educational status (p-value: 0.45), occupation (p-value: 0.45), and marital status (p-value: 0.45).

When the participants were asked about breast self-examination, more than half of them 181 (54.8%) said that they have never heard about it, and of the people who have heard about it, 77 (51.3%) of them said that health education campaigns were their primary source of information about breast self-examination.

Tab. II. Participants knowledge about breast cancer.

Question	Frequency	Percentage
Breast cancer is the most common cancer among females?		
Yes	201	61
No	34	10
I don't know	95	29
Breast cancer is a curable disease?		
Yes	202	61
No	50	15
I don't know	78	24
Early diagnosis of breast cancer increases the chances of treatment?		
Yes	258	78
No	19	6
I don't know	53	16
Unequal breasts size after puberty is normal?		
Yes	209	63
No	89	27
I don't know	32	10
In most of the cases, breast cancer appears as non-painful lumps in the breast?		
Yes	195	59
No	30	9
I don't know	105	32
Normal breast feeding decreases the probability of acquiring breast cancer?		
Yes	153	47
No	64	19
I don't know	113	34
What are the symptoms of breast cancer?		
Axillary lymph nodes enlargement	127	39
Breast redness or change in color	128	39
Nipple discharge	137	42
Sever Weight loss	81	25
I don't know	123	37
Breast cancer can be diagnosed using:		
Tissue biopsy	81	25
Ultra sound	86	26
Mammography	53	16
I do not know	175	53
Risk factors of breast cancer include:		
Smoking	175	53
Alcohol	107	32
Aging:	89	27
Late menopause	58	18
Family history of BC	83	25
Obesity	63	19
Long term use of contraceptive pills	83	25
I don't know	116	35

The majority of the participants 218 (66.3%) had poor knowledge about breast self-examination, and poor knowledge was significantly associated with low educational status (p-value: < 0.001), with housewives as an occupation (p-value: 0.04), and with 18-30 years age group (p-valve: 0.004), however it was not associated with the participants marital status (p-value: 0.27). When the participants were asked about their opinion regarding breast self-examination the majority of them 143 (95.3%) thought that it was important, their attitude was neither associated with their educational status, occupation, marital status, nor their age (p-values: 0.91, 0.15, 0.19, 0.11, respectively). In addition, 315 (95.5%) of the participants had poor practice towards breast self-examination, and their practice was neither associated with their educational status, occupation, nor marital status (p-values: 0.07, 0.71, 0.06, respectively).

Discussion

Breast cancer prevalence is dramatically increasing every year and so both mortality and morbidity of the disease do [10, 13, 20], for this reason early detection which improves the prognosis is becoming more important [22, 23]. These facts reflects the huge role of appropriate knowledge and attitude of women in community regarding the disease and participating in screening programs specially in under-developed countries with limited health care resources like Sudan.

The mean age in this study was 31 (SD: 11.2) which gives a good presentation of the risk group of breast cancer. Other previous studies had similar age groups, 30 is the mean in the study (Delta state – Nigeria 2013) [10], and 31.3 (north Iran,2015), but higher ages in other studies, the mean age was 40.48 (Malaysia, 2010) [20], and 41 (Iran, 2018) and lower ages in other studies like in UAE with mean 23 [11]. Although all these ages are at risk for breast cancer [10], but the variation of age might affect the knowledge, attitude and practice of these women toward the disease.

The majority of our participants (60.9%) correctly answered that breast cancer is the most common cancer among females, and (61.2%) stated that it is a curable disease. comparing these results with a similar previous study in Nigeria [10], higher percentage (84.6%) confirmed the same facts, this might be due to increasing the number of awareness campaigns regarding the breast cancer in the previous years before this study.

Regarding the participants' knowledge about the disease symptoms, (41.5%) had chosen nipple discharge as the most common symptom however women of northern Iran had different opinions considering the presence of the breast mass as the most common symptom (75.4%) [23]. In addition, smoking was the most commonly selected risk factor of breast cancer (53%), however in another study it was the second most commonly chosen after alcohol consumption [23]. Furthermore, the overall participants' knowledge about breast cancer in this study was poor, and it was associated with low education status. This association was also suggested in other studies [10, 22, 23].

More than half of our participants never heard about breast self-examination (BSE) before, the same thing was observed in previous study held in Nigeria [10]. The main source of the participants information about BSE was breast cancer campaigns, few of them get some knowledge from the media. Television was considered as the main source of information among younger participants with tertiary level of education among students in the university of Buea in Cameroon [24].

Overall knowledge about BSE was poor (66.3% of the participants had poor knowledge), similar results were reported in other studies, participants showed poor knowledge in Nigeria and Malaysia with (82.3%) and (86.2%) respectively [10, 20]. The association between the level of education and level of knowledge regarding BSE was positively concluded in our findings, higher level of knowledge about the disease was associated with higher level of education, similar observation was noticed in other studies [10, 20, 24].

Poor practice was the most common result among the majority of the participants in this study (95.3%). It was noted that the participants' scores in questions regarding the knowledge about breast cancer general information like signs, symptoms and risk factors were as bad as their scores in BSE related questions. This was also noted in other study conducted in Nigeria [9]. In addition, a study conducted in Iran revealed that (74.8%) of women never practice BSE ever, and (9.8%) did the first breast examination after they felt pain in the breast [20], also (60.4%) was the percentage of community ignorance- specially the risk group- regarding BSE as it was found in Malaysian study [19]. Moreover, a study performed by Nde et al stated that only (30%) of university female students had practiced BSE at least once, however less than (3%) were practicing BSE regularly with monthly pattern [24]. So, all these studies agreed that there is a poor practice in general but with some degree of variation between communities due to different levels of education and breast cancer related knowledge.

Conclusion

One of the limitations of this study is that it took place in only one facility, which makes it difficult to generalize it's results over the larger population of inmates in Sudan. Despite this limitation, our results revealed that more than half of the participants had poor knowledge about breast cancer and self-examination. The majority of the respondents think about breast self-examination as an important issue but they have poor practice. Thus, we recommend more health

campaigns and educational sessions in such facilities. Additionally, educational broadcasts and mini-videos illustrating the steps of breast-self-examination ought to be delivered via these imprisonments' radios and televisions. Furthermore, more research projects are encouraged to address this issue in other amenities and geographical locations throughout Sudan, also a comprehensive breast cancer screening program is also recommended.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

AOAM: study design, data acquisition , data interpretation, and manuscript writing. MMMN: study design, data acquisition , data interpretation, and manuscript writing. ASMA: data management, data interpretation, and manuscript writing. RAAA: study design, data acquisition , data interpretation, and manuscript writing. ESMK: study design, data interpretation, and manuscript writing.
All the authors participated equally in all of the steps of this research.

References

[1] Anderson BO, Yip CH, Smith RA, Shyyan R, Sener SF, Eniu A, Carlson RW, Azavedo E, Harford J. Guideline implementation for breast healthcare in low-income and middle-income countries: overview of the Breast Health Global Initiative Global Summit 2007. Cancer 2008;113(S8):2221-43. https://doi.org/10.1002/cncr.23844

[2] Jemal A, Center MM, DeSantis C, Ward EM. Global patterns of cancer incidence and mortality rates and trends. Cancer Epidemiol Biomarkers Prev 2010;19:1893-907. https://doi.org/10.1158/1055-9965.EPI-10-0437

[3] Breast cancer: prevention and control [Internet]. World Health Organization. 2019 [cited15October2019]. Availablefrom:http://www.who.int/cancer/detection/breastcancer/en/

[4] Freddie B, Peter M, Maxwell P. The changing global patterns of female breast cancer incidence and mortality. Breast Cancer Res 2004;6:229-39. https://doi.org/10.1186/bcr932

[5] Jemal A, Bray F, Forman D, O'Brien M, Ferlay J, Center M, Parkin DM. Cancer burden in Africa and opportunities for prevention. Cancer 2012;118:4372-84. https://doi.org/10.1002/cncr.27410

[6] Choi JS, Park M. Factors predicting young women's willingness to conduct vulvar self-examinations in Korea. Health Care Women Int 2019;40:653-64. https://doi.org/10.1080/07399332.2018.1531003

[7] Black E, Richmond R. Improving early detection of breast cancer in sub-Saharan Africa: why mammography may not be the way forward. Global Health 2019;15:3. https://doi.org/10.1186/s12992-018-0446-6

[8] Cancer Facts & Figures 2009 | American Cancer Society [Internet]. Cancer.org. 2019 [cited 15 October 2019]. Available from: https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2009.html

[9] Al-Naggar RA, Al-Naggar DH, Bobryshev YV, Chen R, Assabri A. Practice and barriers toward breast self-examination among young Malaysian women. Asian Pac J Cancer Prev 2011;12:1173-8.

[10] Azubuike SO, Okwuokei SO. Knowledge, attitude and practices of women towards breast cancer in Benin City, Nigeria. Ann Med Health Sci Res 2013;3:155-60. https://doi.org/10.4103/2141-9248.113653

[11] Al-Sharbatti SS, Shaikh RB, Mathew E, Al-Biate MA. Breast self-examination practice and breast cancer risk perception among female university students in Ajman. Asian Pacific Journal of Cancer Prevention 2013;14:4919-23. https://doi.org/10.7314/APJCP.2013.14.8.4919

[12] Elgaili EM, Abuidris DO, Rahman M, Michalek AM, Mohammed SI. Breast cancer burden in central Sudan. Int J Womens Health 2010;2:77. https://doi.org/10.2147/ijwh.s8447

[13] Elamin A, Ibrahim ME, Abuidris D, Mohamed KE, Mohammed SI. Part I: cancer in Sudan - burden, distribution, and trends breast, gynecological, and prostate cancers. Cancer Med 2015;4:447-56. https://doi.org/10.1002/cam4.378

[14] Nelson HD, Tyne K, Naik A, Bougatsos C, Chan BK, Humphrey L. Screening for breast cancer: an update for the US Preventive Services Task Force. Ann Intern Med 2009;151:727-37. https://doi.org/10.7326/0003-4819-151-10-200911170-00009

[15] Nystrom L. How effective is screening for breast cancer? Br Med J 2000;321(7262):647-8. https://doi.org/10.1136/bmj.321.7262.647

[16] Siahpush M, Singh GK. Sociodemographic predictors of pap test receipt, currency and knowledge among Australian women. Prev Med 2002;35:362-8. https://doi.org/10.1006/pmed.2002.1086

[17] Karayurt Ö, Özmen D, Çetinkaya AÇ. Awareness of breast cancer risk factors and practice of breast self examination among high school students in Turkey. BMC Public Health 2008;8:359. https://doi.org/10.1186/1471-2458-8-359

[18] Hamad HM. Cancer initiatives in Sudan. Ann Oncol 2006;17(suppl 8): 32-6. https://doi.org/10.1093/annonc/mdl985

[19] Haji-Mahmoodi M, Montazeri A, Jarvandi S, Ebrahimi M, Haghighat S, Harirchi I. Breast self-examination: knowledge, attitudes, and practices among female health care workers in Tehran, Iran. Breast J 2002;8:222–5. https://doi.org/10.1046/j.1524-4741.2002.08406.x

[20] Rosmawati NH. Knowledge, attitudes and practice of breast self-examination among women in a suburban area in Terengganu, Malaysia. Asian Pac J Cancer Prev 2010;11(6):1503-8.

[21] Saadeldin A. Idris, Aamir A. Hamza, Mohamed M. Hafiz, Mohammed Eltayeb A. Ali, Gamal E. H. A. El Shallaly.

Knowledge, attitude and practice of breast self examination among final years female medical students in Sudan. Int J Public Health Res 2013;1:6-10.

[22] Gangane N, Ng N, Sebastian MS. Women's knowledge, attitudes, and practices about breast cancer in a rural district of Central India. Asian Pac J Cancer Prev 2015;16:6863-70. https://doi.org/10.7314/APJCP.2015.16.16.6863

[23] Tilaki KH, Auladi S. Awareness, attitude, and practice of breast cancer screening women, and the associated socio-demographic characteristics, in Northern Iran. Iran J Cancer Prev 2015;8(4). https://doi.org/10.17795/ijcp.3429

[24] Nde FP, Assob JC, Kwenti TE, Njunda AL, Tainenbe TR. Knowledge, attitude and practice of breast self-examination among female undergraduate students in the University of Buea. BMC Rese Notes 2015;8(1):43. https://doi.org/1186/s13104-015-1004-4

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Correspondence: Almegdad Sharafaldin Mohamed Ahmed, Faculty of Medicine, University of Khartoum, Alqaseer avenue, B.O: 11111. Khartoum Sudan - E-mail: almegdadsharaf@gmail.com - Tel. +249116349417

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ORIGINAL ARTICLE

Association between parity and obesity: a cross sectional study on 6,447 Iranian females

MARYAM TAGHDIR[1], YOUSEF ALIMOHAMADI[2,3], MOJTABA SEPANDI[1,4], ABBAS REZAIANZADEH[5], SEPIDEH ABBASZADEH[1], FATIMA MUHAMMAD MAHMUD[6]

[1] Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran; [2] Pars Advanced and Minimally Invasive Medical Manners Research Center, Pars Hospital, Iran University of Medical Sciences, Tehran, Iran; [3] Department of Epidemiology and biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; [4] Department of Epidemiology and Biostatistics, Faculty of Health, Baqiyatallah University of medical sciences, Tehran, Iran; [5] Breast Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; [6] Wilaayah multiconsult limited, Kano, Nigeria

Keywords

Parity • Obesity • Iranian women • Body mass index

Summary

Background. *The aim of this study was to determine the relationship between parity and obesity in Iranian women.*

Methods. *In a cross-sectional study, a total of 6447 urban women aged 40-65 were studied. Parity(number of full-term births), age at menarche, menopausal status, age, height, weight, marital status, education level and occupation were gathered by a checklist. Body Mass Index (BMI) was calculated. Statistical associations between parity and obesity using multiple logistic regression model were examined.*

Results. *The mean age of the enrolled women was 48.40 ± 6.13 years. The mean BMI was 27.55 ± 4.47 kg/m^2. Of the total participants enrolled, 3517 (54.55%) had < 3 parities, while 2930 (45.44%) had ≥ 3 parities. The prevalence of obesity (BMI ≥ 30 kg/m^2) and overweight (30 > BMI ≥ 25) was 27.50 % (95% CI 26.85-28.15) and 43.70% (95% CI 42.98-44.42), respectively. After adjustment for potential confounders, women with ≥ 3 parities were at higher risk of being obese (OR 1.19, 95% CI 1.05-1.35).*

Conclusion. *There was a statistically significant positive association between higher parity and obesity. It is recommended that health policymakers plan appropriate weight loss programs for postpartum.*

Introduction

The global prevalence of overweight has been reported as 39 % among adults by World Health Organization (WHO) [1]. The overweight and obesity are accounted as main contributors of 19.4 million deaths [2]. The risk of some diseases such as dys-lipidemia, type 2 diabetes and hypertension is associated with Obesity [3, 4]. Asians are more susceptible to metabolic syndrome and cardiovascular diseases and percentage of body fat in Asian people is also higher than in Europeans [5, 6]. Iran as a developing country in Persian Gulf region has a high prevalence of obesity [7]. Pregnancy and childbearing are associated with insulin resistance, weight gain and obesity in women [8-11]. Parity(number of full-term births) has been reported to be associated with obesity [12]. Several studies have reported an association between parity and obesity [12-14]. In some longitudinal studies, obesity was only observed after the first childbirth [8, 9], whereas other studies have stated an association with number of pregnancies [12, 13].

However, the results of a prospective study in the United States have shown that childbearing does not increase the risk of obesity in young women [15]. Also, several studies have reported a non-significant statistical relationship between parity and obesity [16, 17]. Current evidence suggests that the parity-obesity association varies among different cultures [18], ethnic groups [14, 19-21] and levels of country development. However, there is not much information about the effect of parity on body size in this ethnic group. In addition, the relationship between parity and obesity has been suggested to depend on the level of development of countries. [22], it is reasonable to assess this relationship in both developed and developing regions. The present study examined the relationship between parity and body mass index (BMI) in 6447 Iranian women aged 40 to 65 years.

Material and methods

A breast cancer screening program, was done between 2005 and 2013 in Shahid Motahhari Breast Clinic affiliated to Shiraz University of Medical Sciences (SUMS). A total of 11,850 women who refer to the hospital for breast cancer screening during an 8-year period were screened. Initially, a clinical examination was performed on all women participating in the screening program. Participants were then subjected to mammography, ultrasound, needle aspiration, biopsy, or surgery, depending on the physician's decision. A face-to-face interview was conducted by trained personnel. In the present study all participants

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over the age of 40 (6447 women) entered the analysis by census. The information (age at menarche, menopausal status, parity, age, height, weight, marital status, education and occupation) were gathered by a checklist. Parity was considered as number of full-term births (any pregnancy lasting longer than 5 months, regardless of outcome). Parity was divided into categories as < 3 and ≥ 3. Each subject was weighed using a digital scale to the nearest 100 grams, while the participant had the least clothing and no shoes. Height was measured with a un-stretchable tape measure, and the BMI was calculated as weight divided by height squared (kg/m^2). Women were classified according to BMI as non-obese/overweight (BMI < 30 kg/m^2), obese (BMI ≥ 30 kg/m^2) or overweight (30 > BMI ≥ 25).

Statistical analysis

All analyses were performed with SPSS software (version 16.0; SPSS, Chicago, Ill., USA). We summarized continuous data as means ± standard deviation (SD) and presented categorical variables as percentages. The difference in the distribution of the women by parity and BMI variables was tested using χ^2 test or used analysis of variance. The multiple logistic regressions were used to determine the effect of factors that are associated with obesity. The odds ratios (OR) were presented together with 95% Confidence Intervals (CIs). Adjusted ORs according to age, marital status, education, occupation, menopausal status and age at menarche were calculated.

Results

The mean (± SD) of age was 48.40 ± 6.13 years (Min:40 y, Max: 65 y). The mean (± SD) of weight, height and BMI for the total population was 67.94 ± 11.12 kg (Min:38,Max:128); 157.13 ± 5.8 cm (Min:130,Max:194), and 27.55 ± 4.47 Kg/m^2(Min:15.23,Max:51.9) respectively. A total of 3517 (54.55%) women had < 3 parities. Table I presents the descriptive characteristics of the study population, by parity. A higher parity was observed among women in 45-49 years group, those currently married, housewives, those that were overweight, and those with a lower level of education. The prevalence of obesity (BMI ≥ 30) and overweight (30 > BMI ≥ 25) was 27.50 % (95% CI 26.85-28.15) and 43.70% (95% CI 42.98-44.42), respectively (Data not shown in Table). The mean BMI of the women by selected variables was shown in Table II. The mean BMI was greater in women who had higher parity (p = 0.0001), were housewives (p = 0.01), married (p = 0.04),were post menopause (p = 0.04) and were uneducated (p = 0.002).The crude

Tab. I. Descriptive characteristics of 6447 women aged 40-65 by parity.

Variables	Parity< 3 (n = 3517)	Parity ≥ 3 (n = 2930)	
	n (%)	n (%)	P-value
Marital status			
Married	3015 (85.7)	2598 (88.7)	0.0001
Single	502 (14.3)	332 (11.3)	
Occupation			
Housewife	2536 (72.1)	2699 (92.1)	0.0001
Employed	981 (27.9)	231 (7.9)	
Education level			
Illiterate	96 (2.7)	440 (15)	
Elementary	929 (26.4)	1682(57.4)	0.0001
High school	1780(50.6)	713(24.3)	
University	711 (20.2)	94 (3.2)	
Age at menarche (yrs)			
< 12	269 (7.6)	202 (6.9)	0.13
≥ 12	3248 (92.4)	2728 (93.1)	
Menopause status			
Pre-menopausal	2689(76.3)	1849 (63.1)	0.0001
Post-menopausal	835(23.7)	1081 (36.9)	
BMI (Kg/m^2)			
< 25	1094 (31.1)	765 (26.2)	0.0001
25-29.9	1541 (43.9)	1264 (43.2)	
≥30	878 (25.0)	894 (30.6)	
Age (yrs)			
40-44	1410 (40.10)	650 (22.2)	
45-49	1057 (30.1)	829 (28.3)	0.0001
50-54	605(17.2)	756(25.8)	
55-59	299 (8.5)	446 (15.2)	
60-65	146 (4.2)	249 (8.5)	

Tab. II. Mean BMI among women aged 40 - 65 according to selected variables.

Variables	n (%)	Mean BMI(SD)	P-Value
Age			
40-44	2060(32)	27.28(4.4)	0.01
45-49	1886(29.30)	27.60(4.5)	
50-54	1361(21.10)	27.78(4.6)	
55-59	745(11.60)	27.77(4.6)	
60-65	395(6.10)	27.55(4.2)	
Parity			
Nulliparous	386(6)	26.7(4.7)	0.0001
1-3	3131(48.6)	27.3(4.4)	
4-6	2467(38.3)	27.8(4.5)	
6+	463(7.2)	28.3(4.5)	
Marital status			
Married	5613(87.10)	27.6 (5.3)	0.04
Single	834(12.90)	27.2 (4.3)	
Occupation			
Housewife	5235(81.20)	27.6 (4.5)	0.01
Employed	1212(18.80)	27.2 (4.4)	
Education level			
Illiterate	538(8.30)	27.9 (4.5)	0.002
Elementary	2611(40.50)	27.6 (4.5)	
High school	2493(38.70)	26.5(4.4)	
University	805(12.50)	25.0 (4.4)	
Age at menarche			
< 12	471(7.30)	28.2 (5.1)	0.0001
≥ 12	5976(92.70)	27.5 (4.5)	
Menopause status			
Pre -menopausal	4531(70.30)	27.4 (4.4)	0.04
Post-menopausal	1916(29.70)	27.7 (4.6)	

Tab. III. The Crude and adjusted Odds Ratio for obesity based on characteristics of 6447 women, aged 40-65 .

Variables	Crude OR (95%CI)	Adjusted OR (95%CI)
Parity		
< 3	1	1
≥ 3	1.32(1.18-1.47)	1.19(1.05-1.35)
Age	1.01(1.008-1.02)	1.01(1.001-1.02)
Marital status		
Married	1.13(0.95-1.33)	1.17(0.99-1.40)
Single	1	1
Occupation		
Housewife	1.17(1.01-1.35)	0.99(0.89-1.20)
Employed	1	1
Education level		
Illiterate	1.45(1.13-1.85)	1.23(0.90-1.67)
Elementary	1.38(1.15-1.66)	1.25(0.98-1.60)
High school	1.18(0.98-1.42)	1.14(0.91-1.43)
University	1	1
Age at menarche		
< 12	1.31(0.07-1.60)	1.32(1.08-1.62)
≥ 12	1	1
Menopause status		
Pre-menopausal	1	1
Post-menopausal	1.16(1.03-1.31)	1.02(0.87-1.19)

OR showed a significant association between parity and obesity (OR 1.32, 95% CI 1.18-1.47, Tab. III). After adjusting for age, age at menarche, employment status, educational level, menopausal and current marital status, higher parity (≥ 3 pregnancies) was associated with obesity and this association was statistically significant (OR 1.19, 95% CI 1.05-1.35, Tab. III).

Discussion

The results of current study show that higher parity in women is related to increased BMI and obesity. In this study, the prevalence of obesity (BMI ≥ 30) of 27.5% is much lower than the 38.3 % reported in Tehran [23]. The mean BMI of 27.55 kg/m^2 observed in present study is similar to the average of 29.1 kg/m^2 reported in Iran by Hajiahmadi et al in 2015(23).

The results of other studies have shown that there is a positive relationship between parity and BMI [12, 13, 23-26] that is consistent with results of the present study. The results of a study conducted in Finland show that there is a direct relationship between parity and BMI [25]. Also, the results of a study in China showed that higher parity in the long run is associated with an increased risk of obesity [27]. A large population-based study found that women who had at least one delivery had an average BMI higher than women with no pregnancy experience [28]. In addition, a U-shaped trend of postpartum weight retention has been reported for women during pregnancy [15]. A significant weight gain during a 10-year follow-up has also been reported in US [21]. However, Martinez et al. [14] reported no association between parity and obesity in Mexican-American women, though nulliparous women hadn't been included in their study. In previous studies, age, level of education, age at menstruation, menopausal status, employment status and marital status have been reported as factors related to BMI in women [7, 24]. The results of current study showed that the mean BMI was greater in women who were housewives, married, post menopause or uneducated. After adjusting other variables(age, age at menarche, employment status, educational level, menopausal and current marital status), higher parity (≥ 3 pregnancies) has a statistically significant association with higher BMI.

The mechanisms of the association between parity and obesity are unclear, although some evidence suggests that high concentrations of glucose, fatty acids and amino acids may contribute to weight gain during pregnancy and postpartum, thus increasing the risk of obesity [12, 14]. During pregnancy, the release of corticotrophin realizing hormone from placenta-drive the hypothalamic-pituitary-adrenal axis and cortisol concentrations in pregnant women which may contribute to pathophysiological mechanism of obesity [29, 30]. In addition, during pregnancy, some factors such as stress, depression and anxiety may also play role in hypothalamic- pituitary-adrenal hyperactivity [27, 30]. Women with several children may have gained weight as a result of their reduced physical activity. Women with further children have less time to focus on health behaviors including weight management. Because obesity increases the risk of diseases such as diabetes, cardiovascular disease, metabolic syndrome and some cancers, it is important to identify the risk factors for obesity in women. Encouraging women to follow a healthy lifestyle may be effective in preventing obesity.

The main limitation of the present study is that the amount of physical activity and the quantity and quality of the diet were not considered. So, further support from longitudinal studies is required. Another limitation is lack of data on BMI, prior to any pregnancy. In this cross-sectional study, it is not possible to determine whether weight gain occurred during pregnancy or at any other time. Because the prevalence of obesity is relatively high in the general population, some women may be obese before their first pregnancy and childbirth, and as a result, parity may have little effect on their obesity. Though, a strength point of present study was measuring variables by trained staff using standard procedures, instead of relying on the self-reporting. Hence, the risk of ascertainment bias was reduced. As a final point, our study had a high precision due to large sample size.

Conclusion

The present study showed that higher parity is a risk factor for obesity in later life. Higher parity was observed among women in 45-49 years group, those currently married, housewives, and those with a lower level of education. Following a healthy lifestyle after childbirth may be helpful in preventing weight gain. The study's findings might help health professionals diagnose women at higher risk for obesity, as well as help health policymakers plan programs to prevent weight gain and postpartum obesity.

Ethical statement

This study was approved by the Ethics Committee of Research at Shiraz University of Medical Sciences, Iran (IR.SUMS.REC.1391.S6422).

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

MS,MT and AR: conceptualization and design; AR,MS and YA: methodology; AR and MS: acquisition of data; AR, MS, MT, YA: formal analysis and interpretation of data; MT, MS, AR, YA,FMM and SA: writing - original draft; SA, FMM and MS: writing - review and editing; AR and MS: supervision and project administration.
All authors have read and agreed to the submitted version of the manuscript.

References

[1] Malik VS, Willett WC, Hu FB. Global obesity: trends, risk factors and policy implications. Nature Reviews Endocrinology 2013;9:13-27. https://doi.org/10.1038/nrendo.2012.199

[2] Wang H, Naghavi M, Allen C, Barber RM, Bhutta ZA, Carter A, Casey DC, Charlson FJ, Chen AZ, Coates MM. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 2016;388(10053):1459-1544. https://doi.org/10.1016/S0140-6736(16)31012-1

[3] Organization WH. Obesity and overweight factsheet from the WHO. Health 2017.

[4] Bastien M, Poirier P, Lemieux I, Després J-P. Overview of epidemiology and contribution of obesity to cardiovascular disease. Prog Cardiovasc Dis 2014;56:369-81.

[5] Heber D. An integrative view of obesity. Am J Clin Nutr 2009;91:280S-3S. https://doi.org/10.1016/j.pcad.2013.10.016

[6] Deurenberg P, Yap M, Van Staveren WA. Body mass index and percent body fat: a meta analysis among different ethnic groups. Int J Obes 1998;22:1164. https://doi.org/10.1038/sj.ijo.0800741

[7] Bahrami H, Sadatsafavi M, Pourshams A, Kamangar F, Nouraei M, Semnani S, Brennan P, Boffetta P, Malekzadeh R. Obesity and hypertension in an Iranian cohort study; Iranian women experience higher rates of obesity and hypertension than American women. BMC Public Health 2006;6:158. https://doi.org/10.1186/1471-2458-6-158

[8] Kirkegaard H, Stovring H, Rasmussen KM, Abrams B, Sørensen TI, Nohr EA. How do pregnancy-related weight changes and breastfeeding relate to maternal weight and BMI-adjusted waist circumference 7 y after delivery? Results from a path analysis. Am J Clin Nutr 2014;99:312-9. https://doi.org/10.3945/ajcn.113.067405

[9] Moll U, Olsson H, Landin-Olsson M. Impact of Pregestational weight and weight gain during pregnancy on long-term risk for diseases. PloS One 2017;12:e0168543. https://doi.org/10.1371/journal.pone.0168543

[10] Dahlgren J. Pregnancy and insulin resistance. Metab Syndr Relat Disord 2006;4:149-52. https://doi.org/10.1089/met.2006.4.149

[11] Linné Y, Barkeling B, Rössner S. Long-term weight development after pregnancy. Obes Rev 2002;3:75-83. https://doi.org/10.1046/j.1467-789x.2002.00061.x

[12] Koch E, Bogado M, Araya F, Romero T, Diaz C, Manriquez L, Paredes M, Román C, Taylor A, Kirschbaum A. Impact of parity on anthropometric measures of obesity controlling by multiple confounders: a cross-sectional study in Chilean women. J Epidemiol Community Health 2008;62:461-70. https://doi.org/10.1136/jech.2007.062240

[13] Mansour A, Ajeel N. Parity is associated with increased waist circumference and other anthropometric indices of obesity. Eat Weight Disord 2009;14(2-3):e50-5. https://doi.org/10.1007/BF03327800

[14] Martínez ME, Pond E, Wertheim BC, Nodora JN, Jacobs ET, Bondy M, Daneri-Navarro A, Meza-Montenegro MM, Gutierrez-Millan LE, Brewster A. Association between parity and obesity in Mexican and Mexican-American women: findings from the Ella Binational breast cancer study. J Immigr Minor Health 2013;15:234-43. https://doi.org/10.1007/s10903-012-9649-8

[15] Robinson WR, Cheng MM, Hoggatt KJ, Stürmer T, Siega-Riz AM. Childbearing is not associated with young women's long-term obesity risk. Obesity 2014;22:1126-32. https://doi.org/10.1002/oby.20593

[16] Brown J, Kaye S, Folsom A. Parity-related weight change in women. Int J Obes Relat Metab Disord 1992;16:627-31.

[17] Heliövaara M, Aromaa A. Parity and obesity. J Epidemiol Community Health 1981;35:197-9. https://doi.org/10.1136/jech.35.3.197

[18] Kopelman PG. Obesity as a medical problem. Nature 2000;404(6778):635. https://doi.org/10.1038/35007508

[19] Mannan M, Doi SA, Mamun AA. Association between weight gain during pregnancy and postpartum weight retention and obesity: a bias-adjusted meta-analysis. Nutr Rev 2013;71:343-52. https://doi.org/10.1111/nure.12034

[20] Lee SK, Sobal J, Frongillo EA, Olson CM, Wolfe WS. Parity and body weight in the United States: differences by race and size of place of residence. Obes Res 2005, 13(7):1263-1269. https://doi.org/10.1038/oby.2005.150

[21] Abrams B, Heggeseth B, Rehkopf D, Davis E. Parity and body mass index in US women: a prospective 25-year study. Obesity 2013;21:1514-8. https://doi.org/10.1002/oby.20503

[22] Kim SA, Yount KM, Ramakrishnan U, Martorell R. The relationship between parity and overweight varies with household wealth and national development. Int J Epidemiol 2007;36:93-101. https://doi.org/10.1093/ije/dyl252

[23] Hajiahmadi M, Shafi H, Delavar MA. Impact of parity on obesity: a cross-sectional study in Iranian women. Med Princ Pract 2015;24:70-4. https://doi.org/10.1159/000368358

[24] Rebholz SL, Jones T, Burke KT, Jaeschke A, Tso P, D'Alessio DA, Woollett LA. Multiparity leads to obesity and inflammation in mothers and obesity in male offspring. Am J Physiol Endocrinol Metab 2012;302:E449-57. https://doi.org/10.1152/ajpendo.00487.2011

[25] Luoto R, Männistö S, Raitanen J. Ten-year change in the association between obesity and parity: results from the National FINRISK Population Study. Gend Med 2011;8:399-406. https://doi.org/10.1016/j.genm.2011.11.003

[26] Weng HH, Bastian LA, Taylor Jr DH, Moser BK, Ostbye T: Number of children associated with obesity in middle-aged women and men: results from the health and retirement study. J Womens Health 2004;13:85-91. https://doi.org/10.1089/154099904322836492

[27] Li W, Wang Y, Shen L, Song L, Li H, Liu B, Yuan J, Wang Y. Association between parity and obesity patterns in a middle-aged and older Chinese population: a cross-sectional analysis in the Tongji-Dongfeng cohort study. Nutr Metab 2016;13:72. https://doi.org/10.1186/s12986-016-0133-7

[28] Mamun A, McIntyre D, Najman J, Williams G, Khatun M, Finlay J, Callaway L. Young adult pregnancy status and the risk of developing overweight and obesity among women and men. Clin Obes 2018. https://doi.org/10.1111/cob.12262

[29] Magiakou MA, Mastorakos G, Rabin D, Margioris AN, Dubbert B, Calogero AE, Tsigos C, Munson PJ, Chrou-

sos GP: The maternal hypothalamic-pituitary-adrenal axis in the third trimester of human pregnancy. Clin Endocrinol 1996;44:419-28. https://doi.org/10.1046/j.1365-2265.1996.683505.x

[30] Pasquali R, Vicennati V, Cacciari M, Pagotto U. The hypothalamic-pituitary-adrenal axis activity in obesity and the metabolic syndrome. Ann N Y Acad Sci 2006;1083:111-28. https://doi.org/10.1196/annals.1367.009

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Correspondence: Mojtaba Sepandi, Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran - E-mail: msepandi@bmsu.ac.ir

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ORIGINAL ARTICLE

Predictors of mortality among hemodialysis patients in Hamadan province using random survival forests

LEILI TAPAK[1,2], VIDA SHEIKH[3], ENSIYEH JENABI[4], SALMAN KHAZAEI[5]
[1] Department of Biostatistics, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran; [2] Modeling of Non-communicable diseases research center, Hamadan University of Medical Sciences, Hamadan, Iran; [3] Clinical Research Development Unit of Shahid Beheshti Hospital, Hamadan University of Medical Sciences, Hamadan, Iran; [4] Autism Spectrum disorders Research Center, Hamadan University of Medical Sciences, Hamadan, Iran; [5] Research Center for Health Sciences, Hamadan University of Medical Sciences, Hamadan, Iran

Keywords

Hemodialysis • Kidney Failure • Survival • Random Survival Forest

Summary

Background. *Hemodialysis patients are at a high risk for morbidity and mortality. This study aimed to find the predictors of mortality and survival in hemodialysis patients in Hamadan province of Iran.*
Methods. *A number of 785 patients during the entire 10 years were enrolled into this historical cohort study. Data were gathered by a checklist of hospital records. The survival time was the time between the start of hemodialysis treatment to patient's death as the end point. Random survival forests (RSF) method was used to identify the main predictors of survival among the patients.*
Results. *The median survival time was 613 days. The number of 376 deaths was occurred. The three most important predictors of survival were hemoglobin, CRP and albumin. RSF method predicted survival better than the conventional Cox-proportional hazards model (out-of-bag C-index of 0.808 for RSF vs. 0.727 for Cox model).*
Conclusions. *We found that positivity of CRP, low serum albumin and low serum hemoglobin were the top three most important predictors of low survival for HD patients*

Introduction

Globally, chronic kidney disease (CKD) is an important health challenge with an alarming increase in incidence as well as prevalence [1]. CKD patients have a lower survival rate, and no significant improvement has been achieved on their survival over the past two decades [2]. In Iran, unlike some other countries, hemodialysis (HD) is the main way of renal replacement therapy in end stage renal disease (ESRD) patients [3]. Limited studies have been conducted in Iran regarding the survival of HD patients, suggesting a low survival rate for these patients [4, 5].
Common independent predictors of survival in HD patients are: age, race, albumin and hemoglobin levels, etiology of kidney failure and presence of certain comorbidities [3, 6-9]. Apart from these accepted prognostic factors, the role of some factors such as dialysis frequency is controversial on morbidity and mortality among hemodialysis patients [10].
In general, the interaction occurs when the effect of one risk factor is dependent on the presence of another risk factor [11]. When resources are limited, assessing interactions provides insight into the mechanisms for the outcome and identifying subgroups would benefit most from interventions [11, 12]. Interactions between some variables like three-way interaction between protein-energy wasting (PEW), inflammation and cardiovascular diseases (CVD) [13], interaction between albumin and phosphor [14] and three-way interaction between BMI, physical activity and smoking [15] on HD patients survival were shown previously. However, the lack of knowledge in this regard is still high.
Identification of survival modifiable factors and interactions between them could help in prioritizing the clinical care of HD patients. Evidences regarding the survival rate of hemodialysis patients in developing countries are rare and most of these studies were conducted in developed countries where patients have more access to health cares, and their results cannot be generalized to developing countries. Therefore, in the present study, we aimed to investigate the survival rate and it`s correlates in HD patients in Hamadan province using random survival forests.

Material and methods

Study Design

We performed a retrospective cohort study to investigate predictors of mortality among hemodialysis patients.

Study settings

We examined data on 758 patients who underwent hemodialysis treatment in Hamadan province in western Iran from March 2007 to March 2017. Hamadan province, with an area of 19,493 square kilometers in extent,

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is located in western Iran. According to the national census held by the Statistical Center of Iran, Hamadan province had a population of 1,758,268 people in 2011. We used information of patients from eight hospitals in the province with a dialysis wards, including: Alimoradian, Besat, Vali-asr, Ghaem, Imam Hossein, Valiasr, Imam Reza, and Shahid-Beheshti in Nahavand, Hamadan, Tuyserkan, Asadabad, Malayer, Razan, Kabudarahang and Hamadan city, respectively.

Eligibility criteria

All ESRD patients who initiated chronic hemodialysis programs at the dialysis units of the above mentioned hospitals during 2007 to 2017 were considered as inclusion criteria. Patients with acute renal failure or under treatment with peritoneal dialysis, patients on transient hemodialysis and patients with incomplete medical records were excluded from study.

Clinical and demographic measures

Data were gathered by a checklist on hospital records of all HD patients in the province. The checklist used in this study included characteristics related to demographic profiles (age (year), gender (male, female), marital status (married, single, divorce, widow), BMI (kg/m^2), residence area (urban, rural), educational level (illiterate, primary, guidance, high school, academic), the history of tobacco use (yes, no) and substance abuse (yes, no)), and clinical information (including Hemoglobin (g/dl), BUN (mg/dL), Creatinine (mg/dL), CRP status (positive, negative), Sodium (mEq/L), Calcium (mg/dL), Phosphor (mg/dL), iPTH (pg/ml), Albumin (g/dl) and ESRD cause (Hypertension, Diabetes, Urologic& obstructive diseases, Polycystic Kidney, Glomerulonephritis, Un-Known)). The clinical and laboratory information of patients at the beginning of their treatment and before receiving the first dialysis treatment was gathered. In order to minimize measurement variability, all two baseline measures (two last measurements before the first dialysis treatment) for each patient were averaged. These records were collected by reviewing patients' medical records.

Outcome

We considered the end point of the patients' follow up as their death. The survival time was the time between the start of HD treatment to patients death. Patients with renal transplantation, or withdrawal of dialysis, or unable to follow them due to transferred another dialysis facility out of province as well as, patients who died because of injury or accident, or other causes unrelated to renal failure were considered as censored cases.

Statistical analysis

We utilized the random survival forest (RF) method that ensembles binary decision trees and extends the RF regression model to right-censored survival data. In this technique, a random bootstrap sample (containing two thirds of the original data on average) is drawn for every decision tree. Thus, the remaining one third of the data known as out-of-bag (OOB) data is excluded. Decision trees are grown based on the bootstrap samples by applying a random node splitting process which works as follows: At each node random candidate variables (mtry determined by the square root of the total number of variables) are selected for random node splitting. Then the variable that maximizes the survival differences between two daughter nodes (determined by a splitting rule like the log-rank statistic) for a special split point is selected for node splitting. The growth of a decision tree is stopped when all the terminal nodes contain only a predefined minimal number of unique events.

To comparison, the stepwise Cox proportional hazards model was used. So, the Harrell's concordance index (C-index) criterion was utilized [16] using out-of-bag (OOB) data. The minimal depths of the covariates were obtained to select predictive variables. Minimal depth is a dimensionless order statistic that measures the predictiveness of a variable in a tree. It can be used to select variables in high-dimensional problems. It assesses the predictiveness of a variable by a depth calculation relative to the root node of a tree. The smaller the minimal depth, the more predictive the variable.

Analyses were performed by using "randomForestSRC", a freely available package from the Comprehensive R Archive Network (CRAN).

Results

Descriptive statistics of the characteristics of the patients were presented in Tab. I. According to the table, the majority of the patients were male (54.9%), married (79.1%), non-smoker (76.7%), non-substance abused, illiterate (54.9%) and lived in rural area (65.6%). About 47.8% of the patients experienced death. The median survival time was 613 days. The mean and standard deviation of other variables were reported in Table I.

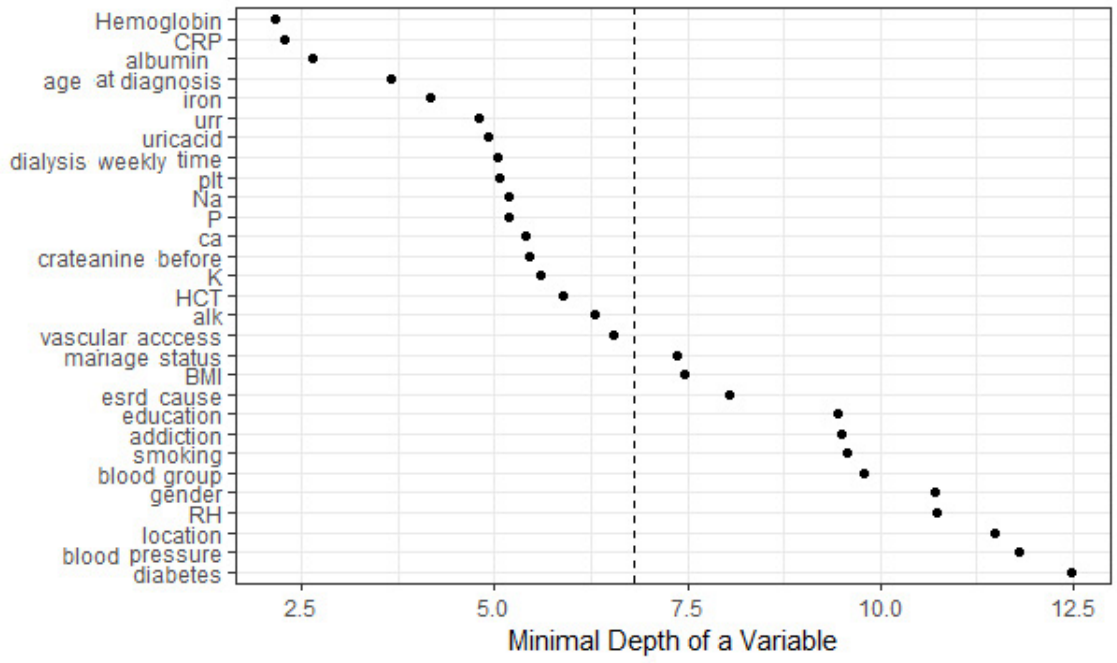
Figure 1 shows the minimal depth of the variables obtained from RSF. According to the figure, hemoglobin, CRP, albumin, age at diagnosis and iron were highly predictive and URR, uric acid, dialysis weekly time, PLT, Na, P, Ca, K, HCT, ALK and vascular access were moderately predictive. Moreover, other used variables were unlikely to be predictive. According to the stepwise Cox PH model, the variables of age at diagnosis, marriage status, BMI, addiction, hemoglobin, iron, albumin and CRP were selected as the most important variables (Tab. II). The performance of the RSF was assessed using Harrel's C-index and compared with the stepwise Cox PH. According to the results, the RSF had a higher c-index (0.808) compared with the Cox model (0.727).

The effects of the most four influential variables found in the RSF with 5-year partial survival plots analysis were demonstrated in Figure 2. The estimated partial survival for a variable shows the estimated survival for different levels of the variable when the effects of all other variables are justified. For example, patients with negative CRP shower a higher 5-year predicted survival

Tab. I. Descriptive statistics of characteristics related to participants.

Variable	Frequency (%)	Variable	Frequency (%)/Mean (SD)
Gender		**ESRD cause**	
Male	431 (54.9)	Hypertension	222 (28.3)
Female	354 (45.1)	Diabetes	193 (24.6)
Marital status		Urologic& obstructive diseases	75 (9.6)
Married	621 (79.1)	Polycystic Kidney	35 (4.5)
Single	81 (10.3)	Glomerulonephritis	56 (7.1)
Divorce	11 (1.4)	Diabetes and Hypertension	79 (10.1)
Widow	72 (9.2)	Unknown	-
The history of tobacco use			
Yes	183 (23.3)	**BMI (kg/m²)**	23.10 (3.97)
No	602 (76.7)	**Hemoglobin (g/dl)**	10.49 (1.92)
Substance abuse		**Creatinine (mg/dL)**	**7.85 (3.18)**
Yes	121 (15.4)	**Sodium (mEq/L)**	138.88 (7.24)
No	664 (84.6)	**Calcium (mg/dL)**	8.71 (0.86)
Residence area		**Phosphor (mg/dL)**	5.11 (1.58)
Urban	487 (62)	**Iron**	99.89 (97.58)
Rural	298 (38)	**Albumin (g/dl)**	3.69 (0.72)
CRP status			
Positive	270 (34.4)		
Negative	515 (65.6)		
Education			
Illiterate	415 (52.9)		
Primary school	229 (29.2)		
High school	114 (14.5)		
Academic	27 (3.4)		

Fig. 1. Minimal depth of variables affecting survival of HD patients obtained using random survival forest.



compared with those with positive CRP. The non-linear nature of the selected variables is evident from the figure. For example, as hemoglobin increases up to about 10 (g/dl (the five-year predicted survival increases very slightly and it tends to increase dramatically after that point up to.

Figure 3 displays interaction between the three important variables of CRP, hemoglobin, and Albumin on 5 year predicted survival. Patients with positive CRP and hemoglobin values lower than 11 have the worst survival (see the first row, second column) and most had low albumin. Survival was best for patients

Tab. II. The results of stepwise Cox proportional Hazards in selecting predictors affecting survival time of HD patients in Hamadan Province from 2007 to 2017.

Variables	B	SE	P.Value	Exp(B)	95% CI for Exp(B)		
					Lower	Upper	
Age at diagnosis	-0.023	0.006	< 0.001	0.977	0.966	0.988	
Marriage status	Married	1.000					
	Single	-1.423	0.615	0.021	0.241	0.072	0.804
	Divorce	-0.174	0.464	0.707	0.840	0.338	2.085
	Widow	0.308	0.486	0.526	1.361	0.525	3.525
Location	Urban	1.000					
	Rural	-0.220	0.110	0.046	0.803	0.647	0.996
BMI status	< 18.5 kg/m^2	1.000		0.010			
	18.5-25 kg/m^2	1.000	0.303	0.001	2.719	1.500	4.926
	25-30 kg/m^2	0.663	0.250	0.008	1.941	1.190	3.165
	> 30 kg/m^2	0.740	0.265	0.005	2.096	1.247	3.523
Addiction	Yes	1.000					
	No	-0.326	0.136	0.017	0.722	0.552	0.942
Hemoglobin	-0.203	0.030	< 0.001	0.816	0.769	0.866	
Iron	-0.003	0.001	< 0.001	0.997	0.996	0.999	
Albumin	-0.421	0.070	< 0.001	0.656	0.572	0.753	
CRP	-1.157	0.119	< 0.001	0.314	0.249	0.397	

Fig. 2. Partial 5-year predicted survival for four most influential variables on survival in HD data. Dashed red lines are ± 2 standard error bars (red dots indicates the estimated survival at the observed levels of each correlate and the black hatches are the smoothed curves based on the loess curves for the estimated survival for each individual.

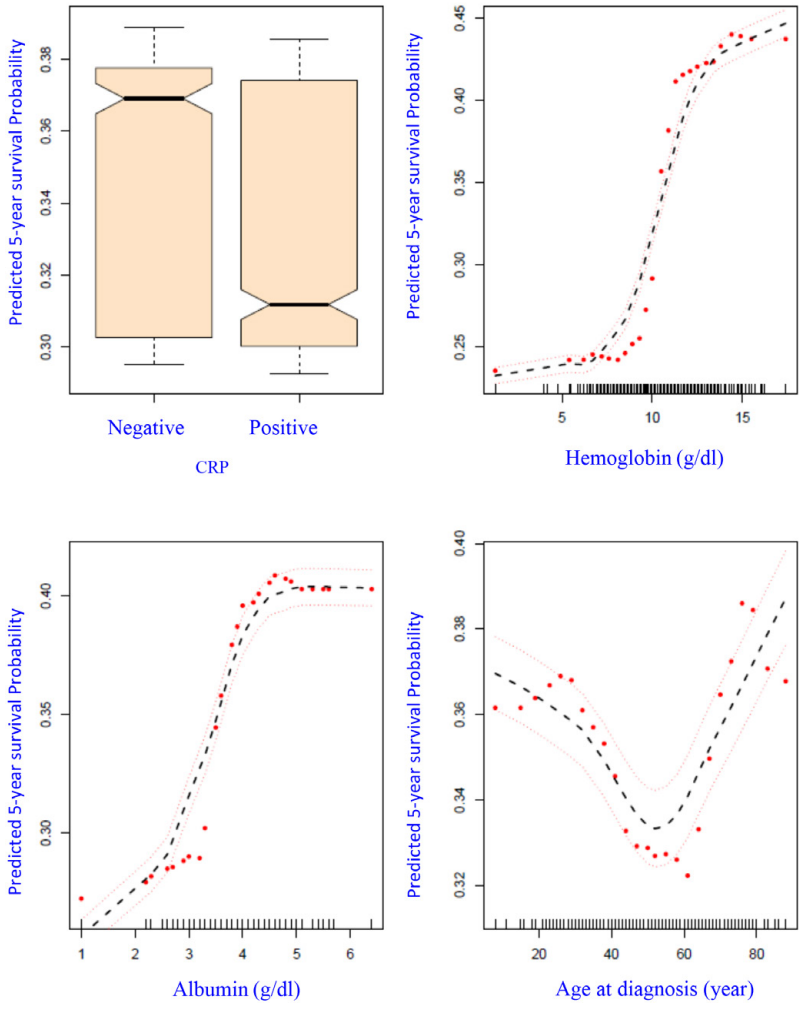
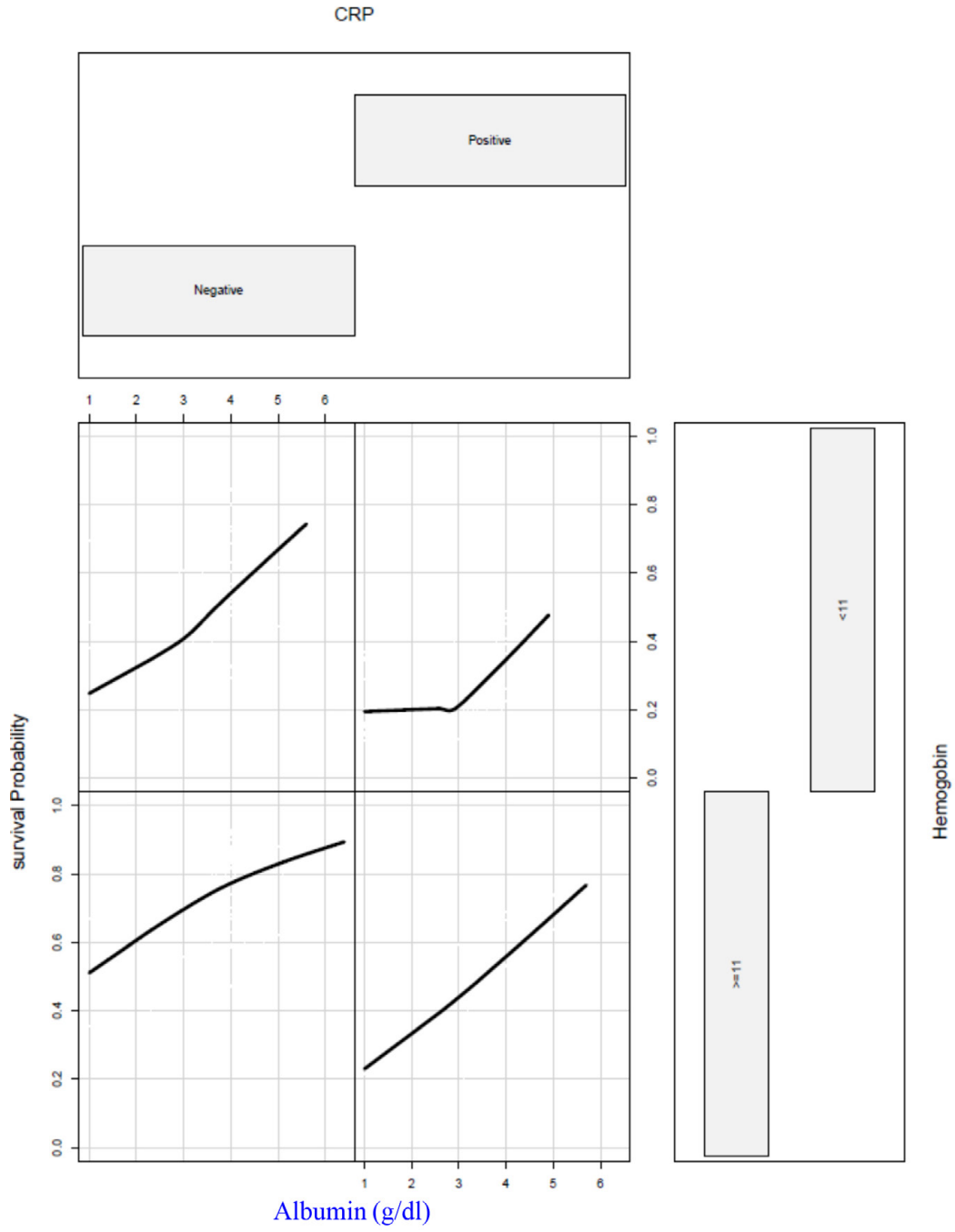


Fig. 3. Random survival forest estimated five-year survival as a function of CRP, Hemoglobin, and Albumin. Smoothed curves are loess curves of the estimated survival for each individual.



with negative CRP and hemoglobin ≥ 11 (see second row, first column) and further dependent on changes in albumin.

In this group the 5 years predicted survival was over 70% for those with albumin values greater than 4, but only about 50% for those with albumin of 1 (g/dl). It is important to note that these interactions and non-linear relationships were identified by the random survival forest, and not prespecified by the analyst. The median survival time in the two categories of low Hemoglobin/positive CRP and high Hemoglobin/negative CRP were 5.38 and 40.53 months, respectively.

Discussion

Hemodialysis is a common treatment modality for ESRD patients in Iran. In this study we investigated the predictors of survival in HD patients. The findings of this study can be used to improve quality of cares provided for the HD patients and better resource allocation. We found that hemoglobin level, albumin level, and CRP status were the top three most important predictors of survival for HD patients in the present study.

Along with the results of this study, the role of low hemoglobin level in increasing the risk of mortality in

HD patients has been shown in previous studies [17, 18]. Anemia is one of the main risk factors in the development of left ventricular hypertrophy (LVH), and consequence cardiac mortality and morbidity in ESRD patients [19]. Early management of anemia is associated with reduction in the severity of comorbid conditions and can slow the progression to renal failure [20]. Robert et al. showed that normalization of hemoglobin through preventing the development of LV dilation, leads to improved quality of life of HD patients [21].

In agreement with our finding, other studies results indicated that low serum albumin is one of the main predictors of poor survival in HD patients [22, 23]. Hypoalbuminemia usually considered as the proxy of malnutrition. Mafra et al. in a Brazilian cohort of hemodialysis patients found that both low BMI and hypo-albuminemia are strong predictors of death [24]. Combe et al. showed that a decrease in serum albumin over time correlated with increased CVD death [25].

One of the other main predictors of mortality in HD patients in this study was CRP status. Similar findings have been obtained in other studies [26, 27]. Lseki et al. in their study showed that regardless of serum albumin and other possible confounders, CRP is a significant predictor of death in HD patients [28]. Inflammation usually is in relation with insulin resistance, oxidative stress, wasting, infections and endothelial dysfunction [29].

Interactions between some modifiable variables on survival of HD patients were seen previously [13, 15]. We found that the combined effect of CRP status with serum Hemoglobin and CRP status has the significant effect on the survival of HD patients. This finding is important because HD patients with a high mortality risk can be identified through regular screening. More studies are needed to determine multiple pathophysiological pathways may underlie these combined effects.

This study had some limitations as well. First, because of the retrospective design of the study, verifying quality control of the data was not possible. Second, the addiction and smoking status of patients was based on their self-report and therefore was prone to information bias and finally quality of the services and technology may vary over time, and also the quality of service provision in the dialysis wards of hospitals is not the same, which could not be considered in this study. Despite these limitations there was strength for this study which was the utilization of the RSF to analyze the data set. RSF can handle the issues with the traditional Cox model like proportionality assumption automatically. This will help analysts to deal with the relationships (i.e. linear, nonlinear) between variables over time without any previous knowledge.

Conclusions

We found that higher levels of CRP, low serum albumin and low serum hemoglobin were the top three most important predictors of poor survival for HD patients.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

SK and LT are responsible for the design of the study. They analyzed the data and wrote the paper. VSH and EJ dealt with the collection of information. All authors reviewed and approved the final version of the manuscript.

References

[1] Grassmann A, Gioberge S, Moeller S, Brown G. ESRD patients in 2004: global overview of patient numbers, treatment modalities and associated trends. Nephrol Dial Transplant 2005;20:2587-93. https://doi.org/10.1093/ndt/gfi159

[2] Collins AJ, Foley RN, Herzog C, Chavers B, Gilbertson D, Ishani A, Kasiske B, Liu J, Mau LW, McBean M, Murray A, St Peter W, Guo H, Gustafson S, Li Q, Li S, Li S, Peng Y, Qiu Y, Roberts T, Skeans M, Snyder J, Solid C, Wang C, Weinhandl E, Zaun D, Arko C, Chen SC, Dalleska F, Daniels F, Dunning S, Ebben J, Frazier E, Hanzlik C, Johnson R, Sheets D, Wang X, Forrest B, Constantini E, Everson S, Eggers P, Agodoa L. US renal data system 2010 annual data report. Am J Kidney Dis 2011;1(57):A8, e1-526. https://doi.org/10.1053/j.ajkd.2010.10.007

[3] Mafra D, Farage NE, Azevedo DL, Viana GG, Mattos JP, Velarde LGC, Fouque D. Impact of serum albumin and body-mass index on survival in hemodialysis patients. Int Urol Nephrol 2007;39:619-24. https://doi.org/10.1007/s11255-007-9201-2

[4] Mousavi SSB, Hayati F, Ansari MJA, Valavi E, Cheraghian B, Shahbazian H, et al. Survival at 1, 3, and 5 years in diabetic and nondiabetic patients on hemodialysis. Iran J Kidney Dis 2010;4:74.

[5] Montaseri M, Yazdani Cherat J, Espahbodi F, Mousavi SJ. Five-year survival rate in hemodialysis patients Attending Sari Imam Khomeini Hospital. Journal of Mazandaran University of Medical Sciences 2013;23(101):78-85.

[6] Bleyer AJ, Tell GS, Evans GW, Ettinger WH, Burkart JM. Survival of patients undergoing renal replacement therapy in one center with special emphasis on racial differences. Am J Kidney Dis 1996;28:72-81. https://doi.org/10.1016/s0272-6386(96)90133-x

[7] Brodowska-Kania D, Rymarz A, Gibin K. First year survival of patients on maintenance dialysis treatment in Poland. Nagoya J Med Sci 2015;77:629.

[8] Akizawa T, Saito A, Gejyo F, Suzuki M, Nishizawa Y, Tomino Y, omino Y, Tsubakihara Y, Akiba T, Hirakata H, Watanabe Y, Kawanishi H, Bessho M, Udagawa Y, Aoki K, Uemura Y, Ohashi Y; JET Study Group. Low hemoglobin levels and hypo-

responsiveness to erythropoiesis-stimulating agent associated with poor survival in incident japanese hemodialysis patients. Ther Apher Dial 2014;18:404-13. https://doi.org/10.1111/1744-9987.12155

[9] Iseki K, Tozawa M, Takishita S. Effect of the duration of dialysis on survival in a cohort of chronic haemodialysis patients. Nephrol Dial Transplant 2003;18:782-7. https://doi.org/10.1093/ndt/gfg145

[10] Slinin Y, Greer N, Ishani A, MacDonald R, Olson C, Rutks I, Wilt TJ. Timing of dialysis initiation, duration and frequency of hemodialysis sessions, and membrane flux: a systematic review for a KDOQI clinical practice guideline. Am J Kidney Dis 2015;66:823-36. https://doi.org/10.1053/j.ajkd.2014.11.031

[11] VanderWeele TJ, Knol MJ. A tutorial on interaction. Epidemiologic Methods. 2014;3:33-72.

[12] de González AB, Cox DR. Interpretation of interaction: a review. Ann Appl Stat 2007;1:371-85.

[13] de Mutsert R, Grootendorst DC, Axelsson J, Boeschoten EW, Krediet RT, Dekker FW; NECOSAD Study Group. Excess mortality due to interaction between protein-energy wasting, inflammation and cardiovascular disease in chronic dialysis patients. Nephrol Dial Transplant 2008;23:2957-64. https://doi.org/10.1093/ndt/gfn167

[14] Zitt E, Lamina C, Sturm G, Knoll F, Lins F, Freistätter O, Kronenberg F, Lhotta K, Neyer U. Interaction of time-varying albumin and phosphorus on mortality in incident dialysis patients. Clin J Am Soc Nephrol 2011;6:2650-6. https://doi.org/10.2215/CJN.03780411

[15] Hallan S, de Mutsert R, Carlsen S, Dekker FW, Aasarød K, Holmen J. Obesity, smoking, and physical inactivity as risk factors for CKD: are men more vulnerable? Am J Kidney Dis 2006;47:396-405. https://doi.org/10.1053/j.ajkd.2005.11.027

[16] Harrell FE, Jr., Califf RM, Pryor DB, Lee KL, Rosati RA. Evaluating the yield of medical tests. JAMA 1982;247:2543-6.

[17] Regidor DL, Kopple JD, Kovesdy CP, Kilpatrick RD, McAllister CJ, Aronovitz J, Greenland S, Kalantar-Zadeh K. Associations between changes in hemoglobin and administered erythropoiesis-stimulating agent and survival in hemodialysis patients. J Am Soc Nephrol 2006;17:1181-91. https://doi.org/10.1681/ASN.2005090997

[18] Gilbertson DT, Ebben JP, Foley RN, Weinhandl ED, Bradbury BD, Collins AJ. Hemoglobin level variability: associations with mortality. Clin J Am Soc Nephrol 2008;3:133-8.

[19] Berweck S, Hennig L, Sternberg C, Dingerkus H, Ludat K, Hampl H. Cardiac mortality prevention in uremic patients. Therapeutic strategies with particular attention to complete correction of renal anemia. Clin Nephrol 2000;53(1 Suppl):S80-5.

[20] Kausz AT, Khan SS, Abichandani R, Kazmi WH, Obrador GT, Ruthazer R, Pereira BJ. Management of patients with chronic renal insufficiency in the Northeastern United States. J Am Soc Nephrol 2001;12:1501-7.

[21] Ofsthun N, Labrecque J, Lacson E, Keen M, Lazarus JM. The effects of higher hemoglobin levels on mortality and hospitalization in hemodialysis patients. Kidney Int 2003;63:1908-14. https://doi.org/10.1046/j.1523-1755.2003.00937.x

[22] Kalantar-Zadeh K, Supasyndh O, Lehn RS, McAllister CJ, Kopple JD. Normalized protein nitrogen appearance is correlated with hospitalization and mortality in hemodialysis patients with Kt/V greater than 1.20. J Ren Nutr 2003;13:15-25. https://doi.org/10.1053/jren.2003.50005

[23] Kalantar-Zadeh K, Kilpatrick RD, Kuwae N, McAllister CJ, Alcorn Jr H, Kopple JD, Greenland S. Revisiting mortality predictability of serum albumin in the dialysis population: time dependency, longitudinal changes and population-attributable fraction. Nephrol Dial Transplant 2005;20:1880-8. https://doi.org/10.1093/ndt/gfh941

[24] Mafra D, Farage NE, Azevedo DL, Viana GG, Mattos JP, Velarde LG, Fouque D. Impact of serum albumin and body-mass index on survival in hemodialysis patients. Int Urol Nephrol 2007;39:619-24. https://doi.org/10.1007/s11255-007-9201-2

[25] Combe C, Chauveau P, Laville M, Fouque D, Azar R, Cano N, Canaud B, Roth H, Leverve X, Aparicio M; French Study Group Nutrition in Dialysis. Influence of nutritional factors and hemodialysis adequacy on the survival of 1,610 French patients. Am J Kidney Dis 2001;37:S81-S8. https://doi.org/10.1053/ajkd.2001.20756

[26] Yeun JY, Levine RA, Mantadilok V, Kaysen GA. C-reactive protein predicts all-cause and cardiovascular mortality in hemodialysis patients. Am J Kidney Dis 2000;35:469-76. https://doi.org/10.1016/s0272-6386(00)70200-9

[27] de Filippi C, Wasserman S, Rosanio S, Tiblier E, Sperger H, Tocchi M, Christenson R, Uretsky B, Smiley M, Gold J, Muniz H, Badalamenti J, Herzog C, Henrich W. Cardiac troponin T and C-reactive protein for predicting prognosis, coronary atherosclerosis, and cardiomyopathy in patients undergoing long-term hemodialysis. JAMA 2003;290:353-9. https://doi.org/10.1001/jama.290.3.353

[28] Iseki K, Tozawa M, Yoshi S, Fukiyama K. Serum C-reactive protein (CRP) and risk of death in chronic dialysis patients. Nephrol Dial Transplant 1999;14:1956-60. https://doi.org/10.1093/ndt/14.8.1956

[29] Furuland H, Linde T, Ahlmén J, Christensson A, Strömbom U, Danielson BG. A randomized controlled trial of haemoglobin normalization with epoetin alfa in pre-dialysis and dialysis patients. Nephrol Dial Transplant 2003;18:353-61. https://doi.org/10.1093/ndt/18.2.353

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Correspondence: Salman Khazaei, Research Center for Health Sciences, Hamadan University of Medical Sciences, Hamadan, Iran - E-mail: salman.khazaei61@gmail.com

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ORIGINAL ARTICLE

Direct detection of free vitamin D as a tool to assess risk conditions associated with chronic plaque psoriasis

TIZIANA GRASSI[1], ALESSANDRA PANICO[1], FRANCESCO BAGORDO[1], GIOVANNI IMBRIANI[1], ISABELLA GAMBINO[1], DEBORA LOBREGLIO[2], GIAMBATTISTA LOBREGLIO[2], MAURIZIO CONGEDO[3], ANTONELLA DE DONNO[1]
[1] Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy; [2] Clinical Pathology Laboratory, Vito Fazzi General Hospital, Lecce, Italy; [3] Dermatology Unit, Vito Fazzi General Hospital, Lecce, Italy

Keywords

Psoriasis • Free vitamin D • Direct immunoassay • Cross-sectional study

Summary

Introduction. *Psoriasis is a major public health problem that results in high social and health costs. New approaches and methods are required to identify any conditions related to the disease and comorbidity development. The vitamin D deficiency is associated to psoriasis and could play an important role in its pathogenesis. However, the serum level of vitamin D is currently measured as total vitamin D, which is affected by wide variability. Therefore, the determination of the free form could be more significant, since it is independent of confounding factors. A cross-sectional study was performed to assess the association between chronic plaque psoriasis and serum level of free vitamin D, detected by a direct analytical method.*
Methods. *The levels of bioavailable vitamin D, total vitamin D and other metabolic parameters whose homeostasis is regulated by vitamin D were evaluated in 72 psoriasis patients and in 48 healthy controls. A direct immunoassay method was used to measure serum free vitamin D level. Analysis of covariance was performed to calculate estimated marginal means (EMM) and 95% confidence interval (CI), after adjustment for age, sex and BMI, within the two groups.*
Results. *Patients showed an EMM of 5.526 ± 0.271pg/ml, 95% CI 4.989-6.063; while controls an EMM of 6.776 ± 0.271 pg/ml, 95% CI 6.115-7.437.*
Conclusions. *Chronic plaque psoriasis patients exhibited a serum level of free vitamin D lower than controls. The direct immunoassay method could represent a useful tool to assess vitamin D status and identify a risk condition associated with the onset of the pathology.*

Introduction

Psoriasis is a chronic inflammatory skin disease characterized by raised, well-demarcated, erythematous oval plaques with adherent silvery scales, resulting from a hyperproliferative epidermis with abnormal differentiation [1, 2].
It is a major public health problem, affecting approximately 125 million people worldwide [3]. Globally, the prevalence of psoriasis varies considerably across different populations, with higher levels in northern countries and in Caucasians. Overall, reported prevalence rates were about 1-3%, up to 8.5 and 11.8% in Norway and the Arctic, respectively. In Italy, the prevalence of psoriasis ranged from 1.8 to 3.1% in the overall general Italian population, varying across regions [4].
The majority of cases were diagnosed in individuals younger than 30 years of age with more than 10 000 cases per year arising in children less than 10 years old [5]. The prevalence of psoriasis among children was up to 2% in Europe and 2.1% in Italy [6].
The cutaneous manifestations of psoriasis have a strong negative impact on quality of life and social relations. In addition to the skin lesions, several adverse effects and comorbid conditions can affect psoriasis patients, including arthritis (which can be severe and deforming), bowel inflammatory disease, cardiovascular diseases, metabolic syndrome, mental distress, depression, which all contribute to the disease burden [7-10].
Psoriasis also places a substantial economic burden on both patient and society [11, 12]. Psoriasis patients are more likely to require urgent care, have greater hospitalization rates and more frequent outpatient visits, and incur greater costs than other patients [13]. In addition, comorbidities result in higher overall pharmacy and medical costs [14]. The financial burden of this disease in the USA has been estimated to be as high as $11.25 billion annually [9].
The pathogenesis of psoriasis involves autoimmune, genetic and environmental factors. In addition, the vitamin D altered pathway may play a role in the pathogenesis of psoriasis since the skin acts as both a site of its biosynthesis and a target organ for its activity [15, 16].
Deficiency of the active form of vitamin D was hypothesized to be implicated in the onset of psoriatic plaques and, for this reason, drugs based on vitamin D and its analogues have been used as standard therapy for decades [17, 18].
Recently, therapies based on biotechnological drugs have been developed; their action is directly performed against specific parts of the immune system associated with psoriasis without altering the serum levels of vitamin D.
The biosynthesis of vitamin D requires that ultraviolet solar rays penetrate the skin and transform 7-dehydro-

cholesterol into pre-vitamin D, which is rapidly converted to vitamin D.
Vitamin D is transported to the liver by Vitamin D Binding Protein (DBP), where it undergoes a first hydroxylation of carbon 25 thus forming 25 hydroxyvitamin D (25(OH) D) which is the major circulating form of vitamin D and whose serum levels are commonly used for the evaluation of vitamin D status. [19]. The 25(OH)D can be found for about 85-90% bound with high affinity to DBP, for 10-15% weakly bound to albumin and less than 1% exists in free form, which is able to perform physiological functions; the sum of free and albumin-bound vitamin D represents the bioavailable vitamin D [20].
Several studies have shown that the serum levels of 25(OH)D were significantly lower among patients with psoriasis compared to healthy controls [21-23]. However this association was often unclear because it took into account the plasma level of total 25(OH) vitamin D which is affected by numerous individual, genetic, environmental and physiological factors which result in a wide variability in the general population.
The level of vitamin D could be more significant if the free form were detected, since it is independent of confounding factors and much better correlates with some pathological conditions. However, in most of the published studies the dosage of free 25(OH)D was carried out by indirect and low accurate methods. Moreover, there are no current studies on the measurement of this parameter in psoriasis patients.
A cross-sectional study was performed to assess the association between chronic plaque psoriasis and serum level of free 25(OH)D, detected by a direct analytical method.
The levels of total vitamin D, bioavailable vitamin D and other metabolic parameters whose homeostasis is regulated by vitamin D were also evaluated either in psoriasis patients and in healthy controls.

Methods

Study design

The study was performed in a three-months period (January-March 2018) in order to avoid the variability of vitamin D levels due to seasons.
The patients were recruited from the 128 subjects diagnosed with chronic plaque psoriasis who, during the study period, went to the Psoriasis Centre of the Dermatology Unit of the Vito Fazzi General Hospital in Lecce for a check-up. The healthy controls were healthcare workers of the hospital who voluntary accepted to participate in the study after an informed invitation. Information about age, sex, drugs or any phototherapy treatments of subjects were obtained from their medical records and were used to select the individuals eligible for the study.
All participating subjects had to be at least 18 years old and of Caucasian race. In addition, for patients the following criteria of inclusion were set: a clinical diagnosis of chronic plaque psoriasis (lasting at least 6 months) regardless of the actual score of the Psoriasis Area and Severity Index (PASI), the absence of systemic or topical anti-psoriasis treatments, including phototherapy and/or topical vitamin D derivatives, for at least 3 months before the study investigations. The only systemic treatment allowed was the therapy with biotechnological drugs. Patients with other types of psoriasis (guttate psoriasis, erythrodermic and pustular), those with concomitant inflammatory bowel disease (i.e. Crohn's disease and ulcerative colitis) and those receiving therapeutic interventions that could affect the status of vitamin D, including bisphosphonates, systemic corticosteroids, vitamin D and calcium supplements were also excluded. The subjects recruited as controls were free from current or previous pathologies, did not take vitamin D or calcium supplements and were not subjected to phototherapy.
Overall, after exclusion of not eligible subjects, seventy-two patients and forty-eight controls were included in the study.
The measurement of analytical parameters was performed on blood samples taken from patients who are subjected to the laid down checks for their pathology and from healthcare workers who routinely undergo health surveillance. Each subject, after being informed about the research purposes, gave his/her consent for processing personal data and the execution of the analytical investigations provided by the present study.
Data regarding anthropometric measurements (weight and height) from both patients and controls, taken according to World Health Organization (WHO) recommendations [24], were used to calculate the body mass index (BMI) (weight [kg]/height [m]squared).
The study was conducted in compliance with Helsinki Declaration and with the Italian Laws concerning the rights of the study participants.

PASI score

The most frequently adopted measure to define the severity of skin involvement is the Psoriasis Area and Severity Index (PASI), a numerical index that combines the extension of the damage with other clinical signs [25]. This index is based on the quantitative assessment of three typical signs of psoriatic lesions: erythema (E), infiltration (I), and desquamation (D), on a scale of 0-4, combined with the skin surface involved (A = affected area), which is divided in four separate body areas: head (h), trunk (t), and upper (u) and lower extremities (l). To calculate PASI, the formula is:

0,1 (Eh + Ih + Dh) Ah + 0,2 (Eu + Iu + Du) Au + 0,3 (Et + It + Dt) At + 0,4 (El + Il + Dl) Al

A numerical value is given to the extent of the lesions in each area: 1 = < 10%; 2 = 10-30%; 3 = 30-50%; 4 = 50-70%; 5 = 70-90%; and 6 = 90-100%. E, I and D are assessed according to a five-point scale (0 = no symptoms; 1 = slight; 2 = moderate; 3 = marked; and 4 = very marked) to obtain a final value between 0 and 72.

Measurement of analytical parameters

After sampling, blood was promptly centrifuged (3000 rpm at 4°C for 10 minutes) and serum was immediately stored at -80°C until the measurement of the analytical parameters. Blood samples from both patients and controls were dosed in a single analytical session carried out in the Clinical Pathology Unit of the Vito Fazzi General Hospital in Lecce and the following parameters were assessed: total 25(OH)D, free 25(OH)D, parathyroid hormone (PTH), albumin, calcium, phosphorus; the bioavailable 25(OH)D was calculated on the basis of total 25(OH)D and albumin levels and the albumin affinity constant for vitamin D [26]. The laboratory uses standard operating procedures, carries out the required internal quality controls and participates in external quality checks for all the services provided.

The method used to measure the free 25(OH)D is described in the following section.

For the total 25(OH)D measurement was used Abbott Architect 25-OH D reagent on i2000 Architect analyzer (Abbott Laboratories, Abbott Park, IL 60064 USA) with a chemiluminescent competitive delayed phase immunoassay (Chemiflex) standardized according to the NIST SRM 2972 (National Institute of Standard & Technology Standard Reference Material 2972).

PTH 1-84 measurement was performed with a two-step chemiluminescent immunoassay on DiaSorin LIAISON XL analyzer (DiaSorin, Stillwater, Mn, USA).

Albumin was measured with an immunoturbidimetric method (Roche Tina-quant Albumin) standardized using reference material ERM-DA470k/IFCC of the IRMM (Institute for Reference Materials and Measurements) on Roche/Hitachi Modular P analyzer (Roche Diagnostic GmbH, Mannheim, Deutschland). The same analyzer was also used to measure calcium and phosphorus levels with a spectrophotometric method, respectively Roche CA2 and Roche Phosphate inorganic ver.2.

Measurement of free 25(OH)D

The measurement of free 25(OH)D was performed with a direct quantitative immunoassay on a microplate developed by Future Diagnostics Solutions (Wijchen, The Netherlands), based on patented monoclonal antibodies from DIAsource ImmunoAssays (Louvain-la-Neuve, Belgium). Briefly, calibrators, controls and samples diluted 1:10 were transferred into the microplate wells coated with the anti-vitamin D antibody and incubated for 90 min at 37°C with shaking at 650 RPM. After washing, 100 µL of biotinylated 25(OH)D were added to each well and the plate was incubated for 30 min at 37°C with shaking at 650 RPM and washed again. Then, a 100 µL aliquot of the streptavidin-peroxidase reagent was added to the wells and further incubated for 20 min at 37°C with shaking at 650 RPM. In the next step, the plate was washed, added with 100 µL of tetramethylbenzidine and incubated for 15 min at room temperature, stationary and protected from light. Finally, 100 µL of HCl 1 M were added to stop the reaction and the absorbance was read on the spectrophotometer at a wavelength of 450 nm. According to the producers, this test has a sensitivity of 0.5-1.0 pg/mL.

Statistical analysis

All data were entered into a Microsoft Excel database and statistically processed using MedCalc Software version 14.8.1 (MedCalc Software bvba, Ostend, Belgium). The chi-square test was used to detect any difference in the distribution of female and male subjects among patients and controls; while the t-student test was used for age and BMI. For all analytical parameters (dependent variable) estimated marginal means (EMM), standard error (SE) and 95% confidence interval (CI), after adjustment for age, sex and BMI, were calculated within the two considered groups by analysis of covariance. In all cases, differences were considered significant when $p < 0.05$.

Results

The study population consisted of 72 patients affected by psoriatic plaque disease (41 males and 31 females) and 48 controls (22 males and 26 females) (Tab. I). On average patients were 50.5 ± 13.0 years old and control 50.0 ± 12.9 years old. The BMI average value was 27.8 ± 5.98 kg/m^2 for patients and 25.4 ± 5.45 kg/m^2 for controls ($p = 0.024$). PASI score calculated for cases was

Tab. I. Descriptive characteristics of patients and controls.

Variables		Unit of measure	Patients (n = 72)	Controls (n = 48)	*p-values*
Age		Average ± DS	50.5 ± 13.0	50.0 ± 12.9	***0.846****
Sex	Male	N (%)	41 (56.9%)	22 (46%)	***0.233*** **
	Female		31 (43%)	26 (54%)	
BMI		Kg/m^2 ± DS	27.8 ± 5.98	25.4 ± 5.45	***0.024****
PASI		Score ± DS	1.88 ± 3.34	0	-
Drugs	Ustekinumab	N (%)	32 (43.7%)	0	-
	Adalimumab		11 (16.6%)	0	-
	Etanercept		14 (18.7%)	0	-
	Secukinumab		9 (12.5%)	0	-
	Golimumab		6 (9%)	0	-

*Differences between groups evaluated by Student t-test; ** differences between groups evaluated by chi square test.

Tab. II. Estimated margin means (EMM) ± standard error (SE) and 95% confidence interval (CI) of serum analytical parameters after adjustment for age, sex and BMI among patients affected by psoriasis and controls.

Variables	Groups	EMM ± SE	95% CI	*p-values*
Total Vitamin D (ng/ml)	Patients Controls	23.051 ± 1.195 25.286 ± 1.473	20.684-25.418 22.369-28.203	***0.2477***
Free Vitamin D (pg/ml)	Patients Controls	5.526 ± 0.271 6.776 ± 0.271	4.989-6.063 6.115-7.437	***0.0049***
Albuminemia (g/dl)	Patients Controls	4.146 ± 0.041 4.197 ± 0.050	4.065-4.227 4.098-4.297	***0.4379***
Calcemia (mg/dl)	Patients Controls	9.665 ± 0.068 9.614 ± 0.083	9.531-9.799 9.449-9.780	***0.6409***
Phosphoremia (mg/dl)	Patients Controls	3.570 ± 0.076 3.728 ± 0.093	3.420-3.720 3.543-3.913	***0.1969***
PHT (pg/ml)	Patients Controls	22.539 ± 1.030 22.633 ± 1.269	20.500-24.579 20.120-25.147	***0.9549***
Bioavailable Vitamin D (ng/ml)	Patients Controls	2.232 ± 0.128 2.522 ± 0.158	1.979-2.486 2.210-2.834	***0.1613***

on average 1.88 ± 3.34. Patients involved in the study were treated only with biotech drugs, among which Ustekinumab was the most used (43.7%).

The measurement of analytical parameters showed that in patients the serum concentration of Total and Free 25(OH)D was on average 22.781 ± 11.410 ng/ml and 5.487 ± 2.238 pg/ml respectively while in controls 25.692 ± 7.482 ng/ml and 6.642 ± 2.379 pg/ml.

The estimated marginal mean (EMM), adjusted for age, sex and BMI, of Total 25(OH)D in patients was 23.051 ± 1.195 ng/ml while in controls was 25.286 ± 1.473 ng/ml (Tab. II). However, differences seemed to be not significant ($p > 0.05$). Overall, 58 (80,5%) patients and 36 (75,0%) controls ($p < 0.05$) had a level of total 25(OH)D lower than the reference threshold of 30 ng/ml (not in table). Also the bioavailable 25(OH)D was detected lower, with non-significant differences, among patients respect to controls. Instead, significant differences ($p < 0.05$) were observed for the free 25(OH)D, whose concentration appeared lower in patients (EMM 5.526 ± 0.271pg/ml; 95% CI 4.989-6.063) compared to controls (EMM 6.776 ± 0.271 pg/ml; 95% CI 6.115-7.437).

PTH, albuminemia, calcemia and phosphoremia showed similar levels both for patients and controls.

Discussion

This study evaluated serum concentration of free 25(OH) vitamin D by a direct immunoassay method in a group of patients with chronic plaque psoriasis compared to a control group. Bioavailable and total 25(OH) vitamin D as well as other metabolic parameters, whose homeostasis is regulated by vitamin D, were also evaluated.

The results showed that the level of free 25(OH)D was significantly lower in patients compared to controls, while total and bioavailable 25(OH)D levels appeared lower but not significantly. The other parameters were similar in the two study groups.

In addition, most of subjects (78.3%) exhibited a level of total vitamin D lower than the value of 30 ng/ml accepted as the reference threshold to define the vitamin D deficiency. In this case, the proportion of subjects with vitamin D deficiency was higher among patients with psoriasis than healthy controls.

Many studies compared serum level of vitamin D in psoriasis patients with corresponding controls and found that the former had significantly lower serum concentrations of 25(OH)D [27, 28]. In addition, the prevalence of subjects with high deficiency of vitamin D (serum 25(OH)D levels < 20 ng/mL) was found to be higher in psoriasis patients (57.8%) than in healthy controls (29.7%) ($p < 0.001$) [21]. Similar results were obtained in our study where the prevalence of 25(OH)D levels < 20 ng/mL was 51.4% among patients and 22.9% among controls.

Although it is not the most active metabolite, the concentrations of total 25(OH) vitamin D in the serum are currently routinely used in clinical practice to assess vitamin D status [20]. To date, no studies compared serum free 25(OH)D levels in psoriasis patients with healthy subjects.

Due to the wide variability of total 25(OH)D, several studies agree that free 25(OH)D is more significant in assessing vitamin D levels, especially when there are physiological or pathological alterations [20]. In fact, liver functions, estrogens, kidney functions and genetic background as well as environmental exposure, might influence total circulating vitamin D levels. Moreover, diseases or conditions that affect the synthesis of DBP or albumin have a huge impact on the amount of total 25(OH)D. DBP and albumin are synthesized in the liver, therefore people with an impairment of liver functions have alterations in their total vitamin D concentrations, while free 25(OH)D levels remain mostly constant [29]. Estrogens stimulate the synthesis of DBP and this explains why total vitamin D concentrations are higher during pregnancy as compared to non-pregnant women, while the concentrations of free 25(OH)D remain similar in both groups of women [30]. The vitamin D-DBP and vitamin D-albumin complexes are filtered through the kidney, hence acute and chronic kidney diseases characterized by a tubular damage, are associated with a loss of

vitamin D-DBP complexes in the urine [31, 32]. Finally, the gene encoding DBP protein is highly polymorphic in different human racial groups [26, 33].
To improve accuracy and precision of vitamin D measurements, free vitamin D could be measured, because it is independent of these confounding factors and thus are much better correlated with pathological conditions, particularly, with liver, kidney, tumor, and allergic diseases as well as in pregnancy [20]. Findings of this study showed that free 25(OH)D is also related to chronic plaque psoriasis.
In addition, the thresholds set to identify the "normal" level of circulating total vitamin D as well as its deficiency [34] are often controversial, therefore, many authors agree that a different set of normal values for total vitamin D should be developed considering all confounding factors. This is also confirmed by the large amount of people who has lower levels of total 25(OH) D in respect of the threshold of 30 ng/ml established to indicate the vitamin D deficiency [35, 36].
From a methodological point of view, free 25(OH)D concentrations can be either measured directly or calculated based on total 25(OH)D, DBP, and albumin serum levels [37, 38].
The indirect method is the most used. However, it presents several problems. The weakness of the calculation method is its relative inaccuracy due to many factors included into the formula. For instance, the binding constants for DBP are known to vary depending on several physiologic and pathologic conditions (for example, in pregnancy or in hyperlipemic conditions, when the binding capability of DBP to vitamin D metabolites can be lowered, since DBP may bind fatty acids as well) [38-42].
Free vitamin D levels obtained by calculation were reported to be overestimated compared to directly measured vitamin D concentrations [38]. A direct free 25(OH)D measurement can be performed either by centrifugal ultrafiltration or by a recently established immunoassay method (ELISA). However, centrifugal ultrafiltration did not become commonly used due to its high costs and technical difficulties in application [39].
ELISA kit for a direct measurement of free 25(OH)D in serum, such that used in our study, is instead characterized by ease of use, cost-effectiveness and sensitivity [43].
However, it is important to underline that the direct measurement of free 25(OH)D by immunoassay method was sometimes reported to have a lower affinity to the vitamin D2 metabolite compared to vitamin D3 (from 60% [42] to 77% [43]), which might result in underestimation of real values of free 25(OH)D.
This study has various strengths. To our knowledge, this is the first study concerning the association of free 25(OH)D with chronic plaque psoriasis. In addition, the methodology used for the detection of free 25(OH)D is innovative since it was carried out by a recently established commercially ELISA kit.
This study has some limits too. First, the control group could be few representative of the general population, since it consisted of healthcare workers recruited in the same hospital where the patients were treated. Second, the model used to assess the association between free 25(OH)D and psoriasis took into account only few factors, while many other variables could influence that relation (i.e. nutrition, work, physical activity, sun exposure, etc.).
Therefore, a lot of work still remains to be done in order to overcome those limits and clearly establish the relationship between free 25(OH)D and disease. It would be interesting to select a cohort of patients and follow the temporal trend of free 25(OH)D levels in respect of the disease status, therapy, seasonality, lifestyles and individual characteristics.

Conclusions

Patients affected by chronic plaque psoriasis exhibited a lower serum level of free 25(OH) vitamin D than healthy controls. In addition, this parameter seemed to be more sensitive than total and bioavailable vitamin D to identity abnormalities in vitamin D pathways in chronic plaque psoriasis patients.
The direct detection of free 25(OH)D by immunoassay method could represent a useful tool to assess vitamin D status and identify a risk condition associated with the onset of chronic plaque psoriasis.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

All authors made substantial contributions to the conception and design of the study and were involved in drafting and critically revising the manuscript in terms of intellectual content. In particular, FB conceived the study and performed the statistical analysis. TG, GL and MC interpreted the results. FB, TG and ADD were involved in the study design and methodology. AP, GI, IG and DL were involved in collection and management of data. ADD, GL and MC were involved in planning and supervising the work.

References

[1] Di Cesare A, Di Meglio P. The IL-23/Th17 axis in the immunopathogenesis of psoriasis. J Invest Dermatol 2009;129:1339-50. https://doi.org/10.1038/jid.2009.59

[2] Boehncke WH, Schön MP. Psoriasis. Lancet 2015;386(9997):983-94. https://doi.org/10.1016/S0140-6736(14)61909-7

[3] International Federation of Psoriasis Associations. World Psoriasis Day 2015. Available at: https://ifpa-pso.com/our-actions/world-psoriasis-day [Accessed on 21/01/2020].

[4] Prignano F, Rogai V, Cavallucci E, Bitossi A, Hammen V, Cantini F. Epidemiology of psoriasis and psoriatic arthritis in Italy - a systematic review. Curr Rheumatol Rep 2018;20:43. https://doi.org/10.1007/s11926-018-0753-1

[5] Gudjonsson JE, Elder JT. Psoriasis. Reference Module in Biomedical Sciences 2014. https://doi.org/10.1016/B978-0-12-801238-3.05622-1

[6] Di Costanzo L, Fattorusso V, Mozzillo E, Patrì A, Di Caprio R, De Nitto E, Balato N, Franzese A. Psoriasis in children with type 1 diabetes: a new comorbidity to be considered? Acta Diabetol 2017;54:803. https://doi.org/10.1007/s00592-017-1000-3

[7] Langley RG, Krueger GG, Griffiths CE. Psoriasis: epidemiology, clinical features, andquality oflife. Ann Rheum Dis 2005;64:ii18-ii23. http://dx.doi.org/10.1136/ard.2004.033217

[8] Gelfand JM, Neimann AL, Shin DB, Wang X, Margolis D, Troxel AB. Risk of myocardial infarction in patients with psoriasis. JAMA 2006;296:1735-41. https://doi.org/10.1001/jama.296.14.1735

[9] Helmick CG, Lee-Han H, Hirsch SC, Baird TL, Bartlett CL. Prevalence of psoriasis among adults in the US: 2003-2006 and 2009-2010 National Health and Nutrition Examination Surveys. Am J Prev Med 2014;47:37-45. https://doi.org/10.1016/j.amepre.2014.02.012

[10] Seremet S, Genc B, Tastan A, Akyildiz ZI, Nazli C, Ozcelik S, Afsar FS, Solak A, Emren V. Are all patients with psoriasis at increased risk for coronary artery disease? Int J Dermatol 2015;54:355-61. https://doi.org/10.1111/ijd.12673

[11] Kim N, Thrash B, Menter A. Comorbidities in psoriasis patients. Semin Cutan Med Surg 2010;29:10-5. https://doi.org/10.1016/j.sder.2010.01.002

[12] Yeung H, Takeshita J, Mehta NN, Kimmel SE, Ogdie A, Margolis DJ, Shin DB, Attor R, Troxel AB, Gelfand JM. Psoriasis severity and the prevalence of major medical comorbidity: a population-based study. JAMA Dermatol 2013;149:1173-9. https://doi.org/10.1001/jamadermatol.2013.5015

[13] Kimball A, Guerin A, Tsaneva M, Yu A, Wu E, Gupta S, Bao Y, Mulani P. Economic burden of comorbidities in patients with psoriasis is substancial. J Uur Acad Dermatol Venereol 2011;25:157-63. https://doi.org/10.1111/j.1468-3083.2010.03730.x

[14] Feldman SR, Zhao Y, Shi L, Tran MH, Lu J. Economic and comorbidity burden among moderate-to-severe psoriasis patients with comorbid psoriatic arthritis. Arthritis Care Res (Hoboken). 2015;67:708-17. https://doi.org/10.1002/acr.22492

[15] Soleymani T, Hung T, Soung J. The role of vitamin D in psoriasis: a review. Int J Dermatol 2015;54383-92. https://doi.org/10.1111/ijd.12790

[16] Umar M, Sastry KS, Al Ali F, Al-Khulaifi M, Wang E, Chouchane AI. Vitamin D and the pathophysiology of inflammatory skin diseases. Skin Pharmacol Physiol 2018;3174-86. https://doi.org/10.1159/000485132

[17] Mason A, Mason J, Cork M, Hancock H, Dooley G. Topical treatments for chronic plaque psoriasis: an abridged cochrane systematic review. J American Acad Dermatol 2013;69:799-807. https://doi.org/10.1016/j.jaad.2013.06.027

[18] Scott LJ, Dunn CJ, Goa KL. Calcipotriol ointment: A review of its use in the management of psoriasis. Am J Clin Dermatol 2001;2:95-120. https://doi.org/10.2165/00128071-200102020-00008

[19] Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. Physiol Rev 2016;96:365-408. https://doi.org/10.1152/physrev.00014.2015

[20] Tsuprikov O, Chen X, Hocher CF, Skoblo R, Yin L, Hocher B. Why should we measure free 25(OH) vitamin D? J Steroid Biochem Mol Biol 2018;180:87-104. https://doi.org/10.1016/j.jsbmb.2017.11.014

[21] Gisondi P, Rossini M, Di Cesare A, Idolazzi L, Farina S, Beltrami G, Peris K, Girolomoni G. Vitamin D status in patients with chronic plaque psoriasis. Br J Dermatol 2012;166:505-10. https://doi.org/10.1111/j.1365-2133.2011.10699.x

[22] Ricceri F, Pescitelli L, Tripo L, Prignano F. Deficiency of serum concentration of 25-hydroxyvitamin D correlates with severity of disease in chronic plaque psoriasis. J Am Acad Dermatol 2013;68:511-2. https://doi.org/10.1016/j.jaad.2012.10.051

[23] Lee YH, Song GG. Association between circulating 25-hydroxyvitamin D levels and psoriasis, and correlation with disease severity: a meta-analysis. Clin Exp Dermatol 2018;43:529-35. https://doi.org/10.1111/ced.13381

[24] World Health Organization (WHO). Physical status: the use and interpretation of anthropometry, 1995. Available at: https://www.who.int/childgrowth/publications/physical_status/en/ [Accessed on 10/02/2019].

[25] Fredriksson T, Pettersson U. Severe psoriasis-oral therapy with a new retinoid. Dermatologica 1978;157:238-44.

[26] Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, Tamez H, Zhang D, Bhan I, Karumanchi SA, Powe NR, Thadhani R. Vitamin-D binding-protein and vitamin D status of black Americans and white Americans. N Engl J Med 2013;369:1991-2000. https://doi.org/10.1056/NEJMoa1306357

[27] Soleymani T, Tracy H, Jennifer S. The role of vitamin D in psoriasis: a review. Int J Dermatol 2015;54:383-92. https://doi.org/10.1111/ijd.12790

[28] Barrea L, Savanelli MC, Di Somma C, Napolitano M, Megna M, Colao A, Savastano S. Vitamin D and its role in psoriasis: An overview of the dermatologist and nutritionist. Rev Endocr Metab Disord 2017;18:195-205. https://doi.org/10.1007/s11154-017-9411-6

[29] Schwartz JB, Lai J, Lizaola B, Kane L, Weyland P, Terrault NA, Stotland N, Bikle D. Variability in free 25(OH) vitamin D levels in clinical populations. J Steroid Biochem Mol Biol 2014;144:156-8. https://doi.org/10.1016/j.jsbmb.2013.11.006

[30] van Hoof HJ, de Sévaux RG, van Baelen H, Swinkels LM, Klipping C, Ross HA, Sweep CGJ. Relationship between free and total 1,25-dihydroxyvitamin D in conditions of modified binding. Eur J Endocrinol 2001;144:391-6.

[31] Grymonprez A, Proesmans W, Van Dyck M, Jans I, Goos G, Bouillon R. Vitamin D metabolites in childhood nephrotic syndrome. Pediatr Nephrol 1995;9:278-81.

[32] Lambert PW, De Oreo PB, Fu IY, Kaetzel DM, von Ahn K, Hollis BW, Roos BA. Urinary and plasma vitamin D3 metabolites in the nephrotic syndrome. Metab Bone Dis Relat Res 1982;4:7-15.

[33] Aloia J, Mikhail M, Dhaliwal R, Shieh A, Usera G, Stolberg A, Ragolia L, Islam S. Free 25(OH)D and the vitamin D paradox in African Americans. J Clin Endocrinol Metab 2015;100:3356-63. https://doi.org/10.1210/JC.2015-2066

[34] Płudowski P, Karczmarewicz E, Bayer M, Carter G, Chlebna-Sokół D, Czech-Kowalska J, Dębski R, Decsi T, Dobrzańska A, Franek E, Głuszko P, Grant WB, Holick MF, Yankovskaya L, Konstantynowicz J, Książyk JB, Księżopolska-Orłowska K, Lewiński A, Litwin M, Lohner S, Lorenc RS, Łukaszkiewicz J, Marcinowska-Suchowierska E, Milewicz A, Misiorowski W, Nowicki M, Povoroznyuk V, Rozentryt P, Rudenka E, Shoenfeld Y, Socha P, Solnica B, Szalecki M, Tałałaj M, Varbiro S, Żmijewski MA. Practical guidelines for the supplementation of vitamin D and the treatment of deficits in Central Europe - recommended vitamin D intakes in the general population and groups at risk of vitamin D deficiency. Endokrynol Pol 2013;64:319-27. https://doi.org/10.5603/EP.2013.0012

[35] Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr 2008;87:1080S-6S. https://doi.org/10.1093/ajcn/87.4.1080S

[36] Cashman KD, Sheehy T, O'Neil CM. Is vitamin D deficiency

a public health concern for low middle income countries? A systematic literature review. Eur J Nutr 2019;58:433-53. https://doi.org/10.1007/s00394-018-1607-3

[37] Chun NRF, Nielson CM. Free vitamin D: Concepts, assays, outcomes, and prospects. In: Feldman D, Pike JW, Bouillon R, Giovannucci E, Goltzman D, Hewison M, editors. Vitamin D. 4th edition. San Diego: Elsevier Press 2017, pp. 925-37. https://doi.org/10.1016/B978-0-12-809965-0.00051-3

[38] Jassil NK, Sharma A, Bikle D, Wang X. Vitamin D binding protein and 25-hy-droxyvitamin D levels: emerging clinical applications. Endocr Pract 2017;23:605-13. https://doi.org/10.4158/EP161604.RA

[39] Bikle DD, Siiteri PK, Ryzen E, Haddad JG, Gee E. Serum protein binding of 1, 25-dihydroxyvitamin D: a reevaluation by direct measurement of free metabolite levels. J Clin Endocrinol Metab 1985;61:969-75. https://doi.org/10.1210/jcem-61-5-969

[40] Bikle DD, Gee E, Halloran B, Kowalski MA, Ryzen E, Haddad JG. Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. J Clin Endocrinol Metab 1986;63:954-9. https://doi.org/10.1210/jcem-63-4-954

[41] Schwartz JB, Lai J, Lizaola B, Kane L, Markova S, Weyland P, Terrault NA, Stotland N, Bikle D. A comparison of measured and calculated free 25(OH) vitamin D levels in clinical populations. J Clin Endocrinol Metab 2014;99:1631-7. https://doi.org/10.1210/jc.2013-3874

[42] Weinstein SJ, Stolzenberg-Solomon RZ, Kopp W, Rager H, Virtamo J, Albanes D. Impact of circulating vitamin D binding protein levels on the association between 25-hydroxyvitamin D and pancreatic cancer risk: a nested case-control study. Cancer Res 2012;72:1190-8. https://doi.org/10.1158/0008-5472.CAN-11-2950

[43] Heureux N, Lindhout E, Swinkels L. A direct assay for measuring free 25-hydro-xyvitamin D. J AOAC Int 2017;100:1318-22. https://doi.org/10.5740/jaoacint.17-0084

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Correspondence: Francesco Bagordo, Department of Biological and Environmental Sciences and Technologies, University of Salento, via Monteroni 165, 73100 Lecce, Italy - E-mail: francesco.bagordo@unisalento.it

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