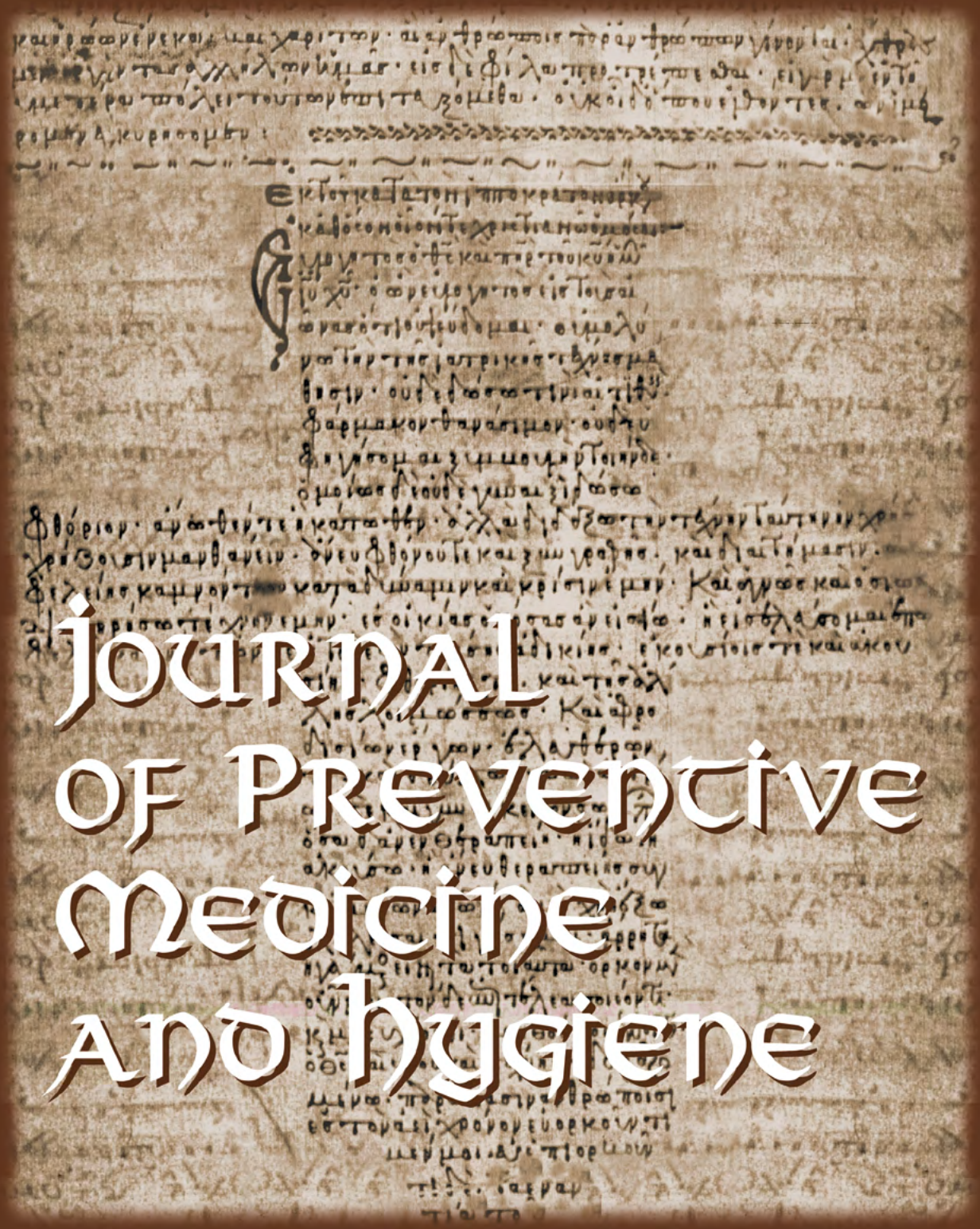
ISSN 2421-4248

vol. 60 n. 4

December 2019

Cited in Index Medicus / Medline
NLM ID 921440 (Pub-Med)



The original document of Hippocrates' oath

Journal of Preventive Medicine and Hygiene

The Journal has been accreditated, on occasion of the 17th December 2004 Meeting of the Executive and Scientific SItI Councils, by the Italian Society of Hygiene, Preventive Medicine and Public Health

PACINI EDITORE MEDICINA

Journal of Preventive Medicine and Hygiene

http://www.jpmh.org

Managing Editor: Patrizia Alma Pacini
Publisher: Pacini Editore Srl, Via Gherardesca 1, 56121 Pisa, Italy

Published online December 2019

Authorization Tribunal of Genoa, Italy n. 507 - 10/6/1960
Journal registered at "Registro pubblico degli Operatori della Comunicazione" (Pacini Editore srl registration n. 6269 - 29/8/2001).

CONTENTS

Overview

Burden of typhoid fever and cholera: similarities and differences. Prevention strategies for European travelers to endemic/epidemic areas

D. AMICIZIA, R.T. MICALE, B.M. PENNATI, F. ZANGRILLO, M. IOVINE, E. LECINI, F. MARCHINI, P.L. LAI, D. PANATTO
Department of Health Sciences, University of Genoa, Italy

Keywords

Salmonella Typhi • Cholera • Typhoid fever vaccines • Cholera vaccines • Travel medicine • International travel

Summary

The burden of diarrheal diseases is very high, accounting for 1.7 to 5 billion cases per year worldwide. Typhoid fever (TF) and cholera are potentially life-threatening infectious diseases, and are mainly transmitted through the consumption of food, drink or water that have been contaminated by the feces or urine of subjects excreting the pathogen. TF is mainly caused by Salmonella typhi, whereas cholera is caused by intestinal infection by the toxin-producing bacterium Vibrio cholerae. These diseases typically affect low- and middle-income countries where housing is overcrowded and water and sanitation are poor, or where conflicts or natural disasters have led to the collapse of the water, sanitation and healthcare systems. Mortality is higher in children under 5 years of age. Regarding their geographical distribution, TF has a high incidence in sub-Saharan Africa, India and south-east Asia, while cholera has a high incidence in a few African countries, particularly in the Horn of Africa and the Arabian Peninsula.

In the fight against these diseases, preventive measures are fundamental.

With modern air travel, transmissible diseases can spread across continents and oceans in a few days, constituting a threat to global public health. Nowadays, people travel for many reasons, such as tourism and business. Several surveys have shown that a high proportion of travelers lack adequate information on safety issues, such as timely vaccination and prophylactic medications. The main objective of this overview is to provide information to help European travelers to stay healthy while abroad, and thus also to reduce the potential importation of these diseases and their consequent implications for public health and society.

The preventive measures to be implemented in the case of travel to countries where these diseases are still endemic are well known: the adoption of safe practices and vaccinations. It is important to stress that an effective preventive strategy should be based both on vaccinations and on hygiene travel guidelines.

Furthermore, the emergence of multidrug-resistant strains is becoming a serious problem in the clinical treatment of these diseases. For this reason, vaccination is the main solution.

Introduction

The burden of diarrheal disease is very high, accounting for 1.7 to 5 billion cases per year worldwide. Specifically, diarrheal diseases are associated with an estimated 1.3 million deaths ann-ually, with most occurring in resource-limited countries. Very young children are the most vulnerable, the incidence of severe gastroenteritis being highest in the first 2 years of life. Indeed, up to 25% of deaths in young children in Africa and south-east Asia are attributable to acute gastroenteritis. In these geographical areas, total mortality has declined in recent decades, owing to the increased use of oral rehydration therapy, improved nutrition, increased breastfeeding, better supplemental feeding, female education, immunization and improvements in hygiene and sanitation. Nevertheless, morbidity due to diarrhea has not declined in the same manner. Indeed, in low-income countries, children under three years old experience, on average, three episodes of diarrhea every year. Although the burden is greatest in low-income populations with poor access to safe water, sanitation and urgent medical care, acute infectious diarrhea is also a common cause of outpatient visits and hospital admissions in high-income regions, and is a major health problem globally [1].

Diarrhea is caused by a wide range of etiological agents, including viruses, bacteria and parasites. Among the etiological agents responsible for diarrheal diseases are *Salmonella Typhi* (*S. typhi)*, *Salmonella Paratyphi* (*S. paratyphi*) and *Vibrio Cholerae*. In this overview, the burden of typhoid and paratyphoid fever and cholera is described in order to provide updated information for European travelers to endemic areas and to identify the best preventive strategies.

Typhoid and paratyphoid fever

Typhoid fever (TF), also known as enteric fever, is a potentially life-threatening multi-systemic illness. It is mainly caused by Salmonella enterica, subspecies enterica serovar *typhi*, and to a lesser extent by serovars *paratyphi* A, B, and C, which are members of the family of *Enterobacteriaceae* [2]. The genus Salmonella is divided into serovars on the basis of surface antigens: O antigen, based on the lipopolysaccharide (LPS) compo-

nent; and H antigen, based on flagellar proteins. Moreover, pathogenic strains of *S. typhi* and *S. paratyphi* C present a Vi antigen polysaccharide component [3].

Clinical features

S. typhi is restricted to human hosts, and chronic carriers constitute the reservoir of infection.

The disease is mainly transmitted through the consumption of food, drink or water that have been contaminated by the feces or urine of subjects excreting bacteria (sick or convalescent people or chronic asymptomatic carriers). After *S. typhi* has been ingested, it reaches the intestinal epithelium, where it colonizes macrophages and dendritic cells in the lamina propria; however, these fail to destroy the bacterium [4]. Subsequently, bacteria invade the bloodstream, multiply and spread to the lymph nodes, spleen and liver, causing multi-systemic disease [5]. The main manifestations of the disease are fever, which can reach 38°-40°C, and abdominal symptoms (such as diarrhea or constipation). Nonspecific symptoms, such as weakness, anorexia, headache and dizziness, may precede the fever. Moreover, rose-colored spots may appear on the trunk, and patients may also experience neuropsychiatric manifestations, hepatomegaly and splenomegaly. The most severe complications are gastrointestinal bleeding, intestinal perforation and typhoid encephalopathy, which occur in 10-15% of patients, generally in the third and fourth weeks of infection [6, 7]. The duration of infection is a major determinant of the risk of severe complications, and a delay in administering appropriate antibiotic treatment may have serious consequences. Isolation of *S. typhi* from blood is the most common method of diagnosis, though the bacterium can also be isolated from bone marrow, feces and duodenal fluid. Blood culture displays suboptimal sensitivity, generally being positive in only about 50% of cases. It also has several limitations, including the volume of blood needed, the need for prompt transport to the laboratory, interference due to prior antibiotic use, limited laboratory expertise and equipment, and expense [8]. Bone marrow culture increases the diagnostic yield to approximately 80% of cases. Stool culture is not usually positive during the earliest phase of the disease [9]. Multiple cultures increase sensitivity and may be required in order to reach a diagnosis. Although the Widal test (based on the detection of agglutinating antibodies to 'O' and 'H' antigens) is unreliable (may give false-positive or false-negative results), it is widely used in developing countries because of its low cost. Newer serologic assays for *S. typhi* infection are occasionally used in outbreak situations, and are somewhat more sensitive and specific than the Widal test [2]. Early diagnosis and the prompt institution of appropriate antibiotic treatment are essential for the optimal management of TF, especially in children. *S. paratyphi* causes paratyphoid fever. *S. paratyphi* is thought to cause milder disease than *S. typhi*, with symptoms being predominantly gastrointestinal [10]. While this is probably true of *S. paratyphi B* infection, there are insufficient data to draw conclusions regarding *S. paratyphi A* [11].

Ciprofloxacin is commonly used as an empiric treatment, as fluoroquinolones are recommended. However, as fluoroquinolone-resistant or multidrug-resistant strains are spreading, third-generation cephalosporins are used when the possibility of resistance is high [6, 12].

Epidemiology

TF is one of the main causes of enteric disease worldwide [13]. The incidence of TF (overall population) is reported in Figure 1. Recent estimates of the global incidence of typhoid and paratyphoid fevers in 2017 indicate 14.3 million people affected (76.3% caused by S. typhi) [14], a 44.6% decline from the 25.9 million in 1990 [5, 8, 15, 16].

The distribution of the disease differs widely throughout the world (Fig. 1). In geographical areas with a high incidence, the main risk factors are inadequate drinking water and inadequate sanitation; indeed, low- and middle-income countries are mainly affected, owing to the lack of clean water and of proper sanitation. Moreover, other risk factors are: high population density, unsanitary living conditions, poor hygiene, low socio-economic status, and recent contact with a patient affected by TF [17]. In 2014, the World Health Organization (WHO) attributed 502,000 deaths to inadequate drinking water and 280,000 to inadequate sanitation [18]. TF has a heavy burden in Asia, with an overall incidence of 170.8 cases per 100,000 people per year, though this estimate varies across the continent [14]. Specifically, Buckle et al. estimated an annual incidence rate of 394.2 per 100,000 in southern Asia. With regard to Africa, the incidence is estimated to be 724.6 cases per 100,000 people per year; however, it is probably underestimated, owing to the lack of information and surveillance systems in the continent [14]. Moreover, Africa suffers many cases of invasive non-typhoid salmonellosis, which are additional confounding factors in estimating the TF burden [17]. TF also affects countries in Latin America, the Caribbean and Oceania, although to a lesser extent, with a median incidence rate of 22.3 cases per 100,000 people per year [5].

In 2014, Mogasale V et al. studied the incidence of TF in various parts of the world, showing that it was considerably higher in low- and middle-income countries (risk-adjusted and corrected for blood culture sensitivity) (Fig. 1) [19].

A 2017 study by Antillón et al. found that the age-group most commonly affected by the disease is in the range between 2 and 14 years. Specifically, incidence peaks in the 2-4 years age-group, while it is lower in children < 2 years of age and adults. It must be stressed that children, even when properly treated, have a high mortality rate. These authors estimated that the expected number of TF cases per year is 17.8 million across all low-income and middle-income countries. According to their analysis, almost 40% of all cases occur in sub-Saharan Africa (7.2 million), although the uncertainty of their estimates is considerable. Figure 2 reports the incidence (per 100,000 person-years) in the Global Burden of Disease

Fig. 1. Typhoid incidence in low- and middle-income countries (risk-adjusted and corrected for blood culture sensitivity), adapted from Mogasale et al. [19]. Colors indicate different incidence values, with darker shades corresponding to higher incidence.

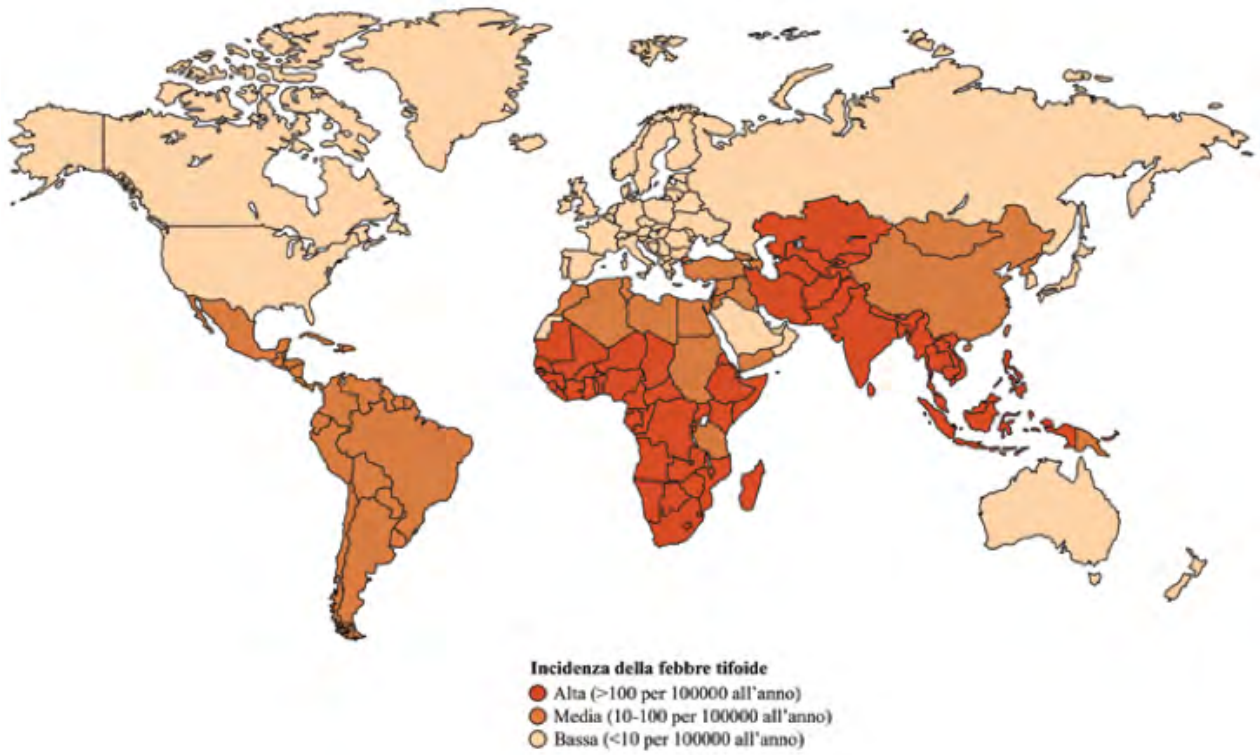
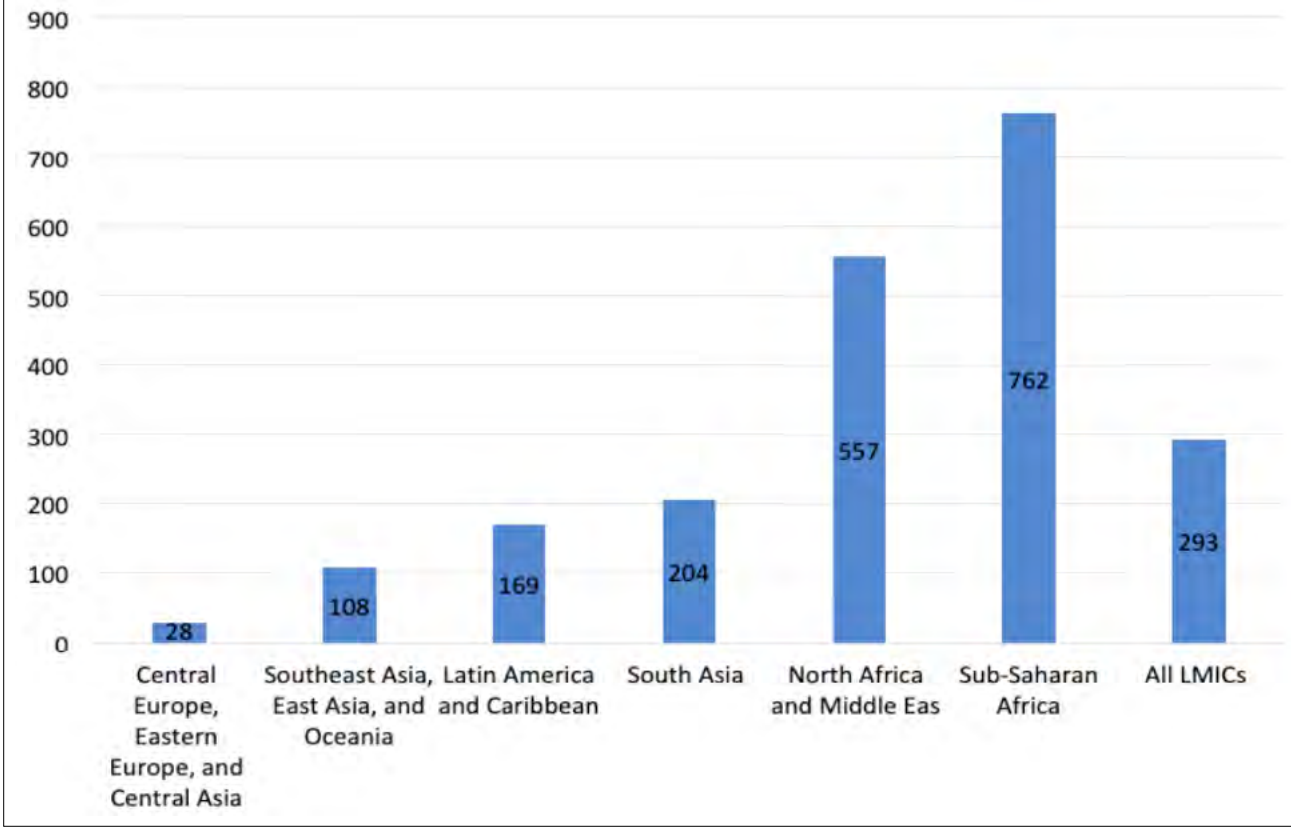


Fig. 2. Incidence in the Global Burden of Disease regions and sub-regions made up of low- and middle-income countries. The incidence is per 100,000 person-years. Adapted from Antillón et al. [20].



regions and sub-regions made up of low- and middle-income countries [20].
In developed countries, the incidence rate per 100,000 per year varies from < 0.1 to 0.3, and the disease mainly affects people who travel to endemic areas located in low- and middle-income countries [15].
The median TF mortality rate varies from region to region: high-income countries such as North America, Europe, Australia and New Zealand register less than 0.1 deaths per 100,000 people per year, while the mortality rate is higher in Sub-Saharan Africa (7.2) and Southern Asia (3.9) [15].
S. paratyphi A has been found to be responsible for a considerable, and increasing, proportion of cases of enteric fever in some Asian regions [8].
A very recent systematic review by Marchello et al. reports the incidence of blood culture-confirmed TF without restrictions on age, country, language or time. The authors identified Africa and Asia as regions with high TF incidence, with a peak in children younger than 15 years old (particularly between 2 and 4 years old). These results confirm the global incidence trend of new cases of TF [21].
The impact of *S. typhi* and *S. paratyphi* disease is probably underestimated, owing to inadequate surveillance systems in the most severely affected areas, the low sensitivity of diagnostic tools, and healthcare inequalities resulting in scant health-seeking behavior among populations at the highest risk [22].

Vaccination

Despite major efforts to prevent and treat cases of enteric fever, millions of new infections of typhoid fever occur in many areas where sanitation is poor and food and water supplies are unsafe, frequently involving travelers to endemic areas [22].
In the fight against this plague, preventive measures are fundamental. Vaccination against typhoid is an effective preventive intervention, especially when coupled with hand-washing, the treatment of household water, and the provision of adequate sanitation [4].
Well-tolerated and effective vaccines are currently available. One of these is based on the use of live attenuated bacteria and is administered orally; the other is based on Vi capsular polysaccharide (Vi-PS), and is administered intramuscularly or subcutaneously [4].

Ty21a vaccine (Vivotif®)

A mutated strain of *Salmonella* (Ty21a) that reproduces the natural infection is contained in Ty21a vaccine (Vivotif®).
The Ty21a strain is a Ty2 mutant, with deficiency of the uridyl diphosphate-galactose enzyme (UDP-Gal) -4-epimerase. The deficiency of this enzyme prevents the conversion of UDP glucose to UDP galactose, one of the components of the lipopolysaccharide membrane of *Salmonella*. The absence of galactose UDP determines the formation of an LPS that does not contain O antigen, which is the most important surface antigen. In this phase, the mutant strain is not immunogenic; however, since the Ty21a strain is fed by galactose, the bacterium becomes capable of generating UDP galactose by means of an alternative route, managing to express a complete and immunogenic LPS. Despite its immunogenic capacity, the mutant strain is not virulent, as the galactose is partially accumulated as galactose-1-phosphate and UDP-galactose, which induce bacterial lysis. *S. typhi* Ty21a is a stable mutant with no possibility of reversion either *in vitro* or *in vivo*. This particular strain causes an abortive infection, stimulating an immune response at the intestinal level and inducing both a humoral, cell-mediated and antibody reaction [23, 24].

Therapeutic indications

Children aged over 5 years and adults can take the Ty21a vaccine orally. The pack contains 3 capsules, to be taken every other day, with cold or lukewarm water, at least an hour before meals. The protection starts between 7 and 10 days after the third dose.
In countries where the risk of contracting the disease is high, vaccination is recommended every 3 years. Similarly, on the basis of new summary of product characteristics, those traveling to an endemic area should be vaccinated every 3 years [25]. The vaccination schedule must be completed at least one week before going to an endemic area.
Vivotif® may be administered concomitantly with yellow fever vaccine and oral polio vaccine.

Immunogenicity, efficacy and safety

It has been shown that the vaccine stimulates a good local production of IgA against the O antigen and that it induces good humoral and cell-mediated immunogenicity against the O antigen in adult male subjects [26].
The immunogenicity of Ty21a was evaluated in 634 Thai children, who underwent a three-dose vaccination schedule [27]. A seroconversion rate of 60% was found in 3-year-old children and of 91% in 6-year-old children ($p < 0.005$); these percentages were higher than the seroconversion rates in unvaccinated children of the same age. The data showed that seroconversion rates increased proportionally to the age of vaccinated children.
Gilman et al. [28] studied 155 male adults vaccinated with Ty21a and observed good seroconversion rates of antibodies to the O antigen, resulting in disease protection.
In another controlled trial, 32,388 children were recruited in order to evaluate Ty21a vaccine (16,486 received the vaccine, 15,902 received oral placebo, and 25,625 did not receive either) and it was reported that, out of 92,675 doses administered, there were 49 cases of vomiting among vaccinees, versus 21 in the placebo group; 1 case of fever after the vaccine and 3 cases in the control group, and finally 14 cases of abdominal pain in the vaccinated group, versus 2 cases in the placebo group [29]
Levine et al. described a randomized, placebo-controlled field efficacy trial in Santiago (Area West); a total of 65,674 schoolchildren (aged 6-17 years) received three doses of vaccine. Volunteers were randomly assigned to two groups, one of which received the three

capsules within 48 hours (22,170 subjects), and the other within 21 days (21,598). Children who received placebo (21,906) served as the comparator group. The study demonstrated that the best protection was seen in the group that received all three doses of the vaccine in enteric-coated capsules within one week; prolonging the interval between doses to 21 days did not enhance efficacy [30].
Subsequently, a liquid formulation was commercialized after it had been shown to provide greater protection than enteric-coated capsules over three years of follow-up in a randomized, placebo-controlled trial in Area South East and Area North, Santiago [31].
Surveillance in the Area West trial was continued for four additional years (i.e., total seven years of follow-up) and in the Area South East and Area North trial for two additional years (i.e., a total of five years of follow-up).
Over the course of a decade, it was possible to conduct separate large-scale trials to evaluate different immunization regimens and programs of Ty21a oral in vivo typhoid vaccine. The results showed that over 3 years of follow-up in Santiago (Area West), there were 68 cases of bacteriologically confirmed typhoid fever in the placebo group and 23 cases in the short-interval vaccine group, yielding a point estimate of vaccine efficacy of 67% (95% CI 47-79%; $p < 0.00001$). There were 34 cases of confirmed typhoid fever in the long-interval group, providing a point estimate of vaccine efficacy of 49% (95% CI 24-66%; $p = 0.0006$).
In a field trial, the three-dose regimen of enteric-coated capsules taken on alternate days was shown to have a protective efficacy of 71% (95% CI 35-87%) during the first year after vaccination, 67% (95% CI 47-79%) over 3 years, and 62% (95% CI 48-73%) over 7 years of follow-up, which definitively supports the efficacy reported in the vaccine summary of product characteristics [31].
Moreover, the data from the Area South East and Area North trial revealed that three doses of liquid formulation conferred 77% (95% CI 60 ± 87%; $p < 0.001$) protection over three years and 79% (95% CI 65 ± 87%; $p < 0.001$) over five years of follow-up, showing the efficacy of three doses of the liquid formulation of Ty21a [31].
Other studies of vaccine immunogenicity, safety and tolerability have demonstrated the good profile of Vivotif® [32-34].

Vi polysaccharide vaccine (Typhim Vi®)

Vi polysaccharide vaccine (Typhim Vi®) contains purified Vi capsular polysaccharide of Salmonella typhi (Ty 2 strain). Immunity appears within 1-3 weeks after injection and lasts around 3 years. A single dose of ViPS vaccine elicits high levels of serum IgG anti-Vi antibodies. The persistence of anti-Vi antibodies depends on endemicity, with a trend towards greater persistence in endemic areas (documented up to 10 years in 83 children at levels equal to or above the serological correlate of protection of 1 µg/mL). In non-endemic areas, anti-Vi antibodies persist for 2 to 3 years [33].

Therapeutic indications

Typhim Vi® can be used in adults and children over 2 years of age, administration being in a single dose of 0.5 ml, with re-administration required every 2–3 years in subjects who remain at risk of typhoid fever. The common route of administration of this vaccine is intramuscular, although it may be given subcutaneously. Vaccination should be carried out at least 2 weeks prior to potential exposure to *S. typhi* infection.
Typhim Vi® may be administered together with other common vaccines (yellow fever, diphtheria, tetanus, poliomyelitis, rabies prepared on Vero cells, meningitis A+C, hepatitis A and hepatitis B) during the same vaccination session [35].

Immunogenicity, efficacy and safety

A double-blind, randomized, controlled efficacy clinical trial was conducted in a highly endemic area of Nepal, in both pediatric and adult populations: 3,457 subjects received Typhim Vi®. The results indicated that the level of protection conferred by a single dose of the vaccine was 74% against blood culture-confirmed cases of TF throughout the 20 months of active surveillance, in comparison with the control group [36].
The seroconversion rate (defined as a 4-fold rise in anti-Vi antibody levels) was recorded in 19 clinical trials involving a total 2,137 pediatric and adult subjects in endemic and non-endemic areas. In the adult population, the seroconversion rate ranged from 62.5% to 100% four weeks after a single injection, with a similar magnitude of anti-Vi immune response in non-endemic areas and endemic areas. Similar results were obtained in the pediatric population.
During clinical development, more than 15,000 people received Typhim Vi® (first or second injection). The most common adverse reaction, in all age-groups, was injection site pain. In adults over 18 years of age, myalgia and fatigue were the most frequently reported systemic reactions. In children and adolescents (from 2 to 17 years of age), myalgia and headache were the most frequently reported systemic reactions. Most adverse reactions appeared within 3 days after vaccination and most resolved spontaneously within 1 to 3 days after onset [33, 37].

Cholera

Cholera is a rapidly-dehydrating diarrheal disease caused by intestinal infection by the toxin-producing bacterium *Vibrio cholerae.*
Vibrio cholerae strains are classified into serogroups on the basis of the structure of their cell surface lipopolysaccharides. Of the over 200 known serogroups of *Vibrio cholerae*, distinguished by the polysaccharides of the somatic (O) antigen, only the O1 and O139 serovars can produce the cholera toxin and cause pandemic disease [38]. There is no proven cross-protection between O1 and O139. On the basis of a number of phenotypic differences, including their susceptibility to polymyxin B and phage infection [39], the O1 serotype is further

classified into 2 biotypes, El Tor and classical O1. Both of these biotypes can be further classified into 2 cross-reacting serovars, Ogawa and Inaba [40]. El Tor persists for a longer time in the environment and is associated with a higher rate of asymptomatic cases. The classical strains are believed to have been responsible for the six previous cholera pandemics in modern history, the first of which started in 1817. The El Tor biotype is responsible for the longest and most severe seventh pandemic, which started in 1961 and continues today [41]. In 1992, a genetic derivative of the El Tor biotype, termed *Vibrio cholerae* O139 Bengal, caused extensive epidemics of cholera in India and Bangladesh and subsequently in other parts of south Asia [41]. The spread of the O139 serogroup is restricted to Asia and over the years its incidence has decreased following the appearance of a new El Tor strain in 1994 [42]. This serogroup switch has occurred several times over the past decade in cholera-endemic regions, suggesting that acquired immunity plays an important role in the emergence of specific serogroups. Moreover, the rapid evolution and genetic rearrangement of O1 and O139 strains contribute to the persistence and re-emergence of this disease.

In recent years, new pathogenic variants of *Vibrio cholerae* have emerged as the genetic backbone of El Tor strains and the higher infectivity of classical strains, and are associated with increased ecological persistence, infectivity, disease severity, and dispersion worldwide [41, 43-47]. This strain is responsible for the epidemic on Hispaniola and may cause more severe episodes of cholera and higher death rates [12].

As *Vibrio cholerae* strains continue to adapt and evolve, understanding the underlying factors that contribute to their enhanced environmental persistence and increased transmission will be essential, in order to predict outbreaks and establish preventive measures [48].

Cholera currently remains a serious public health problem in many countries, occurring as an endemic disease in some regions and causing major epidemics in some low/middle-income countries [49, 50].

Clinical features

Cholera displays an acute nature, leading to severe dehydration within hours, and death if not treated adequately. *Vibrios* are gram-negative, highly motile and comma-shaped, with a single polar flagellum. In affected individuals, *Vibrio cholerae* secretes a toxin (CT) that affects the small intestine. The toxic action of CT depends on a specific receptor, the monosialosyl ganglioside GM1. The binding (B) subunit of the toxin attaches to GM1 and releases the active (A) subunit; this enters the host cell and activates the G protein, which stimulates adenylate cyclase [50]. This activation increases the outflow of chloride and bicarbonate from the cell and reduces sodium influx, causing water molecules to flow into the lumen of the gut. The consequent net fluid loss causes watery diarrhea and rapid dehydration, which, if untreated, can lead to hypotonic shock and death within 12 hours of the first symptoms [38, 49, 51].

After an incubation period of approximately 18 h to 5 days, the illness typically starts suddenly with passage of watery stools and vomiting. Systemic manifestations, such as fever, are absent unless there is a co-infection. Depending on the severity of dehydration, the patient may be thirsty and irritable and, in later stages, display lethargy, a rapid radial pulse, loss of skin turgor, diminished urine output, low blood pressure, rapid breathing and sunken eyes. When severely dehydrated, the patient may progress to hypovolemic shock. Complications of cholera include electrolyte imbalance, including hypokalemia, hyponatremia, hypocalcemia and acidosis. Children can develop hypoglycemia due to depleted hepatic glycogen reserves and insufficient gluconeogenesis, which may cause seizures [52]. Other complications include various manifestations of diminished perfusion of end organs, including acute renal failure, stroke and, in pregnant patients, miscarriage, premature delivery and stillbirth [12].

Susceptibility to infection by *Vibrio cholerae* depends both on adaptive immune responses, induced by previous infection or vaccination, and on innate host factors. A low gastric acid level has been associated with more severe cholera disease. The immune response in individuals with cholera is directed primarily against bacterial surface molecules and against cholera toxin. The response includes intestinal-mucosal secretory IgA (SIgA) and serum IgA, IgG, vibriocidal antibodies, antibody-secreting cells, T cells and, of special importance for long-term protection, memory B cells and T cells [40].

Intriguingly, not all individuals infected with pathogenic *Vibrio cholerae* exhibit symptoms of cholera, and several host factors appear to impact on immunity to the disease. Both retinol deficiency and blood type O have been associated with an increased susceptibility to infection [53-56]. Regardless of blood type, higher transmission rates of cholera are observed between first-degree relatives than between less closely related contacts living in the same household, indicating that additional genetic factors play a role in the susceptibility to cholera [55].

Laboratory tests are not essential for the diagnosis of cholera, as the clinical picture of acute, non-bloody, profuse, watery diarrhea quickly leading to dehydration does not occur in many other scenarios [57]. Cholera is confirmed through culture of a stool specimen or rectal swab [12].

Treatment for cholera is relatively cheap and simple. Intravenous rehydration with Ringer's lactate solution should be administered aggressively in order to restore the circulation. With adequate and timely rehydration, case-fatality rates (CFRs) are < 1%. The oral rehydration solution recommended by the WHO contains glucose as a source of carbohydrates, and has reduced osmolarity, which is associated with reduced stool output. Antiemetics have no role, and might interfere with rehydration because of their sedating effects. Antibiotic treatment should be dictated by local antimicrobial susceptibility profiles. Azithromycin and ciprofloxacin are commonly used, although azithromycin has been shown to be more effective than ciprofloxacin in terms of shortened

duration of diarrhea, reduced stool volume, lower frequency of vomiting and cessation of fecal excretion of vibrios [12, 40].

Epidemiology

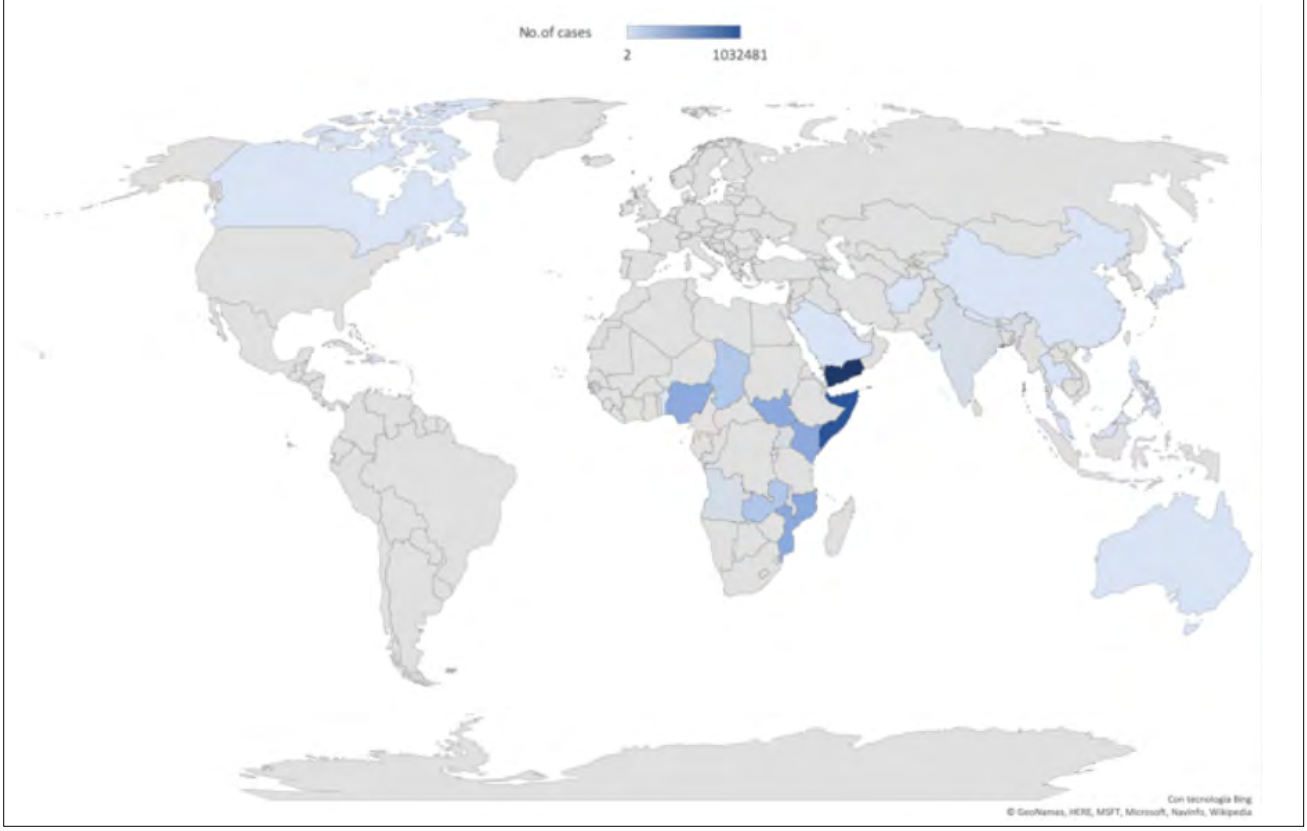
The disease typically affects regions where housing is overcrowded and water and sanitation are poor, or where conflicts or natural disasters have led to the collapse of the water, sanitation and healthcare systems [58]. Access to safe water and improved sanitation facilities has eliminated the transmission of *Vibrio cholerae*, the causative agent, in high-income countries. However, the bacteria continue to afflict millions of people in less developed countries where adequate water and sanitation infrastructure are not widely available [59].

Cholera epidemics are superimposed on the endemic disease in long cycles. These cycles are determined by waning levels of population immunity and periods of climate variability. When introduced into a cholera-naive population, large-scale epidemics can occur, such as the ongoing Haitian epidemic that began in 2010. In epidemic settings where natural immunity is scant or absent, all age-groups are equally affected by the disease although mortality in children < 5 years is higher [59]. Epidemics occur unpredictably and are often associated with natural disasters and humanitarian emergencies that disrupt the supply of water and sanitation. [58].

The global burden of cholera is largely unknown, as the majority of cases are not reported [59]. The WHO estimates that only 5-10% of cases occurring annually are officially reported [60]. The main factors for this low reporting efficiency include the limited capacity of epidemiological surveillance systems and laboratories, and social, political and economic disincentives to reporting [61-63]. While safe drinking water and advanced sanitation systems have made the Global North cholera-free for decades, the disease is still endemic in many regions, including Asia, Africa and the Americas, with 1.3 billion people at risk, Sub-Saharan Africa being the worst affected [64].

As mentioned above, *V. cholera* originated in the Indian subcontinent and caused six pandemics from 1827 through 1923. The seventh pandemic has been ongoing since 1961, reaching South America and most of the Western Hemisphere in 1991 [65]. The WHO estimates 2.8 million cases and 91,000 deaths annually. Recently, cholera has struck vulnerable communities, such as post-earthquake Haiti (2010), Iraq and Yemen, where natural disasters, refugee movements, war and conflict increase the risk of infection and outbreaks [66]. In 2017, the WHO gathered data on cholera from 71 countries worldwide: 34 countries reported a total of 1,227,391 cases and 5,654 deaths (global case-fatality rate (CFR): 0.5%) (Fig. 3), and 37 countries reported cases for the year [62].

Fig. 3. Countries reporting cholera cases in 2017 (Adapted from WHO, 2018) [67]. The colors indicate different values of incidence; a darker shade corresponds to a higher incidence.



Yemen accounted for 84% of all suspected cases reported and for 41% of cholera-attributed fatalities. Excluding cases related to Yemen, an increase of 45% in the number of cases and 33% in the number of deaths over the 2016 global totals was observed in Member States. The increase in 2017 was due largely to severe epidemics in the Democratic Republic of the Congo (DRC), Nigeria, Somalia and South Sudan [68].
Since the last Communicable Disease Threats Report (CDTR), updated on 24 August 2018, the countries reporting the most cases have been Yemen (38,269 cases, 63 deaths), Nigeria (7,966 cases, 224 deaths), the DRC (2,918 cases, 65 deaths), Niger (1,592 cases, 36 deaths) and Ethiopia (1103 cases). Since the last CDTR update, the WHO has declared the cholera outbreaks in Kenya and Uganda to be under control. Two countries have recently reported new cholera outbreaks within their territories: Algeria and Zimbabwe [68].

Natural disasters and epidemics

Epidemics occur unpredictably and are often associated with natural disasters and humanitarian emergencies that disrupt access to water and sanitation supplies. [57].
The WHO defines natural disasters as "catastrophic events with atmospheric, geologic and hydrologic origins", including earthquakes, volcanic eruptions, storm surges, landslides, tsunamis, wildfires, floods and droughts [69, 70].
In humanitarian crises and emergencies, the lack of infrastructures often forces victims to seek refuge in temporary accommodation, without adequate access to food, safe water and sanitation. Obviously, in such situations, public health surveillance systems may be suboptimal, disrupted or even non-existent, exacerbating the risk of transmission of communicable diseases [71]. Furthermore, the lack of electricity, laboratory equipment and supplies can make conventional testing for water-borne pathogens impossible [72].
Moreover, susceptibility to many diseases, including cholera, may be due to pre-existing health conditions, such as malnutrition, especially in infants and children. Indeed, a 2011 study [73] showed that cholera cases (or worse epidemics) are more likely to arise in malnourished populations with limited food availability owing to famine, war or natural disasters [74]. A poor nutritional status may have many causes, such as a deficient macro/micronutrient intake, malabsorption, metabolic disturbances and other health issues (e.g., HIV/AIDS); for example, children with zinc and vitamin A deficiency are more susceptible to cholera [75].
Moreover, natural disasters affect the ecology of pathogens, mainly by increasing the risk of exposure to them, but also by facilitating their growth in aquatic environments, which are the main *reservoir* of pathogenic cholera bacteria [70, 76, 77]. In this regard, changes in the aquatic environment, such as plankton concentration, variations in salinity, temperature, pH, sediments and their re-suspension/transport and other physico-chemical factors, can promote the survival of the pathogen, especially in coastal zones [78].
In recent years, as a consequence of climate change and human activities (e.g., rapid urbanization, deforestation, etc.), the incidence of natural disasters has increased [69, 78, 79]. An estimate derived from the International Disaster Database suggests that about 270 million people each year are affected by natural disasters [70, 80, 81].
However, not all disasters are followed by cholera epidemics; environmental conditions need to be conducive to the rapid growth of this bacterium, the pathogenic strains of which are susceptible to many factors, as seen above. Furthermore, societal structure, prevailing climatic processes and the spatio-temporal seasonal variability of natural disasters play a very important role in predicting cholera outbreaks. Indeed, an already "fragile" area is at greater risk of suffering an epidemic than a high-income area, which, by contrast, will be able to help the victims of a disaster promptly and can recover from it relatively quickly, before an outbreak can occur [70].

Vaccination

In order to prevent cholera, it is important to vaccinate both travelers to endemic areas and people who live in such areas. Currently, the available vaccine for European travelers is cholera inactivated vaccine (Dukoral®), that is administered orally.

Cholera inactivated vaccine (Dukoral®)

Cholera inactivated vaccine (Dukoral®) contains killed whole *Vibrio cholerae* O1 bacteria and the recombinant non-toxic B-subunit of the cholera toxin (CTB) [82].
Bacterial strains of both Inaba and Ogawa serovars and of El Tor and classical biotypes are included in the vaccine. Dukoral® is taken orally together with a bicarbonate buffer, which protects the antigens from the gastric acid. The vaccine acts by inducing antibodies against both the bacterial components and CTB. The intestinal antibacterial antibodies prevent the bacteria from attaching to the intestinal wall, thereby impeding colonization by *Vibrio cholerae* O1. The intestinal anti-toxin antibodies prevent the cholera toxin from binding to the intestinal mucosal surface, thereby preventing the toxin-mediated diarrheal symptoms.
The heat-labile toxin (LT) of enterotoxigenic E. coli (ETEC) is structurally, functionally and immunologically similar to CTB. The two toxins cross-react immunologically.

Therapeutic indications

Dukoral® is indicated for active immunization against disease caused by Vibrio cholerae serogroup O1 in adults and children from 2 years of age who will be visiting endemic/epidemic areas.
As reported in the summary of product characteristics, the use of Dukoral® should be determined on the basis of official recommendations, taking into consideration the variability of epidemiology and the risk of contracting the disease in different geographical areas and traveling conditions. However, Dukoral® should not replace stan-

dard protective measures (such as washing hands, eating well-cooked foods, avoiding raw foods, eating only beverages from sealed bottles, and if not boil them; remember: "boil it, cook it, peel it or forget it).

The standard primary course of vaccination with Dukoral® against cholera consists of 2 doses for adults and children from 6 years of age. Children from 2 to below 6 years of age should receive 3 doses. Intervals of at least one week between doses are necessary. If more than 6 weeks have elapsed between doses, the primary immunization course should be re-started.

For continuous protection against cholera, a single booster dose is recommended within 2 years for adults and children from 6 years of age, and within 6 months for children aged 2 to 6 years. No clinical efficacy data have been generated on repeat booster dosing. However, immunological data and data on the duration of protection suggest that if up to 2 years have elapsed since the last vaccination in adults, and up to 6 months in children aged 2-6 years, a single booster dose should be given. If more than 2 years have elapsed since the last vaccination (more than 6 months in children aged 2-6 years) the primary course should be repeated.

Immunogenicity safety and tolerability

Safety and immunogenicity were studied in Bangladeshi children ($n = 340$) aged 6-18 months. The results showed that two doses of the vaccine were safe and induced antibacterial (vibriocidal) antibody responses in 57% of the children and antitoxin responses in 85%. Immune responses were comparable after the administration of one and two doses. Administering the vaccines without buffer or in water did not affect vibriocidal responses. This study demonstrates that the vaccine is safe and immunogenic in children under 2 years of age and that simple interventions can enhance immune responses in young children [83].

In the Bangladesh Field Trial (1985) protective efficacy against cholera in adults and children aged ≥ 6 years was evaluated, and short-term protection was detected in 85% of subjects [84].

In children aged 2-5 years in Mozambique, Dukoral® provided protection for 6 months [78% protection (CI 39-92%)] [85]. Herd immunity was also registered in non-vaccinated individuals [86, 87].

Recently, Dukoral® was evaluated in a clinical trial: healthy volunteers (n = 21) and renal transplant recipients (n = 30) were vaccinated with the oral whole cell/recombinant B subunit cholera vaccine Dukoral®. The vaccine was administered at the baseline and on day 14. The results showed that more than half of the transplant recipients seroconverted, and adverse events were mild to moderate and transient [88].

Several studies have investigated the cross-protection of Dukoral® against enterotoxigenic Escherichia coli [84, 89, 90]. In particular, one study found that the protective efficacy against cholera was 85% and protection against the toxin of enterotoxigenic Escherichia coli reached 67% [90].

S. typhi and cholera: similarities and differences

Concerning similarities, TF and cholera are both transmitted through the oro-fecal route. Owing to poor sanitary conditions and lack of safe drinking water, which are often associated with poverty and deprivation, developing countries are most severely affected by these diseases [91].

In the 19th and 20th centuries, oro-fecal transmissible diseases were endemic in many areas of the world, including Europe and the Americas. After the widespread introduction of chlorination, sand filtration and other practical preventive methods of water sanification, the spread of oro-fecal diseases decreased drastically worldwide. Today, the WHO is actively involved in reducing the transmission of diseases caused by critical health conditions, particularly in middle- and low-income countries.

The new WHO Guidelines on Sanitation and Health [92] summarize the evidence on the effectiveness of a range of sanitation interventions and provide a comprehensive framework for health-protecting sanitation; this covers policy and governance measures, the implementation of sanitation technologies, systems and behavioral interventions, and monitoring approaches.

Critically, the guidelines detail the role of the health sector in identifying gaps in sanitation interventions, in order to guide future research efforts and to improve and maximize the effectiveness of sanitation interventions on the health of the population (a WHO study in 2012 calculated that for every US$ 1.00 invested in sanitation there was a return of US$ 5.50 in lower health costs, increased productivity, and fewer premature deaths). Indeed, the benefits of improved sanitation extend well beyond reducing the risk of diarrheal disease.

Importance of vaccination in fighting antimicrobial resistance

Today, antimicrobial resistance has grown enormously, and many pathogenic bacteria are resistant to multiple antibiotics, including such micro-organisms as *Vibrio cholerae* and *S. typhi*. Consequently, multi-drug resistance is now one of the most alarming emerging problem associated with infectious diseases. On the other hand, vaccines can prevent infectious diseases and can yield a much longer-lasting control of infections. Indeed, vaccines can control infections over a long period of time without becoming obsolete. This characteristic is due to the fact that vaccines work prophylactically and prevent the start of infections, while drugs work therapeutically on an ongoing infection in which bacteria proliferate and mutate, allowing the drug to select resistant variants [93].

Regarding *S. typhi*, since 2001, the complete genome sequence of multiple drug-resistant *S. typhi* has been mapped and the genes of resistance to the antibiotics commonly used in the treatment of typhoid fever, especially fluoroquinolones, have been identified [94]. Infection caused by multiple drug-resistant strains has

been documented to be associated with more severe illness and higher rates of complications and death, and with a higher rate of prolonged asymptomatic carrier status [95].
In 2016 in Pakistan, an outbreak of Salmonella enterica (ssp. enterica serovar typhi), resistant to chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, fluoroquinolones and third-generation cephalosporins, was observed. More than 1000 cases were registered and laboratory confirmed. The outbreak was caused by the H58 clade, a multidrug-resistant haplotype of S. typhi, common in Asia and in some African areas. As reported by the authors, multi-resistant *S. typhi* involved a densely populated area of Asia where adequate sanitation, water and infrastructure were lacking [96].
With regard to cholera, multidrug-resistant isolates are emerging, particularly in Southern Asia, with resistance to quinolones, trimethoprim-sulfamethoxazole and tetracycline.
A variety of mechanisms of antimicrobial resistance have been identified in *Vibrio cholerae*, including efflux pumps, chromosomal mutations and mobile genetic elements such as plasmids and SXT elements. Antibiotics are often used in combination with rehydration therapy, as they are believed to relieve the symptoms of cholera faster than rehydration treatment alone, and because a shorter disease duration lessens the transmission of infectious *Vibrio cholerae*. Because antibiotics are widely used as part of the cholera treatment regimen, the number of pathogenic *Vibrio cholerae* strains resistant to one or more antibiotics is increasing [97]. To prevent the spread of resistance, it is crucial to limit the use of antibiotics in cholera patients and to implement alternative strategies and novel approaches in managing this disease.
The WHO does not advise the prophylactic administration of antibiotics in travelers coming from or going to a country affected by cholera. Indeed, routine treatment with antibiotics, or preventive chemoprophylaxis, has no effect on the spread of cholera. On the contrary, the use of antibiotics can have adverse effects by increasing antimicrobial resistance.
Although TF and cholera seem to be similar diseases, they display some different features in terms of geographical distribution, pathogenesis, clinical presentation, prognosis and mortality.
Regarding their geographical distribution, TF has a high incidence in sub-Saharan Africa, India and south-east Asia, while cholera has a high incidence in a few African countries, particularly in the Horn of Africa and the Arabian Peninsula (Fig. 1 and Fig. 3).
Cholera has a worse prognosis than TF during the acute phase of the disease, mainly owing to profuse watery diarrhea and vomiting, which cause massive dehydration. If untreated, 50% of severe cases are fatal, while proper treatment and fluid replacement reduce mortality to less than 1% [98]. Mortality is higher in children, especially those under 5 years of age.

International travel and migration

With modern air travel, transmissible diseases can spread across continents and oceans in a few days, constituting a threat to public health. Indeed, it takes only 36 hours to travel around the world by plane; a time much shorter than the incubation period of most infectious diseases [99].
In 1950, international travelers numbered just over 25 million, while according to estimates by the United Nations World Tourism Organization (UNWTO) they could become 1.3 billion by 2020. Over 700 million travelers could be exposed to an increased risk of contracting infectious diseases, owing to changes in their habits, different weather conditions (cold, heat, humidity, exposure to the sun or wind) and the consumption of unsafe food. Asia and the Pacific area, which account for 37% of the world's international tourism expenditure and nearly one-fourth of global arrivals, play a vital role in global tourism, as both an inbound and an outbound market. The increase in international travel in these areas is due to major infrastructure and socio-economic development [100].
Nowadays, people travel for many reasons, such as tourism and business. Several surveys have shown that a high proportion of travelers, whether tourists or businesspeople, lack adequate information on safety issues, such as timely vaccination and prophylactic medications. Indeed, only a small number of travelers seek advice from the Travel Medicine clinic, particularly with regard to vaccinations. Notably, the pre-travel planning of vaccinations is a complex operation that requires adequate medical support and proper timing.
In a cross-sectional, multicenter study, the European Travel Health Advisory Board (ETHAB) used a self-administered anonymous questionnaire to evaluate current travel health knowledge, attitudes and practices and to determine where travelers to developing countries obtained travel health information, what information they received, and what preventive health measures they implemented. The survey was conducted at several airports in Europe, Asia, South Africa and the United States. The questionnaire [101], which was distributed at the departure gate, gathered information on: personal characteristics (age, gender, nationality, country of residence and profession); the journey undertaken (destination countries, type of region, purpose, duration, travel companions) and travelers' knowledge, attitudes and practices (timing of travel preparation, source and timing of travel health information, planned food habits and restrictions, perceived risk of specific infectious diseases, perception and status of vaccinations, contents of travel health kit) regarding malaria and vaccine-preventable travel-related diseases. The results obtained from a total of 5,465 questionnaires showed that the majority of travelers (73.3%) had sought general information about their destination prior to departure, but only just over half of the respondents (52.1%) had sought travel health advice. Tourists and people traveling for religious reasons had sought travel health advice more often, whereas those visiting

friends and relatives were less likely to do so. Hepatitis A was perceived as the most common infectious disease, followed by HIV and hepatitis B. When all participants were asked to score the risk of vaccine-preventable diseases, between one-quarter and one-third of respondents stated that they did not know the risk concerning the respective diseases; some 40% could not assess the risk at all, and 10% to 15% did not answer this question. Ignorance was highest with regard to rabies, TF and cholera [101].

Many characteristics of the journey (duration, destination, etc.) can influence decisions regarding the preventive and prophylactic strategies to adopt. For example, the strongest and most consistent predictor of typhoid risk in travelers is the trip destination: 1/3,000 in travelers to South Asia (high risk), 1/50,000-100,000 Sub-Saharan Africa, North Africa and the Middle East or South America (intermediate risk) and < 1/300,000 in travelers to the Caribbean and Central America (low risk) [102].

A review published in 2005 confirmed that the risk to travelers appears to vary by geographic region visited, with travel to the Indian subcontinent accounting for the greatest risk of acquiring typhoid fever. The overall risk of contracting TF during travel to the Indian subcontinent was 18 times higher than in any other geographic area [103]. Many cases acquired in the Indian subcontinent were multidrug-resistant, as fluoroquinolone resistance is on the rise in this geographical area.

A special category at higher risk of contracting TF is that of travelers who visit relatives (emigrants returning to their homeland). These subjects are less likely to have received pre-travel advice, less likely to adopt food and water precautions and, perhaps most importantly, by and large do not perceive their risk or undergo typhoid vaccination before traveling [104].

Regarding the risk of cholera, with the growth of international travel by sea and air, cases of food-borne transmission of cholera have increased. Indeed, in addition to contaminated water, certain foodstuffs are particularly at risk of transmitting the disease: seafood, including fish, shellfish, crabs, oysters and clams, and other foods that can easily be contaminated during preparation in unhygienic food factories or by infected food handlers [105]. Transportation systems and trade routes have greatly improved and expanded worldwide, and the ease and speed of migration mean that cholera is still a global health challenge. Confirmation that cholera is still a public health threat associated with migration and the rapid movement of people is provided by the recent outbreak reported in Algeria. As referred by the European Centre for Disease Prevention and Control, as of 30 August 2018, Algeria reported 74 confirmed cholera cases in six northern and coastal areas of the country. This was the first cholera outbreak reported in Algeria in more than 20 years. Cases were reported in both rural and urban areas, including the capital. A water source was found to be contaminated with *Vibrio cholerae*. On the basis of the number of cases and the geographical extension of the outbreak, additional cases are expected to be reported.

Conclusion

Not only are cholera and TF a severe threat to the populations of low- and middle-income countries, these diseases may also affect travelers to endemic areas. The preventive measures to be adopted in the case of travel to countries where these diseases are still endemic are well known: the adoption of safe practices and vaccinations [24]. It is important to stress that an effective strategy should be based both on vaccinations and on hygiene travel guidelines. It is commonly believed that chances of developing gastrointestinal illness will be reduced considerably by being counselled to "boil it, cook it, peel it, or forget it." However, surveys of returning travellers have shown that receiving advice about food and drink safety appears to have no significant effect on rates of diarrhoea. Indeed, many travellers will commit a food and beverage indiscretion within 72 hours after arrival in a developing country, despite pre-departure counselling. Standard protective measures are always recommended such as: wash your hands thoroughly before eating food, avoid consuming raw foods (meat, fish, shellfish, vegetables and more), with the exception of personally peeled fruits, take well-cooked foods and not re-warmed; avoid milk that has not been previously boiled, pasteurized or sterilized, ice creams, cakes with cream, cream, raw egg sauces (like mayonnaise), fresh cheeses. Don't buy food and drinks from street vendors and drink only from sealed bottles (if this is not possible, boil the water) [106, 107].

TF is one of the main causes of death due to food-borne infections, and results in the greatest loss of Disability-Adjusted Life years (DALYs) worldwide [24]. Given that man is the only host of *S. typhi*, an effective vaccination strategy could limit the spread of disease and reduce its burden, especially in endemic areas [6]. Furthermore, the emergence of multidrug-resistant strains is becoming a serious problem in the clinical treatment of the disease. For this reason, vaccination is the main solution.

The WHO has set the goal of eliminating cholera by 2030. To achieve this objective, it is essential to support the efforts of low-income countries to strengthen their capacities for preparedness, early detection, laboratory confirmation and immediate effective response to outbreaks. In addition, travelers must be fully informed of the risk of disease and be vaccinated before their departure for at-risk areas. Although safe drinking water and advanced sanitation systems have made cholera a treatable and limited illness in Europe and North America, the emergence of new *Vibrio cholerae* strains, the ease of travel and the increased migration of possibly infected individuals have raised serious public health concerns.

Today, people of all ages commonly travel to developing countries for a variety of different reasons. The common objective should be to help travelers stay healthy while abroad, and thus also to reduce the potential importation of infectious diseases and its consequent implications for public health and society [108]. Indeed, the consequences of returning from abroad with an infectious disease can extend beyond the infected individual, in that

they may also involve travelers' relatives, people with whom they have close contact, or the wider community. Thus, prophylactic travel health measures do not only benefit individuals, but also public health [101, 109].
Initiatives to enhance the awareness of travelers should target all groups, including business travelers, those visiting friends and relatives, and the elderly. Nowadays, travelling isn't just a matter for young and adult people: in the last years, the number of older travelers have increased. Older travelers have been encouraged by the greater ease of access to cheap and rapid modes of transport [110].
Obviously, older travels are often patients, with chronic diseases and other medical conditions: for example, lower respiratory tract infections, urinary tract infections and cardiovascular disease. in order to taking account of the special considerations for safe prescription, general practitioners, geriatricians and other healthcare professionals should cooperate with specialist travel medicine clinics [111]. Moreover, the immunosenescence in elderly people is also important in travel medicine, because it could reduce vaccines immunogenicity and could increase the risk of travel-related infectious diseases. For this reason older travelers, need a pre-travel consultation, preferably earlier than others travelers, to have an adequate time to respond to vaccinations, even if they are healthy, without immunosuppressing/immunocompromising conditions [112]. Additionally, the providers of travel health advice should continue to urge travelers to comply with the pertinent recommendations. A large and growing number of "free and independent travelers", identifiable as a "consumer class", especially young travelers aged 15-34 years ("millennials"), use online travel agencies and mobile technology to make faster and simpler bookings through online (and more affordable) tourism platforms. These changes in travel habits should prompt different information strategies, e.g. the use of social media and collaboration between public health organizations and the most widely used travel websites.

Acknowledgements

Funding sources: the Department of Health Sciences, University of Genoa, received a grant from Emergent, Italy.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

DA and DP conceived and designed the overview. All the authors contributed to the literature search and the writing of the manuscript. DA, DP and RTM critically revised the manuscript. All authors read and approved the final version of the manuscript.

References

[1] GBD Diarrhoeal Diseases Collaborators. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Infect Dis 2017;17:909-48. https://doi.org/10.1016/S1473 3099(17)30276-1

[2] CDC. Newton AE, Routh JA, Mahon BE. Infectious Diseases Related Travel. Chapter 3. Typhoid & Paratyphoid fever. Available at: https://wwwnc.cdc.gov/travel/yellowbook/2016/infectious-diseases-related-to-ravel/typhoid-paratyphoid-fever [Accessed on 30/05/2019].

[3] Popoff MY, Bockemühl J, Gheesling LL. Supplement 2002 (no. 46) to the Kauffmann-White scheme. Res Microbiol 2004;155:568-70.

[4] World Health Organization (WHO). Guidelines on the quality, safety and efficacy of typhoid conjugate vaccines. Available at: http://www.who.int/biologicals/areas/vaccines/TYPHOID_BS2215_doc_v1.14_WEB_VERSION.pdf?ua=1&ua=1 [Accessed on 21/02/2019].

[5] Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive Salmonella infections. Clin Microbiol Rev 2015;28:901-37. https://doi.org/10.1128/CMR.00002-15

[6] Anwar E, Goldberg E, Fraser A, Acosta CJ, Paul M, Leibovici L.Vaccines for preventing typhoid fever. Cochrane Database Syst Rev 2014;(1):CD001261. https://doi.org/10.1002/14651858.CD001261.pub3

[7] CDC. Health information for international travel. The yellow book 2016. Oxford University Press. Available at: https://wwwnc.cdc.gov/travel/page/yellowbook-home-2014/ [Accessed on 30/05/2019].

[8] Crump JA, Mintz ED. Global trends in typhoid and paratyphoid fever. Clin Infect Dis 2010;50:241-6. https://doi.org/10.1086/649541

[9] World Health Organization. The diagnosis, treatment and prevention of typhoid fever. WHO/V&B/03.17. Geneva, Switzerland: WHO, 2003.

[10] Bhan MK, Bahl R, Bhatnagar S. Typhoid and paratyphoid fever. Lancet 2005;366:749-62.

[11] Maskey AP, Day JN, Tuan PQ, 2 Thwaites GE, Campbell JI, Zimmerman M, Farrar J, Basnyat B. Salmonella enterica Serovar Paratyphi A and S. enterica Serovar typhi cause indistinguishable clinical syndromes in Kathmandu, Nepal. Clinical Infectious Diseases 2006;42:1247-53.

[12] CDC. Health information for international travel. The yellow book 2016. Oxford University Press. Available at: https://wwwnc. cdc.gov/travel/page/yellowbook-home-2014/ [Accessed on 20/01/2017].

[13] Steele AD, Hay Burgess DC, Diaz Z, Carey ME, Zaidi AK. Challenges and opportunities for typhoid fever control: a call for coordinated action. Clin Infect Dis 2016;62(Suppl 1):S4-8. https://doi.org/10.1093/cid/civ976

[14] GBD 2017 Typhoid and Paratyphoid Collaborators. The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Infect Dis 2019;19:369-81. https://doi.org/10.1016/S1473-3099(18)30685-6

[15] Buckle GC, Walker CL, Black RE. Typhoid fever and para- typhoid fever: Systematic review to estimate global morbidity and mortality for 2010. J Glob Health.2012;2:010401. https://doi.org/10.7189/jogh.02.010401

[16] Kirk MD, Pires SM, Black RE, Caipo M, Crump JA, Dev-

leess- chauwer B, Döpfer D, Fazil A, Fischer-Walker CL, Hald T, Hall AJ, Keddy KH, Lake RJ, Lanata CF, Torgerson PR, Havelaar AH, Angulo FJ. World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis. PLoS Med 2015;12:e1001921. https://doi.org/10.1371/journal.pmed.1001921. eCollection 2015

[17] Wain J, Hendriksen RS, Mikoleit ML, Keddy KH, Ochiai RL. Typhoid fever. Lancet 2015;385:1136-45. https://doi.org/10.1016/S0140-6736(13)62708-7

[18] Prüss-Ustün A, Bartram J, Clasen T, Colford JM Jr, Cumming O, Curtis V, Bonjour S, Dangour AD, De France J, Fewtrell L, Freeman MC, Gordon B, Hunter PR, Johnston RB, Mathers C, Mäusezahl D, Medlicott K, Neira M, Stocks M, Wolf J, Cairncross S. Burden of disease from inadequate water, sanitation and hygiene in low- and middle-income settings: a retrospective analysis of data from 145 countries. Trop Med Int Health 2014;19:894-905. Epub 2014 Apr 30. https://doi.org/10.1111/tmi.12329

[19] Mogasale V, Maskery B, Ochiai RL, Lee JS, Mogasale VV, Ramani E, Kim YE, Park JK, Wierzba TF. Burden of typhoid fever in low-income and middle-income countries: a systematic, literature-based update with risk-factor adjustment. Lancet Glob Health 2014;2:e570-80. https://doi.org/10.1016/S2214-109X(14)70301-8

[20] Antillón M, Warren JL, Crawford FW, Weinberger DM, Kürüm E, Pak GD, Marks F, Pitzer VE. The burden of typhoid fever in low- and middle-income countries: a meta-regression approach. PLoS Negl Trop Dis 2017;11:e0005376. https://doi.org/10.1371/journal.pntd.0005376

[21] Marchello CS, Hong CY, Crump JA. Global typhoid fever incidence: a systematic review and meta-analysis. Clin Infect Dis 2019;68(S2):S105-16. https://doi.org/10.1093/cid/ciy1094

[22] Franco-Paredes C, Khan MI, Gonzalez-Diaz E, Santos-Preciado JI, Rodriguez-Morales AJ, Gotuzzo E. Enteric fever: a slow response to an old plague. PLoS Negl Trop Dis 2016;10:e0004597. https://doi.org/10.1371/journal.pntd.0004597

[23] Amicizia D, Arata L, Zangrillo F, Panatto D, Gasparini R. Overview of the impact of Typhoid and Paratyphoid fever. Utility of Ty21a vaccine (Vivotif®). J Prev Med Hyg 2017;58:E1-E8.

[24] Levine MM. Typhoid fever vaccines. In: Plotkin SA, Orenstein WA, Offit PA, eds. Vaccines. 6t^{h} ed. Philadelphia, PA: Elsevier Saunders 2012, pp. 812-36.

[25] AIFA. Summary of the product Characteristic. Available at: https://farmaci.agenziafarmaco.gov.it/aifa/servlet/PdfDownloadServlet?pdfFileName=footer_004969_025219_RCP.pdf&retry=0&sys=m0b1l3. [Accessed on 23/07/2019].

[26] Nisini R, Biselli R, Matricardi PM, Fattorossi A, D'Amelio R. Clinical and immunological response to typhoid vaccination with parenteral or oral vaccines in two groups of 30 recruits. Vaccine 1993;11:582-6.

[27] Cryz SJ, Jr, Vanprapar N, Thisyakorn U, Olanratmanee T, Losonsky G, Levine MM, Chearskul S. Safety and immunogenicity of Salmonella typhi Ty21a vaccine in young Thai children. Infection Immunity 1993;61:1149-51.

[28] Gilman RH, Hornick RB, Woodard WE, DuPont HL, Snyder MJ, Levine MM, Libonati JP. Evaluation of a UDP-glucose-4-epimeraseless mutant of Salmonella typhi as a liver oral vaccine. J Infect Dis.1977;136:717-23.

[29] Wahdan MH, Serie C, Germanier R, Lackany A, Cerisier Y, Guerin N, Sallam S, Geoffroy P, el Tantawi AS, Guesry P. A controlled field trial of live oral typhoid vaccine Ty21a. Bulletin of the World Health Organization 1980;58:469-74.

[30] Levine MM, Ferreccio C, Black RE, Germanier R. Large-scale field trial of Ty21a live oral typhoid vaccine in enteric-coated capsule formulation. Lancet 1987;1:1049-52.

[31] Levine MM, Ferreccio C, Abrego P, Martin OS, Ortiz E, Cryz S. Duration of efficacy of Ty21a, attenuated Salmonella typhi live oral vaccine. Vaccine 1999;17(Suppl 2):S22-27.

[32] Bhuiyan TR, Choudhury FK, Khanam F, Saha A, Sayeed MA, Salma U, Lundgren A, Sack DA, Svennerholm AM, Qadri F. Evaluation of immune responses to an oral typhoid vaccine, Ty21a, in children from 2 to 5 years of age in Bangladesh. Vaccine 2014;32:1055-60. https://doi.org/10.1016/j.vaccine.2014.01.001

[33] Jackson BR, Iqbal S, Mahon B; Centers for Disease Control and Prevention (CDC). Updated recommendations for the use of typhoid vaccine - Advisory Committee on Immunization Practices, United States, 2015. MWR Morb Mortal Wkly Rep 2015;64:305-8.

[34] Date KA, Bentsi-Enchill A, Marks F, Fox K. Typhoid fever vaccination strategies. Vaccine 2015;33(Suppl 3):C55-61. https://doi.org/10.1016/j.vaccine.2015.04.028.

[35] FDA Typhoid VI polysaccharide vaccine Typhim vi®. Available at https://www.fda.gov/media/75993/download [Accessed on 20/02/2019].

[36] Acharya IL1 Lowe CU, Thapa R, Gurubacharya VL, Shrestha MB, Cadoz M, Schulz D, Armand J, Bryla DA, Trollfors B. Prevention of typhoid fever in Nepal with the Vi capsular polysaccharide of Salmonella typhi: a preliminary report. N Engl J Med 1987;317:1101-4.

[37] Tacket CO, Ferreccio C, Robbins JB, Tsai CM, Schulz D, Cadoz M, Goudeau A, Levine MM. Safety and immunogenicity of two Salmonella typhi Vi capsular polysaccharide vaccines. J Infect Dis 1986;154:342-5.

[38] Kaper JB, Morris JG, Levine MM. Cholera. Clin Microbiol Rev 1995;8:48-86.

[39] Faruque SM, Albert MJ, Mekalanos JJ. . Epidemiology, genetics, and ecology of toxigenic Vibrio cholerae. Microbiol Mol Biol Rev 1998;62:1301-14.

[40] Clemens JD. Cholera vaccines. In: Plotkin SA, Orenstein WA, Offit PA, eds. Vaccines. 7th edition. Philadelphia, PA: WB Saunders Company 2017, Chapter 14.

[41] Mukhopadhyay AK, Takeda Y, Nair GB. Cholera outbreaks in the El Tor biotype era and the impact of the new El Tor variants. Curr Top Microbiol Immunol 2014;379:17-47.

[42] Faruque AS, Fuchs GJ, Albert MJ.Changing epidemiology of cholera due to Vibrio cholerae O1 and O139 Bengal in Dhaka, Bangladesh. Epidemiol Infect 1996;116:275-8.

[43] Siddique AK, Zaman K, Akram K, Mutsuddy P, Eusof A, Sack RB. Emergence of a new epidemic strain of Vibrio cholerae in Bangladesh. An epidemiological study. Trop Geogr Med 1994;46:147-50.

[44] Grim CJ, Hasan NA, Taviani E, Haley B, Chun J, Brettin TS, Bruce DC, Detter JC, Han CS, Chertkov O, Challacombe J, Huq A, Nair GB, Colwell RR. Genome sequence of hybrid *Vibrio cholera* O1 MJ-1236, B-33, and CIRS101 and comparative genomics with *V. cholerae*. J Bacteriol 2010;192:3524-33.

[45] Kanungo S, Sah BK, Lopez AL, Sung JS, Paisley AM, Sur D, Clemens JD, Nair GB. Cholera in India: an analysis of reports, 1997-2006. Bull World Health Organ 2010;88:185-91.

[46] Siddique AK, Nair GB, Alam M, Sack DA, Huq A, Nizam A, Longini IM, Qadri F, Faruque SM, Colwell RR, Ahmed S, Iqbal A, Bhuiyan NA, Sack RB. El Tor cholera with severe disease: a new threat to Asia and beyond. Epidemiol Infect. 2010;138:347-52.

[47] Piarroux R, Barrais R, Faucher B, Haus R, Piarroux M, Gaudart J, Magloire R, Didier R. Understanding the Cholera Epidemic, Haiti. Emerg Infect Dis 2011;17:1161-7.

[48] Conner JG, Teschler JK, Jones CJ, Yildiz FH. Staying alive: vibrio cholerae's cycle of environmental survival, transmission, and dissemination. Microbiol Spect. 2016;4(2). https://doi.org/10.1128/microbiolspec

[49] Barr AJ. The biochemical basis of disease. Essays Biochem 2018;62:619-42. https://doi.org/10.1042/EBC20170054

[50] Cholera vaccines: WHO position paper – August 2017. Wkly Epidemiol Rec 201;92:477-98.

[51] Charles RC, Ryan ET. Cholera in the 21st century. Curr Opin Infect Dis 2011;24:472-7.

[52] Clemens JD, Nair GB, Ahmed T, Qadri F, Holmgren J. Cholera. Lancet 2017;390:1539-49. https://doi.org/10.1016/S0140-6736(17)30559-7

[53] Chowdhury F, Khan AI, Harris JB, LaRocque RC, Chowdhury MI, Ryan ET, Faruque ASG, Calderwood SB, Qadri F. A comparison of clinical and immunologic features in children and older patients hospitalized with severe cholera in Bangladesh. Pediatr Infect Dis J 2008;27:986-92.

[54] Harris JB, Khan AI, LaRocque RC, Dorer DJ, Chowdhury F, Faruque ASG, Sack DA, Ryan ET, Qadri F, Calderwood SB. Blood group, immunity, and risk of infection with Vibrio cholerae in an area of endemicity. Infect Immun 2005;73:7422–7.

[55] Harris JB, LaRocque RC, Chowdhury F, Khan AI, Logvinenko T, Faruque ASG, Ryan ET, Qadri F, Calderwood SB. Susceptibility to Vibrio cholerae infection in a cohort of household contacts of patients with cholera in Bangladesh. PLoS Negl Trop Dis 2008;2:e221. https://doi.org/10.1371/journal.pntd.0000221

[56] Holmner A, Mackenzie A, Krengel U. Molecular basis of cholera blood-group dependence and implications for a world characterized by climate change. FEBS Lett 2010;584:2548-55.

[57] Hannah G. Davies, Conor Bowman, Stephen P. Luby. Cholera – management and prevention. J Infect 2017;74: S66-S73.

[58] Watson JT, Gayer M, Connolly MA. Epidemics after natural disasters. Emerg Infect Dis 2007;13:1-5.

[59] Ali M, Nelson AR, Lopez AL, Sack DA. Updated global burden of cholera in endemic countries. PLoS Negl Trop Dis 2015;9:e0003832. https://doi.org/10.1371/journal.pntd.0003832

[60] WHO (2014) Cholera surveillance and number of cases. Geneva: World Health Organization.

[61] Griffith DC, Kelly-Hope LA, Miller MA. Review of reported cholera outbreaks worldwide, 1995-2005. Am J Trop Med Hyg 2006;75:973-7.

[62] Zuckerman JN, Rombo L, Fisch A. The true burden and risk of cholera: implications for prevention and control. Lancet Infect Dis 2007;7:521-30.

[63] Masuet Aumatell C, Ramon Torrell JM, Zuckerman JN Review of oral cholera vaccines: efficacy in young children. Infect Drug Resist 2011;4:155-60. https://doi.org/10.2147/IDR.S10339

[64] Legros D; Partners of the Global Task Force on Cholera Control. Global cholera epidemiology: opportunities to reduce the burden of cholera by 2030. J Infect Dis 2018;218(suppl_3):S137-S140. https://doi.org/10.1093/infdis/jiy486

[65] Hamilton KL. Robert K. Crane-Na(+)-glucose cotransporter to cure? Front Physiol 2013;4:53.

[66] Dutta D, Chowdhury G, Pazhani GP, Guin S, Dutta S, Ghosh S, Rajendran K, Nandy RK, Mukhopadhyay AK, Bhattacharya MK, Mitra U, Takeda Y, Nair GB, Ramamurthy T. Vibrio cholerae non-O1, non-O139 serogroups and cholera-like diarrhea, Kolkata, India. Emerging Infect Dis 2013;19:464-7.

[67] World Health Organization. Cholera, 2017. Wkly Epidemiol Rec 2018;93:489-500.

[68] Communicable Disease Threats Report Week 38, 16-22 September 2018.

[69] World Health Organization. Communicable diseases following natural disasters [Internet]. 1211 Geneva 27 Switzerland; 2006. Report No.: WHO/CDS/NTD/DCE/2006.4. Available at: http://www.who.int/diseasecontrol_emergencies/guidelines/CD_Disasters_26_06.pdf. [Accessed on 30/03/2019].

[70] Jutla A, Khan R, Colwell R. Natural disasters and cholera outbreaks: current understanding and future outlook. Curr Environ Health Rep 2017;4:99-107. https://doi.org/10.1007/s40572-017-0132-5

[71] Outbreak surveillance and response in humanitarian emergencies WHO guidelines for EWARN implementation. Geneva, 2012.

[72] Amar PK. Ensuring safe water in post-chemical, biological, radiological and nuclear emergencies. J Pharm Bioallied Sci 2010;2:253-66. https://doi.org/10.4103/0975-7406.68508

[73] Hove-Musekwa SD, Nyabadza F, Chiyaka C, Das P, Tripathi A, Mukandavire Z. Modelling and analysis of the effects of malnutrition in the spread of cholera. Mathematical and Computer Modelling 2011;53:1583-95. https://doi.org/10.1016/j.mcm.2010.11.060

[74] Cholera: risk factors. Available at: http://www.mayoclinic.com/health/cholera/ds00579/dsection=risk-factors. [Accessed 20/02/2019].

[75] Gaffga NH, Tauxe RV, Mintz ED. Cholera: a new homeland in Africa? Am J Trop Med Hyg 2007;77:705-13.

[76] Alam M, Hasan NA, Sadique A, Bhuiyan NA, Ahmed KU, Nusrin S, Nair GB, Siddique AK, Sack RB, Sack DA, Huq A, Colwell RR. Seasonal cholera caused by Vibrio cholerae serogroups O1 and O139 in the coastal aquatic environment of Bangladesh. Appl Environ Microbiol 2006;72:4096-104. https://doi.org/10.1128/AEM.00066- 06

[77] Singleton FL, Attwell RW, Jangi MS, Colwell RR. Influence of salinity and organic nutrient concentration on survival and growth of Vibrio cholerae in aquatic microcosms. Appl Environ Microbiol 1982;43:1080-5.

[78] Lara RJ, Neogi SB, Islam MS, Mahmud ZH, Yamasaki S, Nair GB. Influence of catastrophic climatic events and human waste on vibrio distribution in the Karnaphuli Estuary, Bangladesh. Eco Health 2009;6:279. https://doi.org/10.1007/s10393-009-0257-6

[79] Leaning J, Guha-Sapir D. Natural disasters, armed conflict, and public health. N Engl J Med 2013;369:1836-42. https://doi.org/10.1056 /NEJMra1109877

[80] McMichael A. Human population health: sentinel criterion of environmental sustainability. Curr Opin Environ Sustain. 2009;1:101- 6. https://doi.org/10.1016/j.cosust.2009.07.001

[81] EM DAT. The OFDA/CRED International Disaster Database [Internet]. 2016. Available at: http://www.emdat.be/classification [Accessed on 30/03/2019].

[82] EMA. Summary of the product Characteristic. Available at: https://ec.europa.eu/health/documents/community-register/2015/20150408131570/anx_131570_en.pdf. [Accessed on 20/02/2019].

[83] Ahmed T, Svennerholm AM, Al Tarique A, Sultana GN, Qadri F. Enhanced immunogenicity of an oral inactivated cholera vaccine in infants in Bangladesh obtained by zinc supplementation and by temporary withholding breast-feeding.Vaccine 2009;27:1433-9. https://doi.org/10.1016/j.vaccine.2008.12.036

[84] Clemens JD, Sack DA, Harris JR, Chakraborty J, Khan MR, Stanton BF, Kay BA, Khan MU, Yunus M, Atkinson W. Field trial of oral cholera vaccines in Bangladesh. Lancet 1986;2:124-7. https://doi.org/10.1016/S0140-6736(86)91944-6.

[85] Lucas ME, Deen JL, von Seidlein L, Wang XY, Ampuero J, Puri M, Ali M, Ansaruzzaman M, Amos J, Macuamule A, Cavailler P, Guerin PJ, Mahoudeau C, Kahozi-Sangwa P, Chaignat CL, Barreto A, Songane FF, Clemens JD. Effectiveness of mass oral cholera vaccination in Beira, Mozambique. N Engl J Med 2005;352:757-67.

[86] Ali M, Emch M, von Seidlein L, Yunus M, Sack DA, Rao M, Holmgren J, Clemens JD. Herd immunity conferred by killed oral cholera vaccines in Bangladesh: a reanalysis. Lancet 2005;366:44-9.

[87] Longini IM Jr, Nizam A, Ali M, Yunus M, Shenvi N, Clemens JD. Controlling endemic cholera with oral vaccines. PLoS Med 2007;4:e336.

[88] Jonker EFF, Uijlings MAC, Visser LG, Soonawala D. Comparison of the immunogenicity of Dukoral® oral cholera vaccine between renal transplant recipients on either a calcineurin inhibitor or mycophenolate - A controlled trial. Vaccine

2019;37:3133-9. https://doi.org/10.1016/j.vaccine.2019.04.010

[89] Clemens JD, Harris JR, Sack DA, Chakraborty J, Ahmed F, Stanton BF, et al. Field trial of oral cholera vaccines in Bangladesh: results of one year of follow-up. J Infect Dis 1988;158:60–9. https://doi.org/10.1093/infdis/158.1.60

[90] Jeline T, Kollaritsch H. Vaccination with Dukoral against travelers' diarrhea (ETEC) and cholera. Expert Rev Vaccines 2008;7:561-7. https://doi.org/10.1586/14760584.7.5.561

[91] WHO Water, sanitation and hygiene interventions and the prevention of diarrhea. Available at https://www.who.int/elena/titles/bbc/wsh_diarrhoea/en/. [Accessed on 22/03/2019].

[92] WHO Guidelines on sanitation and health. Available at: https://www.who.int/water_sanitation_health/sanitation-waste/sanitation/sanitation-guidelines/en/ [Accessed on 30/03/2019].

[93] Tagliabue A, Rappuoli R. Changing Priorities in Vaccinology: Antibiotic Resistance Moving to the Top. Front Immunol 2018; 9:1068. https://doi.org/10.3389/fimmu.2018.01068

[94] Parkhill J, Dougan G, James KD, Thomson NR, Pickard D, Wain J, Churcher C, Mungall KL, Bentley SD, Holden MT, Sebaihia M, Baker S, Basham D, Brooks K, Chillingworth T, Connerton P, Cronin A, Davis P, Davies RM, Dowd L, White N, Farrar J, Feltwell T, Hamlin N, Haque A, Hien TT, Holroyd S, Jagels K, Krogh A, Larsen TS, Leather S, Moule S, O'Gaora P, Parry C, Quail M, Rutherford K, Simmonds M, Skelton J, Stevens K, Whitehead S, Barrell BG. Complete genome sequence of a multiple drug resistant Salmonella enterica serovar Typhi CT18. Nature 2001;413:848-52.

[95] WHO. Typhoid vaccines: WHO position paper. Wkly Epidemiol Rec 2008;83:49-59.

[96] ClinicalTrials.gov Available at: https://clinicaltrials.gov/ct2/show/NCT03220737?term=CVD103-HgR&cond=Cholera&rank=8

[97] Kitaoka M, Miyata ST, Unterweger D, Pukatzki S. Antibiotic resistance mechanisms of Vibrio cholerae. J Med Microbiol 2011;60(Pt 4):397-407. https://doi.org/10.1099/jmm.0.023051-0

[98] Saulat J. Cholera - Epidemiology, Prevention and Control 2016 Capter 6. https://doi.org/0.5772/63358

[99] Epicentro. Salute in viaggio. Available at: https://www.epicentro.iss.it/viaggiatori/. [Accessed on 22/03/2019].

[100] World Tourism Organization and Global Tourism Economy Research Centre (2018), UNWTO/GTERC Asia Tourism Trends – 2018 Edition, UNWTO, Madrid, DOI: https://doi.org/10.18111/9789284420032

[101] Van Herck K, Zuckerman J, Castelli F, Van Damme P, Walker E, Steffen R; European Travel Health Advisory Board. Travelers' knowledge, attitudes, and practices on prevention of infectious diseases: results from a pilot study. J Travel Med 2003;10:75-8.

[102] Statement on international travelers and typhoid: an Advisory Committee Statement (ACS) Committee to Advise on Tropical Medicine and Travel (CATMAT). Available at: http://publications.gc.ca/collections/collection_2014/aspc-phac/HP40-98-2014-eng.pdf [Accessed on 22/02/2019].

[103] Connor BA, Schwartz E. Typhoid and paratyphoid fever in travellers. Lancet Infect Dis 2005;5:623-8.

[104] Angell SY, Cetron MS. Health disparities among travelers visiting friends and relatives abroad. Ann Intern Med 2005;142: 67-72.

[105] Awofeso N, Aldabk K. Cholera, migration, and global health – a critical review. Int J Travel Med Glob Health 2018;6:92-9. https://doi.org/10.15171/ijtmgh.2018.19

[106] International travel health guide 2019 online edition. Disponibile all'indirizzo: https://www.travmed.com/pages/health-guide-chapter-6-travelers-diarrhea. [ultimo accesso 27/11/2019.

[107] Kozicki M, Steffen R, Schar M. 'Boil it. Cook it. Peel it or forget it': does this rule prevent travellers' diarrhoea? Int J Epidemiol 1985;14:169-72.

[108] Van Herck K, Van Damme P, Castelli F, Zuckerman J, Nothdurft H, Dahlgren AL, Gisler S, Steffen R, Gargalianos P, Lopéz-Vélez R, Overbosch D, Caumes E, Walker E. Knowledge, attitudes and practices in travel-related infectious diseases: the European airport survey. J Travel Med 2004;11:3-8.

[109] Leggat PA. Sources of health advice given to travelers. J Travel Med 2000;7:85-8.

[110] Lee TK, Hutter JN, Masel J, Joya C, Whitman TJ. Guidelines for the prevention of travel-associated illness in older adults. Trop Dis Travel Med Vaccines.2017;3:10.

[111] Flaherty GT, Rossanese A, Steffen R, Torresi J. A golden age of travel: advancing the interests of older travellers. J Travel Med 2018;25(1).

[112] Han CT, Flaherty G. Profile of travelers with preexisting medical conditions attending a specialist travel medicine clinic in Ireland. J Travel Med 2015;22:312-7.

Received on June 21, 2019. Accepted on September 4, 2019.

Correspondence: Donatella Panatto Department of Health Sciences, University of Genoa, via Pastore 1, 16132 Genoa, Italy - Tel. +39 01 08109 - E-mail: panatto@unige.it

How to cite this article: Amicizia D, Micale RT, Pennati BM, Zangrillo F, Iovine M, Lecini E, Marchini F, Lai PL, Panatto D. Burden of typhoid fever and cholera: similarities and differences. Prevention strategies for European travelers to endemic/epidemic areas. J Prev Med Hyg 2019;60:E271-E285. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1333

OPEN ACCESS J PREV MED HYG 2019; 60: E286-E292

ORIGINAL ARTICLE

Measles surveillance activities in the Metropolitan Area of Milan during 2017-2018

S. BIANCHI[1], M. FACCINI[2], A. LAMBERTI[2], S. SENATORE[2, 3], G. CICERI[1], E.R. FRATI[1], D. COLZANI[1], M. GORI[1], D. CEREDA[3], M. GRAMEGNA[3], F. AUXILIA[1], E. TANZI[1, 4, 5], A. AMENDOLA[1, 4, 5]

[1] Department of Biomedical Sciences for Health, University of Milan, Italy; [2] Health Protection Agency, Metropolitan Area of Milan, Italy; [3] DG Welfare, UO Prevenzione, Lombardy Region, Milan, Italy; [4] Coordinated Research Center "EpiSoMI", University of Milan, Italy; [5] CIRI-IV, Department of Health Sciences, University of Genoa, Italy

Keywords

Measles surveillance • Measles laboratory confirmed cases • Measles epidemiological investigations • Indicators to monitor quality of measles laboratory surveillance

Summary

Introduction. *In Italy, the transmission of measles is still endemic, and 7,919 cases were reported to the National Surveillance System between January 2017 and December 2018. Aim of this study is to report the results of the measles surveillance activities in the Metropolitan City of Milan from March 2017 to December 2018, and to evaluate the surveillance performance WHO indicators.*

Methods. *The Local Health Units (LHUs) carried out case investigations and collected specimens to send to the EpiSoMI Lab (Subnational Reference Laboratory, SRL) of the University of Milan for cases/outbreaks confirmation and genotyping performed according to the WHO Guidelines.*

Results. *Overall, 610 suspected measles cases were reported by the surveillance system of the Metropolitan City of Milan. A total of 439 out of 540 cases with adequate specimens were laboratory-confirmed by molecular and/or serological assays. Two-hundred and thirty-six cases were notified as sporadic and 203 as related to 94 outbreaks. The most confirmed cases were aged 15–39 years, almost all not vaccinated. Overall, 282 cases were genotype D8 and 118 genotype B3.*

The evaluation of a set of indicators to monitor the quality of surveillance activities demonstrated the proficiency of the EpiSoMI Lab.

Conclusions. *A well-done investigation of cases and outbreaks by the surveillance local system, in a timely manner, in order to notify and investigate suspected cases and to laboratory confirm or discard cases is fundamental to reduce morbidity, to prevent further virus transmission and to achieve measles elimination.*

Introduction

Measles is a highly contagious disease caused by measles virus (Mv). Humans are the only reservoir of this infection and an effective vaccine is available. These characteristics make this disease eradicable. The World Health Organization (WHO) planned to get the elimination of Mv, but the rapid succession of several measles outbreak in the European Region in the last years made this goal impossible to achieve in a short-term [1-3]. This is one of the leading immunization priorities of the European Region as outlined in the European Vaccine Action Plan 2015-2020 [4]. Elimination of measles will depend on achieving high coverage and closing immunity gaps and ensuring high-quality, case-based surveillance [5, 6].

In Italy, the transmission of measles is still endemic, and 7,919 cases were reported to the National Surveillance System between January 2017 and December 2018 [7, 8], including eight deaths. The median age of the cases was 26 years, but the highest incidence was recorded in children under one year of age, too young to be vaccinated. The main settings involved were hospital, family, school (included nursery and university), workplace and the community. In particular, numerous nosocomial outbreaks have been reported [9, 10], highlighting both the problem of low vaccination coverage among health workers (among which 450 cases were reported) and the need to implement protocols for the prevention of measles transmission in healthcare [11].

In Italy, the Measles and Rubella Surveillance Network (Mo.Ro.Net), consisting of one National Reference Laboratory (NRL) and 14 Subnational Reference Laboratories (SRL) that meet rigorous standards to provide accurate results, was established in March 2017 [12]. Laboratories taking part in the network are required to participate in annual proficiency testing in selected techniques and are evaluated through the WHO accreditation program. The Laboratory of the Coordinated Research Center for the Epidemiology and Molecular Surveillance of Infections EpiSoMI (EpiSoMI Lab) of the University of Milan is one of the two SRL of the Lombardy Region (Northern Italy). The EpiSoMI Lab is a fully WHO-accredited laboratory and, from March 2017, set up a rapid and active surveillance for the complete characterization of the Mv in the Metropolitan City of Milan and surrounding areas.

A set of eight core indicators to monitor the quality of surveillance blend both field and laboratory activities [13]. Moreover, four of the eight indicators are di-

https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1269

rectly related to the management and the performance of the laboratory. These four indicators are the reporting rate of discarded non-measles non-rubella cases, the laboratory confirmation, the viral detection, and the timeliness of reporting laboratory results [14]. These standard performance indicators should be monitored to identify weakness in the laboratory surveillance system so that corrective action can be taken [15].

Aim of this study is to report the results of the measles surveillance activities in the Metropolitan City of Milan and surrounding areas from 1 March 2017 to 31 December 2018. Furthermore, we want to evaluate the four indicators, directly related to the management and the performance of the laboratory, in order to demonstrate whether the routine surveillance laboratory activities provide accurate and timely data.

Methods

Epidemiological surveillance data

In Lombardy Region, according to the National Surveillance Guidelines, all suspected measles cases must be promptly notified to the Local Health Units (LHUs). A suspected Mv case is defined as a subject with clinical evidence of "fever and rash" [6].

The LHUs carry out case investigations to determine source, risk factors and transmission settings, and conduct contact tracing to identify contacts, evaluate their immunity status, and vaccinate susceptible subjects. Moreover, LHUs collect specimens to send to the SRL for the case confirmation.

Notified cases are systematically reported to the Lombardy Regional database that provides, for each case, personal data, clinical details, all information collected during the epidemiological investigation as well as the SRL results including virus genotype.

Descriptive information on measles cases in this study were obtained from the Lombardy Regional database.

Laboratory surveillance data

Specimen collection

Before collecting samples, informed consent was obtained by suspected Mv cases (or their legal tutors in case of minors). Collection of adequate specimens, therefore, may include collection of specimens to test for virus-specific immunoglobulin M (IgM) (by Enzyme ImmunoAssay, EIA, on serum, blood or Dried Blood Spot, DBS) and for measles RNA detection [by Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) on urine and throat or nasopharyngeal swabs, Oral Fluid (OF)]. These biological samples must be collected during the acute phase of the disease, specifically between 4 and 10 days after the exanthema onset (between 4 and 28 days for DBS) for serological test, up to 14 days for OF or up to 21 days for urine for virus detection using molecular techniques, or in any case at the first contact with medical care [13-16].

Measles virus-specific IgM detection

Blood, serum and DBS samples received for IgM analysis were processed and tested as soon as possible after receipt in the laboratory. Serum samples were analyzed for serological testing using MV IgM capture Enzyme ImmunoAssay (EIA, Euroimmun AG, Luebeck, Germany), following the Manufacturing instructions.

Measles RNA detection

Total RNA was extracted from 1.5-15 ml of urine and/or 0.2-1 ml of OF, depending on the timing of specimen collection, using the NucliSENS® easyMAG™ automated platform (bioMérieux bv, Lyon, France) according to the off-board lysis protocol. Extracted RNA was analyzed for molecular testing using a One-Step Real-time PCR targeting the hemagglutinin (H) gene, as previously described [17].

Measles genotyping

The genotype of Mv strains was identified by sequencing the highly variable region of the nucleoprotein gene (N-450) [18]. RT-PCR products were purified with the NucleoSpin® Gel and PCR Clean-Up (Macherey-Nagel GmbH & Co. KG, Germany), and nucleotide sequences were obtained by automated DNA sequencing based on fluorescent dye terminator on genetic analyzer ABI PRISM 3100 Genetic Analyser (Applied Biosystem, Thermo Fisher, USA). N-450 Mv sequences detected during the seasons 2017-2018 were analyzed using the Basic Local Alignment Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify similarities with previously reported strains and to define the belonging genotype. Virus genotypes were designated according to the official WHO nomenclature and sequences have been submitted to the WHO's MeaNS (Measles Nucleotide Surveillance) database [19, 20].

Laboratory confirmed case definition

A confirmed laboratory case was defined as a patient with serological and/or virological evidence of acute measles infection.

Indicators to monitor the quality of laboratory surveillance

The quality of laboratory surveillance and a sensitive system for detection and investigation of suspected cases of measles are evaluated by four performance indicators:

1. Reporting rate of discarded non-measles non-rubella cases: target: ≥ 2 cases per 100000 population per year. This indicator is calculated as the number of non-measles/non-rubella cases in a year divided by the average population in the studied area;
2. Laboratory confirmation: meaning the proportion of suspected cases with adequate specimens for detecting acute measles infection collected and tested in a proficient laboratory (target: ≥ 80%). This indicator is calculated as the proportion between the cases confirmed/discarded by each method of detection and the number of the suspected cases;

3. Viral detection: meaning the proportion of laboratory-confirmed chains of transmission (outbreaks) with samples adequate for detecting measles collected and tested in an accredited laboratory (target: ≥ 80%). This indicator reflects the fundamental contribution of the molecular characterization of the Mvs and is calculated as the percentage of all chains of transmission, identified during a calendar year, that have been successfully characterized by genetic analysis;
4. Timeliness of reporting laboratory results: meaning the proportion of results reported by the laboratory within 4 days of specimen receipt (target: ≥80%).

Statistical analysis

The comparison of two proportions was analyzed using the Chi square test. Two-sided p values < 0.05 were considered statistically significant. All analyses were conducted using the OpenEpi software [21].

Results

Laboratory surveillance data

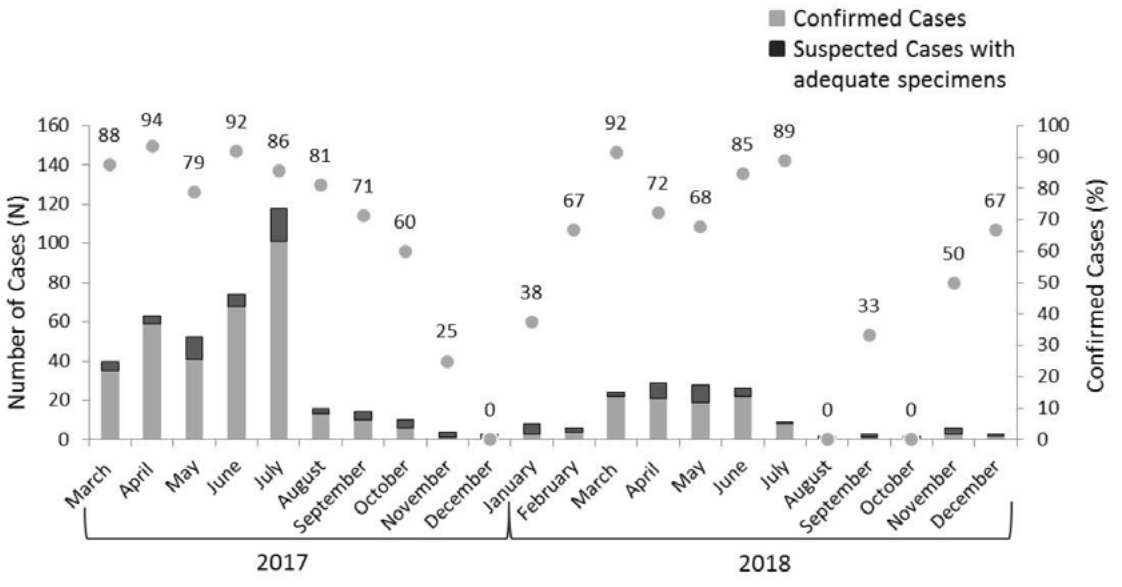
From March 2017 to December 2018, 610 suspected measles cases were reported by the surveillance system of the Metropolitan City of Milan and surrounding areas. For 540 (88.5%, 540/610) measles suspected cases were collected adequate specimens for serological and/or virological confirmation by the SRL. Of the 540 suspected cases, 80 (14.8%) had specimens only to be tested by molecular tests, whereas 6 (1.1%) only for serological evaluation. A total of 439 (439/540, 81.3%) reported cases were laboratory-confirmed by molecular and/or serological assays, in accordance with the WHO guidelines [12] (Fig. 1). The 59.2% (260/439) of confirmed cases tested positive by both serological and molecular assays; the 39.9% (162/439) was confirmed only by molecular tests, and the 3.9% (17/439) exclusively for anti-Measles IgM (Tab. I).

In Figure 2 is described the number of confirmed and discarded cases by age groups and year of surveillance. During the considered period, the proportion of discarded measles cases was 45.2% (38/84) for the age groups 0-4 years, 17.9% (7/39) for the age groups 5-14 years, 11.03% (32/290), 10.7% (12/112) and 75.0% (9/12) for the age groups 15-39, 40-64, and ≥ 65 years respectively. A statistical significance was found in the rate of discarded non-measles cases of age groups 0-4 and ≥ 65 years (both $p < 0.005$).

Overall, 58.8% (258/439) of the confirmed cases (median age: 28 years; range: 1 day-7 years) were aged 15-39 years, 22.8% (100/439) belonged to the 40-64 age group, 10.5% (46/439) were aged 0-4 years, and 3.4% were ≤ 1 year old. The 93.3% of confirmed cases were not vaccinated. During the considered period, the epidemiologic investigation identified 94 different outbreaks/chains of transmission: 77 during 2017 and 17 during 2018 epidemic. The 53.76% of the confirmed cases was notified as sporadic.

Four-hundred and thirty-four out of 439 confirmed cases had adequate specimens for viral detection and 402 out of 434 (92.6%, 402/434) cases were genotyped. In 2 subjects, vaccinated as susceptible during the epidemic period, was identified genotype A vaccine strain (0.5%). These subjects were excluded, since did not meet the case definition (genotype identification is required to distinguish wild type from vaccine strain if vaccinated within 21 days of rash onset). Two different genotypes were identified, D8 and B3. Genotypes D8 and B3 have co-circulated during the whole period (Fig. 3).

Fig. 1. Measles cases investigated during the period of surveillance activity to the enhanced measles surveillance system (data from the official data base of Lombardy Region) and cases confirmed by the SRL EpiSoMI, March2017-December 2018.

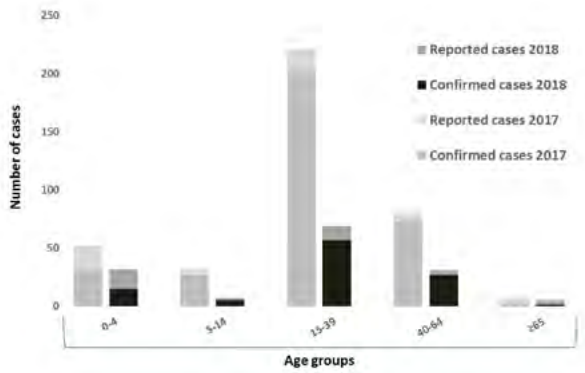


Tab. I. Reported suspected cases, suspected measles cases with adequate specimens and cases confirmation distribution during the study period (2017 and 2018).

Parameters		2017*	2018	Study period, N
Reported suspected measles cases, N (%)		440 (72.1)	170 (27.9)	610
Suspected measles cases with adequate specimens to be tested, N (%)		394 (73.0)	146 (27.0)	540
Number of different specimen type investigated, n (%)	Serum/Blood/DBS	372 (68.9)	168 (31.1)	540
	Urine	413 (69.3)	183 (30.7)	596
	Oral fluid	405 (69.8)	175 (30.2)	580
Measles cases confirmed by lab, N (%)		335 (76.3)	104 (23.7)	439
Assays used to confirm measles case (%)	Serological	11 (64.7)	6 (35.3)	17
	Virological	125 (77.2)	37 (22.8)	162
	Serological and virological	199 (76.5)	61 (23.5)	260

* in 2017 the parameters evaluation started on March.

Fig. 2. Distribution by age groups for confirmed/discarded cases during surveillance activity of SRL EpiSoMI, March 2017- December 2018.



The most common genotype was D8 (70.5%, 282/400 cases), while B3 genotype was identified in the 29.5% (118/400) of cases. D8 genotype was mainly observed during 2017 epidemic (92.6%, 261/282), whereas during 2018 the most circulating genotype was the B3 (76.4%, 68/89).

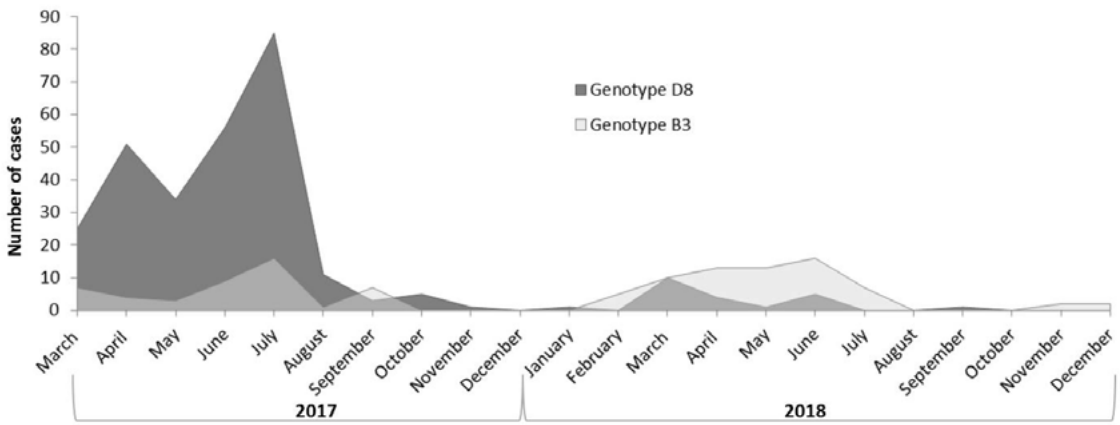
Overall, D8 genotype was identified in 277 (98.2%, 277/282) autochthonous and 4 imported cases. In particular, 152 (53.9%, 152/282) were notified as sporadic cases, while 130 (46.0%, 130/282) were involved in 68 outbreaks. B3 genotype was identified as imported in 13 cases out of 118 (11.0%, 13/118), as imported-related in 1 case and as autochthonous in 105 (89.0%, 105/118) cases. Otherwise, epidemiological investigation notified 61 (51.7%, 61/118) cases as sporadic and 57 (48.3%, 57/118) cases as related to 24 outbreaks.

Indicators to monitor quality of laboratory surveillance

The four indicators to monitor the quality of laboratory surveillance are calculated by period (March 2017-December 2017 and January 2018-December 2018) and the results are shown in Table II.

The number of suspected cases that met the clinical case definition but were not laboratory confirmed was 60 during 2017 and 39 during 2018. Considering a mean population of 4000000 inhabitants included in the Metropolitan City of Milan and surrounding areas, the reporting rate of discarded cases was about 1.5 per 100000 popu-

Fig. 3. Genotypes circulation in the Metropolitan Area of Milan, from March 2017 to December 2018.



lation per year in 2017 and about 1 per 100000 population per year in 2018.
In the considered period, more than the 80% of all cases that met the clinical definition had the collection of adequate specimens and were laboratory confirmed/discarded. Viral detection and genetic characterization of measles virus responsible of a chain of transmission was about 99% (only two chains of transmission were not genetically characterized). The 89.6% (484/540) of laboratory confirmed cases were reported to LHU by the laboratory within 4 days of specimen receipt.

Discussion and conclusions

Monitoring progress toward measles elimination requires high-quality case-based surveillance that is able to, in a timely manner, detect, notify and investigate suspected measles cases and outbreaks, correctly classify them as confirmed or discarded, and prevent further virus transmission [5, 22]. A key role of the high-quality case-based surveillance is represented by the laboratory activities.
In order to increase the performance level requested by the WHO, including timeliness and completeness of data, in March 2017 a sub-national network of accredited laboratories for measles and rubella surveillance (Mo.Ro.Net) coordinated by the NRL was formalized [12, 23].
As SRL, EpiSoMI Lab undergoes regular and thorough processes for monitoring the accuracy and performance of its procedures and the operators who performing them (WHO accreditation). The EpiSoMI Lab must maintain that status for the forthcoming calendar year, thus the accreditation assessment is based on the laboratory's performance during the preceding 12 months. EpiSoMI Lab achieved full accreditation for both investigated years, 2017 and 2018.
In the Lombardy Region, from September 2013 to May 2014 were collected biological samples from 80 suspected cases and the 57.5% were laboratory confirmed. In that period, in Lombardy were noticed 880 suspected cases [24], while in 2016, 165 Mv cases were notified and 114 (69.1%) were confirmed (unpublished data). From March 2017 (date of Mo.Ro.Net establishing) to December 2018, a total of 959 Mv cases, including confirmed, possible and probable cases, were reported in Lombardy Region [25]. In particular, 610 out of 959 Mv cases was related to a large measles outbreak in the Metropolitan City of Milan and surrounding areas, a highly populated area with about 4 million inhabitants. In this area, 439 measles cases (81.3% of cases investigated) were laboratory-confirmed. This highlighted how a well-organized accredited laboratory improves the quality of the surveillance system. The laboratory needs adequate clinical samples to be timely and accurate in reporting confirmation/discard for clinically suspected cases. The detection of virus-specific IgM is the standard method for case confirmation. However, the use of RT-PCR for direct detection of measles specific RNA is fundamental to complement IgM antibody detection. Using PCR method has become necessary to perform a rapid case/outbreak investigation and to adopt the "fever and rash" case definition by collecting specimens at the first encounter with the healthcare system, and performing molecular testing for virus detection in a "fast and aggressive" way [13, 26, 27]. Remarkably, with this strategy, the EpiSoMi Lab, during 2017-2018 period, have confirmed by molecular methods a relevant proportion (13.2%) of seronegative cases (< 4 days from rash). These results prove the value of molecular analysis as tool to identify Mv cases otherwise unrecognized.
Overall, the 439 confirmed cases were classified by epidemiological investigations: 236 as sporadic cases and 203 as related to 94 outbreaks. Four hundred cases were genotyped: 282 were genotype D8 and 118 were genotype B3. These two genotypes co-circulated in Europe during the study period [28].
A set of indicators was identified by the WHO [14] to monitor the quality of surveillance activities, including the reporting rate of discarded non-measles non-rubella cases (≥ 2 cases per 100000 population per year). The 88.57% of suspected Mv cases were laboratory confirmed with a reporting rate of discarded non-measles cases between 1-1.5 per 100000 population in the two years, whereas at national level the reported rates were of 0.67 per 100000 population per year in 2017 and of 0.37 per 100000 population per year in 2018. The rate of discarded Mv cases were significantly higher in 0-4 years and ≥ 65 years age groups. These results could be explained by a higher attention and sensitivity for pediatric population (at higher

Tab. II. Indicators of the quality of laboratory surveillance.

Laboratory indicators	Results		Target
	March 2017 - December 2017	January 2018 - December 2018	
1. Reporting rate of discarded non-measles non-rubella cases	1.5 cases per 100000 population per year	1 case per 100000 population per year	≥ 2 cases per 100000 population per year
2. Laboratory confirmation	89.5%	85.9%	≥ 80%
3. Viral detection	100%	97.4%	≥ 80%
4. Timeliness of reporting laboratory results	88.9%	92.4%	≥ 80%

risk of clinical complications, also neurological), and by a very low measles incidence in the elderly.
Another critical indicator of surveillance performance is the viral detection and genotyping (≥ 80% of laboratory-confirmed chains of transmission) [29]. The mean rate of genomic characterization performed during the observed period is 98.7%, much more than the national average (61.5% for 2017) [11]. The genetic characterization has enabled us to identify or confirmed epidemiological links.
The evaluation of these indicators is intended to demonstrate the proficiency of the accredited laboratories and whether adequate surveillance laboratory activities are implemented and documented for verification purposes. These data demonstrate that the EpiSoMI Lab has supported Mv cases ascertainment in the Lombardy Region in these two years of activity in Mo.Ro.Net in a proficient way, confirming outbreaks/cases and determining Mv circulating genotypes.
Achieving indicator targets provides assurance that public health authorities can detect, locate and describe potential Mv transmission in a timely manner [26].
In conclusion, a well-done investigation of cases and outbreaks by the surveillance local system, in order to notify and investigate suspected cases and promptly laboratory confirm or discard cases, is fundamental to reduce morbidity, to prevent further virus transmission and to achieve measles elimination.

Acknowledgements

The authors wish to thank: all the staffs of Local Health Units involved in outbreaks investigation; the General Directorate of Welfare of Lombardy Region, in particular Marco Mentasti, for his help in retrieving the epidemiological data.
Funding sources: this work was supported by funds from Lombardy Region.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

SB Study design, project and protocol development, coordinated and contributed to the laboratory testing, data analysis and manuscript writing.
MF coordinated the activity of the Health Protection Agency of Metropolitan Area of Milan (Milan, Italy), and critically revised the manuscript.
SS and AL coordinated the epidemiological surveillance activities, contributing substantially to the acquisition of the epidemiological data, and contributed to the writing of the manuscript.
DCo and ERF protocol development, laboratory testing and critically revised the manuscript
GC and MGo laboratory testing and critically revised the manuscript.
MGr and DCe secured study funding, acquisition and analysis of the epidemiological data.
FA contributed to the development of the local surveillance activities and critically revised the manuscript.
ET contributed to the conception and design of the study and critically revised the manuscript.
AA coordinated and supervised the research, designed the study and write the manuscript.
All authors revised the manuscript and contributed to improving the paper. All authors read and approved the final manuscript.

References

[1] European Centre for Disease Prevention and Control. Risk of measles transmission in the EU/EEA, 21 March 2018. Stockholm, ECDC. Available at: https://ecdc.europa.eu/sites/portal/files/documents/Measles-rapid-risk-assessment-European-Union-countries.pdf (Accessed on 16 January 2019).

[2] World Health Organization. Global Measles and Rubella Strategic Plan: 2012–2020. 2012. Available at: http://apps.who.int/iris/bitstream/10665/44855/1/9789241503396_eng.pdf (Accessed on 10 January 2019).

[3] European Centre for Disease Prevention and Control (2018) Monthly measles and rubella monitoring report, July 2018. Stockholm: ECDC; Available at: https://ecdc.europa.eu/sites/portal/files/documents/Monthly-Measles-Rubella-monitoring-report-July-2018-1.pdf (Accessed on 17 January 2019).

[4] World Health Organization Regional Office for Europe (2014) European Vaccine Action Plan 2015-2020. Copenhagen: WHO. Available at: http://www.euro.who.int/en/health-topics/disease-prevention/vaccines-and-immunization/publications/2014/european-vaccine-action-plan-20152020-2014 (Accessed on 16 January 2019).

[5] World Health Organization. Regional Office for Europe Eliminating measles and rubella. Framework for the verification process in the WHO European Region. 2014. Available at: http://www.euro.who.int/__data/assets/pdf_file/0009/247356/Eliminating-measles-and-rubella-Framework-for-the-verification-process-in-the-WHO-European-Region.pdf (Accessed on 24 January 2019).

[6] Sniadack DH, Crowcroft NS, Durrheim DN, Rota PA. Roadmap to elimination standard measles and rubella surveillance. Wkly Epidemiol Rec.2017;92:97-105.

[7] Magurano F, Baggieri M, Mazzilli F, Bucci P, Marchi A, Nicoletti L; MoRoNet Group. Measles in Italy: viral strains and crossing borders. Int J Infect Dis 2018; pii: S1201-9712(18)34583-1. https://doi.org/10.1016/j.ijid.2018.11.005

[8] Morbillo & Rosolia News, January 2019. Available at: https://www.epicentro.iss.it/morbillo/bollettino/RM_News_2018_48%20def.pdf (Accessed on 16 March 2019).

[9] Amendola A, Bianchi S, Frati ER, Ciceri G, Faccini M, Senatore S, Colzani D, Lamberti A, Baggieri M, Cereda D, Gramegna M, Nicoletti L, Magurano F, Tanzi E. Ongoing large measles outbreak with nosocomial transmission in Milan, northern Italy, March-August 2017. Euro Surveill 2017;22(33). pii: 30596. https://doi.org/10.2807/15607917.ES.2017.22.33.30596.

[10] Porretta A, Quattrone F, Aquino F, Pieve G, Bruni B, Gemignani G, Vatteroni ML, Pistello M, Privitera GP, Lopalco PL. A nosocomial measles outbreak in Italy, February-April 2017. Euro Surveill 2017;22(33). pii: 30597. https://doi.org/10.2807/1560-7917.ES.2017.22.33.30597

[11] Adamo G, Sturabotti G, Baccolini V, de Soccio P, Prencipe GP, Bella A, Magurano F, Iannazzo S, Villari P, Marzuillo C. Re-

gional reports for the subnational monitoring of measles elimination in Italy and the identification of local barriers to the attainment of the elimination goal. PLoS One 2018;13:e0205147. https://doi.org/10.1371/journal.pone.0205147

[12] Mo.Ro.Net–liberi dal morbillo e dalla rosolia [Mo.Ro.Net.–free from measles and rubella]. Italian. Available from: http://moronetlab.it/ (Accessed on 16 April 2019).

[13] World Health Organization. Manual for the Laboratory-based Surveillance of Measles, Rubella, and Congenital Rubella Syndrome. Third edition, June 2018. Available at: https://www.who.int/immunization/monitoring_surveillance/burden/laboratory/manual/en/ (Accessed on 16 January 2019).

[14] World Health Organization. Manual for the laboratory diagnosis of measles and rubella virus infection - Second edition. WHO/IVB/07.01. 2007. Available at www.who.int/vaccines-documents/ (Accessed on 16 January 2019).

[15] WHO Vaccine-Preventable Diseases Surveillance Standards: Measles. Available at: https://www.who.int/immunization/monitoring_surveillance/burden/vpd/WHO_SurveillanceVaccinePreventable_11_Measles_BW_R2.pdf?ua=1 (Accessed on 16 February 2019).

[16] WHO. Surveillance Guidelines for Measles, Rubella and Congenital Rubella Syndrome in the WHO European Region Update December 2012. Available from: http://www.euro.who.int/ data/assets/pdf file/0018/79020/e93035-2013%20pdf?ua=1 (Accessed on 6 February 2019).

[17] Hübschen JM, Kremer JR, De Landtsheer S, Muller CP. A multiplex TaqMan PCR assay for the detection of measles and rubella virus. J Virol Methods 2008;149:246-50. https://doi.org/10.1016/j.jviromet.2008.01.032

[18] Bianchi S, Frati ER, Lai A, Colzani D, Ciceri G, Baggieri M, Lamberti A, Senatore S, Faccini M, Mazzilli F, Gramegna M, Zehender G, Magurano F, Tanzi E, Amendola A. Genetic characterization of Measles virus variants identified during a large epidemic in Milan, Italy, March-December 2017. Epid Infec 2019;1-5. https://doi.org/10.1017/S0950268818003606

[19] Rota PA, Brown K, Mankertz A, Santibanez S, Shulga S, Muller CP, Hübschen JM, Siqueira M, Beirnes J, Ahmed H, Triki H, Al-Busaidy S, Dosseh A, Byabamazima C, Smit S, Akoua-Koffi C, Bwogi J, Bukenya H, Wairagkar N, Ramamurty N, Incomserb P, Pattamadilok S, Jee Y, Lim W, Xu W, Komase K, Takeda M, Tran T, Castillo-Solorzano C, Chenoweth P, Brown D, Mulders MN, Bellini WJ, Featherstone D. Global distribution of measles genotypes and measles molecular epidemiology. J Infect Dis 2011;204:S514-S23. https://doi.org/10.1093/infdis/jir118

[20] Santibanez S, Hübschen JM, Ben Mamou MC, Muscat M, Brown KE, Myers R, Donoso Mantke O, Zeichhardt H, Brockmann D, Shulga SV, Muller CP, O'Connor PM, Mulders MN, Mankertz A. Molecular surveillance of measles and rubella in the WHO European Region: new challenges in the elimination phase. Clin Microbiol Infect 2017;23:516-23. https://doi.org/10.1016/j.cmi.2017.06.030

[21] www.openepi.com (Accessed on 21 March 2019).

[22] World Health Organization (WHO). Genetic diversity of wild-type measles viruses and the global measles nucleotide surveillance database (MeaNS). Wkly Epidemiol Rec 2015;90:373-80.

[23] Magurano F, Baggieri M, Filia A, Del Manso M, Lazzarotto T, Amendola A, D'Agaro P, Chironna M, Ansaldi F, Iannazzo S, Bucci P, Marchi A, Nicoletti L; Measles Surveillance Group. Towards measles elimination in Italy: Virological surveillance and genotypes trend (2013-2015). Virus Res 2017;236:24-9. https://doi.org/10.1016/j.virusres.2017.05.009

[24] Amendola A, Bubba L, Piralla A, Binda S, Pariani E, Ranghiero A, Premoli M, Pellegrinelli L, Coppola L, Gramegna M, Baldanti F, Zanetti A. (2015) Surveillance and vaccination coverage of measles and rubella in Northern Italy, Hum Vaccin Immunother 2015;11:206-13. https://doi.org/10.4161/hv.35865

[25] Available at: http://www.epicentro.iss.it/morbillo/bollettino (Accessed on 29 March 2019).

[26] David HS, Crowcroft NS, Durrheim DN, Rota PA. Roadmap to elimination standard measles and rubella surveillance. Wkly Epidemiol Rec. 2017;92(9-10):97-105.

[27] Hübschen JM, Bork SM, Brown KE, Mankertz A, Santibanez S, Ben Mamou M, Mulders MN, Muller CP. Challenges of measles and rubella laboratory diagnostic in the era of elimination. Clin Microbiol Infect. 2017;23(8):511-515. doi: 10.1016/j.cmi.2017.04.009.

[28] Available at: http://www.who-measles.org/Public/Data_Mnt/who_map.php (Accessed on 27 March 2019).

[29] Dabbagh A, Patel MK, Dumolard L, Gacic-Dobo M, Mulders MN, Okwo-Bele JM, Kretsinger K, Papania MJ, Rota PA, Goodson JL. Progress toward regional measles elimination—worldwide, 2000–2016. MMWR Morb Mortal Wkly Rep 2017;66:1148–53. pmid:29073125. https://doi.org/10.15585/mmwr.mm6642a6

Received on May 15, 2019. Accepted on December 2, 2019.

Correspondence: Silvia Bianchi, Department of Biomedical Sciences for Health, University of Milan, via Carlo Pascal 36, 20133 Milan, Italy - Tel. +39 02 50315122 - Fax +39 02 50315120 - E-mail: silvia.bianchi@unimi.it

How to cite this article: Bianchi S, Faccini M, Lamberti A, Senatore S, Ciceri G, Frati ER, Colzani D, Gori M, Cereda D, Gramegna M, Auxilia F, Tanzi E, Amendola A. Measles surveillance activities in the Metropolitan Area of Milan during 2017-2018. J Prev Med Hyg 2019;60:E286-E292. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1269

ORIGINAL ARTICLE

Dangerous passengers: multidrug-resistant bacteria on hands and mobile phones

P.F. MARTINA[1, 2], M. MARTINEZ[3], C.K. CENTENO[2], M. VON SPECHT[1, 3], J. FERRERAS[1, 2]
[1] Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones, Argentina; [2] Instituto de Biología Subtropical (IBS), CONICET-UNaM, CCT-Nordeste, Argentina; [3] Hospital Provincial de Pediatría Dr. F. Barreyro, Posadas, Misiones, Argentina

Keywords

Bacterial contamination • Mobile phones • Hand hygiene • Antibiotic resistance

Summary

Introduction. *It is recognized that mobile phones may play a role in microorganism transmission and that hand hygiene, is considered the most important action for preventing infections and the spread of pathogens. The objective of this study was to determine presence and circulation bacteria on hands and mobile phones capable of causing infections in people and also determine if disinfection with gel-alcohol is useful to reduce the bacterial colonization.*
Methods. *The bacterial evaluation included 596 hands of participants and 256 mobile phones. Isolated colonies were identified by biochemical test and confirmed by gene 16S rRNA sequencing. Antimicrobial susceptibility was performed using the automated instrument Vitek®2-Compact and disk-diffusion-method.*
Results. *In total, 92.9% of mobile phones and 98.3% of participants in study demonstrated evidence of bacterial contamination with different types of bacteria. Surprisingly, we observed that 18.6% plaques inoculated with disinfected fingers showed bacterial growth. In general, Gram negative isolates showed resistance to a higher number of antibiotics tested than Gram positive isolates.*
Conclusions. *Our results could help to raise awareness in our society about the importance of hand hygiene, as well as frequently used devices, reducing bacterial contamination and limiting the possibility of transmission of resistant multi-drug bacteria.*

Introduction

Hand hygiene, is considered the most important habit to prevent infections and the spread of microorganisms pathogens [1]. The common of people often believe that microbes are only present in rubbish and dumps, in research labs, in sick people, in hospitals and clinics and thus they have a misleading feeling of security in other places. Lack of knowledge about where germs occur and how are they transmitted could be the cause of health problems [2]. In fact, microorganisms are found almost everywhere in air, water, soil, food, plants and animals, including humans and may be transmitted, either directly, through hand-to-hand contact, or indirectly via food or other inanimate objects such as cell phones, money and coins [3].
Nowadays, mobile phones have become one of the most essential devices for professional and social life, and they can act as a vehicle for the spread of pathogenic bacteria and other microorganisms [2, 4]. One of the first studies reported that more than 90 % of cell phones of healthcare workers were contaminated with microorganisms and 14 % of them carry pathogenic bacteria that commonly cause nosocomial infections [5]. Predominantly Gram-positive cocci (*Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., *Micrococcus* spp.), but also spore-forming rods (*Bacillus* spp.) or Gram-negative bacteria, can be transmitted through devices like mobile phones or computer keyboards [2, 6].

The purpose of this study was to determine presence and circulation of antibiotic-resistant bacteria on mobile phone and hands capable of causing systemic infections in healthy people and also determine if disinfection with gel-alcohol is useful to reduce the bacterial colonization.

Materials and methods

Study design

This cross-sectional study was conducted in a hand hygiene stand during massive exhibition Tecnopolis Federal, for a period of 2 week (May 2017), at the convention center of Posadas city (Misiones, Argentina). A total of 852 samples were collected from the touch surfaces of mobile phones (256 samples) and ventral surface of finger dominant (single hand) of apparently healthy volunteers (596 samples).
The protocol was approved by the ethical committee of Pediatric Hospital, and an oral informed consent was obtained from the participants or if they were minors, of their legal guardians.

Sample collection

Samples of mobile phones were collected using the fingers previously disinfected with gel-alcohol or sterile swab, were immediately transferred into LB agar plates.

The fingers (two group: with or without disinfection) were supported onto plates for 5-7 seconds. Plates were incubated aerobically at 37°C for 48 h. Bacteria recovered from all plate were pooled and frozen in 20% medium glycerol.

Bacterial identification and antibiotic susceptibility

Colonies obtained in each processed plate were screened by their resistances, using broad spectrum antibiotics-10 µg ampicillin, 4 µg gentamicin and 5 µg chloramphenicol (Britania SA, Argentina) placed separately on Muller–Hinton agar plates and incubated aerobically at 37°C for 18 hours. The number of colony forming units (CFU) for the sample pool was estimated by plaque count.
Isolated microorganisms were identified using Gram stain, colony morphology, standard biochemical tests and confirmed by the automated ID/AST instrument Vitek® 2 Compact (Biomerieux) both Gram positive (GP ID card) and Gram negative (GN ID card) cards (Biomerieux, France) were used. Minimal inhibitory concentration (MIC) was performed using the automated ID/AST instrument Vitek® 2 Compact (Biomerieux) and the Gram positive and Gram negative susceptibility test cards (AST-P577; AST-N117; Biomerieux, France). Diffusion method according to Kirby-Bauer was used for the antibiotics aztreonam, minocycline and levofloxacin. The breakpoints were interpreted following CLSI guidelines [7].

DNA extraction, amplification and sequencing

Culture of each isolate was suspended in sarcosil (0.01%) and DNA extracted using a combination of heating and centrifuged. Universal 16S rRNA bacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') were used to amplify this gene using 10 ng of genomic DNA isolated from each strain. PCR products were purified and sequencing using primers 27F and 1492R [8].

Results

Five hundred thirty-one (98.3%) out of 538 volunteers in study had hands contaminated with bacteria. Also, 238 (92.9%) out of 256 cell phones of volunteers were contaminated with bacteria. Surprisingly, we observed that 18.6% of the 58 plaques inoculated with disinfected fingers showed bacterial growth. Filamentous fungi were observed in 3% of the plates.
Twenty one different appearance colonies were recovered on plates with antibiotic. The organisms identificated with 96-100% probability belonged to seven species of Gram positive bacteria (*Bacillus subtilis, B. pumilus, Lysinibacillus sphaericus, Staphylococcus cohnii ssp urealyticus*, *S. warneri*, *S. saprophyticus* and *Enterococcus durans*) and seven species of Gram negative bacteria (*Serratia marcescens*, *Pseudomonas putida*, *Sphingomonas paucimobilis*, *Acinetobacter baumannii* complex, *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae ssp pneumonia,* and *Ochrobactrum anthropi*).
Results of antibiotic resistance of Gram positive and Gram negative bacterial isolates are listed in Table I (Antibiotic susceptibility results for all tested antibiotics are shown in supplementary data Table SI and Table SII, respectively). A strain *S. cohnii* was resistant to ERI and intermediate to NIT. *S. warneri* was resistant to ERI and GEN. *S. saprophyticus* resistant to OXA, ERI, GEN and STX, plus positive cefoxitin screen. *E. durans* was resistant and intermediate to SXT and NIT, respectively. *Bacillus* sp evidenced intermediate resistance to CLI. In general, Gram negative bacteria exhibit increased resistance to antibiotics. *P. putida* strains were resistant to AMP, SAM, CTX, NAL, NIT, SXT and intermediate to TZP. *S. marcescens* strains were resistant to AMP, SAM, CF, COL and NIT. *S. paucimobilis* was resistant to NAL, GEN and NIT. *A. baumannii* complex was resistant to AMP, CTX, NIT. *S. maltophilia* was resistant to GEN, IPM and AZT. *K. pneumonia* was resistant only to AMP. *O. anthropi* was resistant to AMP, SAM, NAL, NIT and SXT and intermediate to TZP and CTX. All the observed resistances respond to natural mechanisms of resistance.

Discussion

Our study – first of its kind in our region – carried out with aimed to determine presence and circulation of antibiotic-resistant bacteria on mobile phone and hands of healthy people. The most organisms recovered, do not typically cause infections in healthy people rather they have been known to cause significant infections in those with depressed immune systems, including those infected with HIV, patients undergoing cancer chemotherapy, or taking other medications that depress the immune system (transplanted) [9, 10]. However, other belong to species that have been protagonist in both nosocomial and community acquired infections [11, 12].
In recent years, dozens of publications report the presence of microorganisms on money, coins and mobile phones. While the studies can vary, due to the methods used, local community flora, environmental conditions, including the socio-cultural levels of the population, in general, Gram positive bacteria were the most predominant [13]. Staphylococci found in the mucous membranes and normal skin flora has recently got attention as a potential pathogen, specifically for hospital infections where are a major cause of septicemia and bacteremia, especially for the patients who have immune deficiency. Locally, *Staphylococcus aureus* (MRSA) emerged at the Pediatric Hospital, in 2003 as a cause of community-acquired (CA) infections [14]. In several studies, high resistance ratios against erythromycin, gentamicin and trimethoprim-sulfamethoxazole, which is an alternative medicine in the treatment of methicillin-resistant staphylococci infections were reported [15]. Some species of genus *Enterococcus* have currently a particular medical significance, considering their notable ability of acquire and disseminate antimicrobial resistance de-

Tab. I. Minimum inhibitory concentration in bacteria antibiotic resistant recovered in this study.

Species - isolated	OXA	GEN	ERI	NIT	SXT	AMP	SAM	TZP	CF	CTX	CAZ	CEF	IPM	MEM	NAL	COL	AZT
S. cohnii N132			≥ 8	64*													-
S. warneri G62		4	≥ 8														-
S. saprophyticus N4	≥ 4		≥ 8														-
S. saprophyticus N3	≥ 4				≥ 320												-
E. durans G152				64*	10												-
E. durans G153				64*	10												-
S. paucimobilis G61		≥ 16		256											≥ 32		≥ 16
P. *putida* Ch7				≥ 512	80	≥ 32	≥ 32	32*		16					≥ 32		-
P. putida Ch8				≥ 512	80	≥ 32	≥ 32	32*		16					≥ 32		-
A. baumannii Ch16				512		16				8							-
A. baumannii A10B				256		≥ 32		4		8	16						-
S. marcescens M2				256		≥ 32	16		≥ 64							≥ 16	-
S. marcescens A12				256		≥ 32	16		≥ 64							≥ 16	-
S. maltophilia G151		≥ 16											≥ 16	≥ 16			≥ 32
K. pneumoniae M11						2											-
O. anthropi N13				256		≥ 32	≥ 32	≥ 128*		≥ 64*	≥ 64	32					-

OXA, oxalin; GEN, gentamicin; ERI, erythromycin; NIT, nitrofurantoin; SXT, trimethoprim/sulfamethoxazole; AMP, ampicillin, SAM, Ampicillin/Sulbactam; TZP, Piperacillin/Tazobactam; CF, cefalotin; CTX, cefotaxime; CAZ, ceftazidime; CEF, cefepime; IPM, imipenem, MEM, meropenem, NAL, nalidic acid; COL, colistin; AZT, azteronam. (-) no tested;* Intermed according to CLSI [7].

terminants [16]. *Bacillus pumilus* and *B. subtilis* are environmental "non-pathogenic" bacteria that have rarely been associated with clinical infections [17]. *Lysinibacillus sphaericus* (best known as *Bacillus sphaericus*) is first bacteria with insecticidal activity against mosquito larvae were reported in the 1960s [18]. The majority of Bacillus species are susceptible to aminoglycosides, fluoroquinolones, vancomycin, clindamycin and carbapenems while penicillin and cephalosporin susceptibility is variable [19], unlike our isolates (*B. pumilus - A10* and *B. subtilis - A9*) that evidenced intermediate resistance to clindamycin. Previous studies have shown that Bacillus species should be recognized as true pathogens, especially in neonates and other immunosuppressed host [9, 17, 19]. *In vitro* susceptibility testing has shown that strains of *S. paucimobilis* unlike our observed are susceptible to aminoglycosides and quinolones [20, 21]. In dissidence, one-third of *S. paucimobilis* strains recovered cell phone's health worker were resistant to ampicillin, first and second generation cephalosporins, gentamicin and nitrofurantoin. Unfortunately, no antibiotic resistance mechanisms have yet been elucidated [22]. *S. marcescens* is natural sensitivity to aztreonam and naturally resistant to ampicillin, macrolides, and first-generation cephalosporins, expressing chromosomally-encoded AmpC β-lactamases [11]. *O. anthropi* was resistant to all β-lactams which is consistent with the reported expression of an AmpC β-lactamase [23]. *S. maltophilia* is naturally resistant to aminoglycosides, tetracycline, and quinolonas due to the high level of expression of *smeA* or *smeD*. β-lactam resistance is due to the expression of two β-lactamases that hydrolyzes all β-lactams with the exception of aztreonam [24]. Species of Acinetobacter exhibit mechanisms of resistance to all existing antibiotic classes as well as a prodigious capacity to acquire new determinants of resistance [12]. *P. putida* is usually susceptible to fluoroquinolones, aminoglycosides, monobactams, and extended-spectrum cephalosporins. However, acquisittion metallo- β-lactamases that confer resistance to most β-lactams, including carbapenems, have been reported [25].

Our results revealed permanent colonization of bacteria on the mobile phones, which are very close to the hand of users. For this fact, contaminated phones can play a potential role in the spread of drug-resistant bacteria into the community. Food manipulators have been implicated in various outbreaks of food-borne diseases and human occupational activities could introduce the risk of food contamination. Food manipulators (workers) can be infected by pathogens from multiple sources and them in turn become potential sources of contamination in food processing and preparation facilities [26]. Equally important is way as parents tend to use their mobile phones at the bedside to communicate while touching, change diapers or holding their baby, increasing the risk of transmission [27].

Disinfection with gel-alcohol was effective in reducing bacterial colonization [4], however, it does not ensure complete disinfection, according to our results. We speculate the more likely explanation is related to the

inadequate application of gel-alcohol. Developing active preventive strategies like routine decontamination of mobile phones with gel-alcohol containing disinfectant materials might reduce cross-infection. Another way of reducing bacterial contaminations on mobile phones might be the use of antimicrobial additive materials, today available for medical applications [28]. We could easily avoid dispersion of bacterial infections just by using regular cleaning agents, such as gel-alcohol and rearranging our habits. This could include educating to children in schools and parents on the risk that a contaminated mobile phone poses for their baby, mobile phone hygiene and proper hand antimicrobial-gel application before and after mobile phone usage at the baby's bedside [27]. In the future mobile phones and devices could be produced by using protective material against the microbial contamination [29].

This activity was novel, Tecnopolis exhibition gave us the opportunity to interact with the community – especially students of initial and middle school levels to show the microscopic world we carry with itself. Highlighted the occurrence of pathogens bacteria on objects outside the health care environment in order to and raise awareness people on the necessity of improving the habit of washing their hands and employ appropriate disinfection methods to tactile electronic device in order to reduce microbial transmission. However, study presents some limitations. First of all we conducted a descriptive analysis, during an optional intervention, of sample obtained from non-probabilistic sampling thus no generalization of the results can be proposed. Questions to participants regarding the level of knowledge about microorganisms were not properly registered. Furthermore, the design not explore socio-demographic characteristics of the study sample. In future research, these variables should be considered for a more complete analysis of the thematic which allows identifying the determinants of health acting in hand washing adherence of the community to a more effectiveness intervention on specific population groups.

Conclusion

The present study shows that the mobile phones and hands of people even without symptoms of disease harbored a variety of pathogenic organisms with resistance to some of the therapeutic antibiotics used which can cause serious diseases. Our results could help to raise awareness in our society about the importance of hand hygiene and frequently used devices, decreasing bacterial contamination and limiting the transmission of pathogens.

Acknowledgements

The authors are grateful to the fellow-staff of Research Group in Applied Genetics (GIGA).

Funding sources: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

PFM and JF designed and coordinated the design and data acquisition. CKC, PFM and JF collected, analyzed and interpreted the results. MM and MVS were responsible for the microbiology analysis. PFM and MM drafted the manuscript. All authors read and approved the manuscript.

References

[1] Pittet D, Allegranzi B, Sax H, Dharan S, Pessoa-Silva CL, Donaldson L, Boyce J M. Evidence-based model for hand transmission during patient care and the role of improved practices. Lancet Infect Dis 2006;6:641-52. https://doi.org/10.1016/S1473-3099(06)70600-4

[2] Koscova J, Hurnikova Z, Pistl J. Degree of bacterial contamination of mobile phone and computer keyboard surfaces and efficacy of disinfection with chlorhexidine digluconate and triclosan to its reduction. Public Health 2018;15:2238. https://doi.org/10.3390/ijerph15102238

[3] Angelakis E, Azhar EI, Bibi F, Al-ghamdi AK, Ashshi AM, Elshemi AG. Paper money and coins as potential vectors of transmissible disease. Most 2014;9:249-61. https://doi.org/10.2217/fmb.13.161

[4] Brady R, Fraser S, Dunlop M, Paterson-Brown S, Gibb A. Bacterial contamination of mobile communication devices in the operative environment. J Hosp Infect 2007;66:397-8. https://doi.org/10.1016/j.jhin.2007.04.015

[5] Zakai S, Mashat A, Abumohssin A. Bacterial contamination of cell phones of medical students at King Abdulaziz University, Jeddah, Saudi Arabia. J Microsc Ultrastruc 2016;4:143-6. https://doi.org/10.1016/j.jmau.2015.12.004

[6] Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis 2006;6:1-8. https://doi.org/10.1186/1471-2334-6-130

[7] CLSI. Clinical and Laboratory Standards Institute 2018. Performance standards for antimicrobial susceptibility testing, Twenty-Fourth Informational Supplement, CLSI document M100-S28. Wayne; 2018:230.

[8] Srinivasan R, Karaoz U, Volegova M, MacKichan J, Kato-Maeda M, Miller S, Nadarajan R, Brodie EL, Lynch SV. Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. PloS One 2015;10:e0117617. https://doi.org/10.1371/journal.pone.0117617

[9] Castagnola E, Fioredda F, Barretta MA, Pescetto L, Garaventa A, Lanino E, Micalizzi C, Giacchino R, Dini G. Bacillus sphaericus bacteraemia in children with cancer: case reports and literature review. J Hosp Infect 2001;48:142-5. https://doi.org/10.1053/jhin.2001.0995

[10] Lamichhane J, Adhikary S, Gautam P, Maharjan R, Dhakal B. Risk of handling paper currency in circulation chances of potential bacterial transmittance. J Sci Techn 2009;10:161-6. https://doi.org/org/10.3126/njst.v10i0.2952

[11] Stock I, Grueger T, Wiedemann B. Natural antibiotic susceptibility of strains of Serratia marcescens and the S. liquefaciens complex: S. liquefaciens sensu stricto, S. proteamaculans and S. grimesii. Int J Antimicrob Agents 2013;22: 5-47. https://doi.org/10.1016/s0924-8579(02)00163-2

[12] Perez F, Hujer A, Hujer K, Decker B, Rather P, Bonomo R. Global challenge of multidrug-resistant Acinetobacter baumannii. Antimicrob Agents Chemother 2007;51:3471-84. https://doi.org/10.1128/AAC.01464-06

[13] Gedik H, Voss TA, Voss A. Money and transmission of bacteria Money and transmission of bacteria. Infect Control 2013;2:22. https://doi.org/10.1186/2047-2994-2-22

[14] Von Specht MH, Gardella N, Ubeda C, Grenon S, Gutkind G, Mollerach M. Community-associated methicillin-resistant Staphylococcus aureus skin and soft tissue infections in a pediatric hospital in Argentina. J Infect Dev Ctries 2014;8:1119-28. https://doi.org/10.3855/jidc.4271

[15] Koksal FÃ, Yasar H, Samasti M. Antibiotic resistance patterns of coagulase-negative staphylococcus strains isolated from blood cultures of septicemic patients in Turkey. Microbiol Res 2009;164:404-10. https://doi.org/10.1016/j.micres.2007.03.004

[16] Medeiros AW, Pereira RI, Oliveira DV, Martins PD, d'Azevedo PA, Van der Sand S, Frazzon J, Frazzon APG. Molecular detection of virulence factors among food and clinical *Enterococcus faecalis* strains in south Brazil. Braz J Microbiol 2014;332:327-32. https://doi.org/10.1590/S1517-83822014005000031

[17] Kimouli M, Vrioni G, Papadopoulou M, Koumaki V, Petropoulou D, Gounaris A, Friedrich AW, Tsakris A. Case report. Two cases of severe sepsis caused by Bacillus pumilus in neonatal infants. J Med Microbiol 2012;61:596-9. https://doi.org/10.1099/jmm.0.033175-0

[18] Berry C. The bacterium, Lysinibacillus sphaericus, as an insect pathogen. J Invert Pathol 2012;102:1-10. https://doi.org/10.1016/j.jip.2011.11.008

[19] Wenzler E, Kamboj K, Balada-Llasat JM. Severe sepsis secondary to persistent Lysinibacillus sphaericus, Lysinibacillus fusiformis and Paenibacillus amylolyticus Bacteremia. Int J Infect Dis 2015;35:93-5. https://doi.org/10.1016/j.ijid.2015.04.016

[20] Nandy S, Dudeja M, Das AK, Tiwari R. Community acquired bacteremia by Sphingomonas paucimobilis: two rare case reports. J Clin Diagn Res 2013;7:2947-9. https://doi.org/10.7860/JCDR/2013/6459.3802

[21] Benevides GN, Hein N, Lo DS, Esposito Ferronato A, Lopes Betta Ragazzi S, Ryoka Miyao Yoshioka C, Hirose M, Morais Cardoso D, dos Santos SR, Gilio AE. Otomastoiditis caused by Sphingomonas paucimobilis: case report and literature review. Autops Case Rep 2014;4:13-20. https://doi.org/10.4322/acr.2014.024

[22] Kumar BV, Hobani YH, Abdulhaq A, Jerah AA, Hakami OM, Eltigani M, Bidwai AK. Prevalence of antibacterial resistant bacterial contaminants from mobile phones of hospital inpatients. Libyan J Med (Cincinnati) 2014;1:1-4. https://doi.org/10.3402/ljm.v9.25451.

[23] Higgins CS, Murtough SM, Williamson E, Hiom SJ, Payne DJ, Russell AD, Walsh TR. Resistance to antibiotics and biocides among non-fermenting Gram-negative bacteria. Clin Microbiol Infect 2001;7:308-15. https://doi.org/10.1046/j.1198-743x.2001.00253.x

[24] Mojica MF, Papp-wallace KM, Taracila MA, Barnes MD, Rutter JD, Jacobs MR, LiPuma JJ, Walsh TJ, Vila AJ, Bonomo RA. Avibactam restores the susceptibility of clinical isolates of Stenotrophomonas. Antimicrob Agents Chemother 2017;61:13-7. https://doi.org/10.1128/AAC.00777-17

[25] Bhattacharya D, Dey S, Kadam S, Kalal S, Jali S, Koley H, Sinha R, Nag D, Kholkute SD, Roy S. Isolation of NDM-1-producing multidrug-resistant Pseudomonas putida from a paediatric case of acute gastroenteritis, India. N Microb and N Infect 2015;5:5-9. https://doi.org/10.1016/j.nmni.2015.02.002

[26] Todd E, Greig J, Bartleson C, Michaels B. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 6. Transmission and survival of pathogens in the food processing and preparation environment. J Food Prot 2009;72:202-19. https://doi.org/10.4315/0362-028x-72.1.202

[27] Beckstrom AC, Cleman PE, Cassis-ghavami FL, Kamitsuka MD. Surveillance study of bacterial contamination of the parent's cell phone in the NICU and the effectiveness of an antimicrobial gel in reducing transmission to the hands. J Perinatol 2013;33:960-3. https://doi.org/10.1038/jp.2013.108

[28] Polívková M, Hubáček T, Staszek M, Švorčík V, Siegel J. Antimicrobial treatment of polymeric medical devices by silver nanomaterials and related technology. Int J Mol Sci 2017;18:419. https://doi.org/10.3390/ijms18020419

[29] Ulger F, Esen S, Dilek A, Yanik K, Gunaydin M, Leblebicioglu H. Are we aware how contaminated our mobile phones with nosocomial pathogens? Ann Clin Microbiol Antimicrob 2009;4:4-7. https://doi.org/10.1186/1476-0711-8-7

Received on May 15, 2019. Accepted on October 24, 2019.

Correspondence: P.F. Martina, Jujuy 1745, Posadas (3300), Misiones, Argentina - Tel. +54 376 444 0967- E-mail: pfmartina@hotmail.com

How to cite this article: Martina PF, Martinez M, Centeno CK, Von Specht M, Ferreras J. Dangerous passengers: multidrug-resistant bacteria on hands and mobile phones. J Prev Med Hyg 2019;60:E293-E299. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1283

Tab. SI. Determination of minimal inhibition concentration in Gram positive bacteria recovered in this study.

Antibiotic	*S. cohnii* N132		*S. cohnii* N13M		*S. warneri* G62		*S. saprophyticus* N3		*S. saprophyticus* N4		*E. durans* G152		*E. durans* G153		*B. pumilus* A10		*B. subtilis* A9		*L. sphaericus* Ci14		*L. sphaericus* Ci5	
Oxacilin	0.5	S	0.5	S	0.25	S	4	R	4	R	2	S	2	S	-	-	-	-	-	-	-	-
Gentamicin	0.5	S	0.5	S	4	R	0.5	S	0.5	S	-	-	-	-	-	-	-	-	-	-	-	-
Ciprofloxacin	0.5	S	0.5	S	0.5	S	0.5	S	0.5	S	-	-	-	-	-	-	-	-	-	-	-	-
Levofloxacin	1	S	0.5	S	0.12	S	0.5	S	1	S	0.5	S	0.5	S	0.5	S	0.5	S	0.5	S	0.5	S
Moxifloxacin	0.25	S	0.25	S	0.25	S	0.25	S	0.25	S	0.5	S	0.5	S	-	-	-	-	-	-	-	-
Erythromycin	≥ 8	R	0.5	S	8	R	0.5	S	8	R	0.25	S	0.25	S	-	-	-	-	-	-	-	-
Clindamycin	0.25	S	0.25	S	0.25	S	0.25	S	0.25	S	-	-	-	-	2	I	1	I	0.5	S	-	-
Quinupristin/ Dalfopristin	1	S	0.5	S	0.5	S	1	S	0.5	S	1	S	1	S	-	-	-	-	-	-	-	-
Linezolid	4	S	2	S	2	S	4	S	4	S	2	S	2	S	-	-	-	-	-	-	-	-
Teicoplanin	4	S	4	S	0.5	S	4	S	0.5	S	0.5	S	0.5	S	-	-	-	-	-	-	-	-
Vancomycin	1	S	1	S	1	S	1	S	1	S	0.5	S	0.5	S	0.25	S	0.25	S	0.25	S	1	S
Minocycline	0.5	S	0.5	S	0.5	S	0.5	S	0.5	S	0.5	S	0.5	S	-	-	-	-	-	-	-	-
Tetracycline	2	S	1	S	1	S	1	S	1	S	1	S	1	S	-	-	-	-	-	-	-	-
Nitrofurantoin	64	I	32	S	16	S	16	S	16	S	64	I	64	I	-	-	-	-	-	-	-	-
Rifampicin	0.5	S	0.5	S	0.5	S	0.5	S	0.5	S	-	-	-	-	-	-	-	-	-	-	-	-
Trimethoprim/ Sulfamethoxazole	10	S	10	S	10	S	320	R	10	S	10	R	10	R	-	-	-	-	-	-	-	-

(-) no tested. S, sencible. I, intermed. R, resistant according to CLSI [7].

Tab. SII. Determination of minimal inhibition concentration in Gram negative bacteria recovered in this study.

Antibiotic	*S. paucimobilis* G61		*P. putida* Ch7		*P. putida* Ch8		*A. baumannii* Ch16		*A. baumannii* A10B		*S. marcescens* M2		*S. marcescens* A12		*S. maltophilia* G151		*K. pneumoniae* M11		*O. anthropi* N13	
Ampicillin	2	S	≥ 32	R	≥ 32	R	16	R	16	R	32	R	32	R	-	-	2	R	≥ 32	R
Ampicillin/ Sulbactam	2	S	≥ 32	R	≥ 32	R	2	S	2	S	16	R	16	R	-	-	2	S	≥ 32	R
Piperacillin/ Tazobactam	4	S	32	I	32	I	4	S	4	S	4	S	4	S	-	-	4	S	≥ 128	I
Cefalotin											64	R	64	R	-	-	2	S		
Cefotaxime	1	S	16	R	16	R	8	R	8	R	1	S	1	S	-	-	1	S	≥ 64	I
Ceftazidime			4	S	4	S	4	S	4	S	1	S	1	S	4	S	1	S	≥ 64	S
Cefepime	4	S	1	S	1	S	2	S	2	S	1	S	1	S	-	-	1	S	32	S
Imipenem			1	S	1	S	0.25	S	0.25	S	0.5	S	0.5	S	≥ 16	R	0.25	S	1	S
Meropenem	4	S	1	S	1	S	0.25	S	0.25	S	0.25	S	0.25	S	≥ 16	R	0.25	S	1	S
Amikacim	2	S	2	S	2	S	2	S	2	S	2	S	2	S			2	S	16	S
Gentamicin	16	R	1	S	1	S	1	S	1	S	1	S	1	S	≥ 16	R	1	S	2	S
Nalidixic acid	32	R	≥ 32	R	≥ 32	R	2	S	2	S	2	S	2	S	-	-	2	S	4	R
Ciprofloxacin	1	S	0.25	S	0.25	S	0.25	S	0.25	S	0.25	S	0.25	S	-	-	0.25	S	0.25	S
Nitrofurantoin	256	R	512	R	512	R	512	R	512	R	256	R	128	R	-	-	32	S	256	R
Colistin	-	-	0.5	S	0.5	S	0.5	S	0.5	S	16	R	16	R	-	-	0.5	S		
Trimethoprim/ Sulfamethoxazole	-	-	80	R	80	R	20	S	20	S	20	S	20	S	20	S	20	S	≤ 20	S
Levofloxacin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	S	-	-	-	-
Minocycline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	≤ 4	S	-	-	-	-
Aztreonam	≥ 16	R	-	-	-	-	-	-	-	-	-	-	-	-	≥ 32	R	-	-	-	-

(-) no tested; ( ) no detected. S, sensitive. I, intermed. R, resistent according to CLSI [7].

ORIGINAL ARTICLE

Regional indices of socio-economic and health inequalities: a tool for public health programming

R. LILLINI[1], G. MASANOTTI[2], F. BIANCONI[2], A. GILI[2], F. STRACCI[2], F. LA ROSA[2], M. VERCELLI[3]
[1] Department of Health Sciences, University of Genoa. Analytical Epidemiology and Health Impact Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan; [2] Section of Public Health, Department of Experimental Medicine, University of Perugia, Italy; [3] Department of Health Sciences, University of Genova, Italy

Keywords

Public health • Socio-economic status • Socio-economic indices • Inequalities in health • Health care resources

Summary

Objectives. *The aim was to provide an affordable method of computing socio-economic (SE) deprivation indices at the regional level, in order to reveal the specific aspects of the relationship between SE inequalities and health outcomes. The Umbria Region Socio-Health Index (USHI) was computed and compared with the Italian National Deprivation Index at the Umbria regional level (NDI-U).*

Methods. *The USHI was computed by applying factor analysis to census tract SE variables correlated with general mortality and validated through comparison with the NDI-U.*

Results. *Overall mortality presented linear positive trends in USHI, while trends in NDI-U proved non-linear or non-significant. Similar results were obtained with regard to specific causes of death according to deprivation groups, gender and age.*

Conclusions. *The USHI better describes a local population in terms of health-related SE status. Policy-makers could therefore adopt this method in order to obtain a better picture of SE-associated health conditions in regional populations and to target strategies for reducing health inequalities.*

Introduction

Over the last fifty years, most countries have investigated the relationships between socio-economic (SE) status and inequalities in the utilisation and distribution of healthcare resources and patient outcomes [1-6]. These studies have been carried out at the national or individual level, and have examined the relationships between the distribution of demographic characteristics (gender and age), SE factors (income and occupation), cultural factors (educational level), living conditions (marital status, household composition, domestic overcrowding and tenure, etc.) and health outcomes in areas ranging from the macro to the micro level [5, 6]. Indeed, an SE classification that takes the patient's neighbourhood into account provides a useful starting point in describing and improving the effectiveness of local public health interventions [4, 6].

The definition of "neighbourhood" is debated in the literature, the most common being that of the smallest official administrative area [5-9], usually the census tract (CT), which approximates the SE and health features of the area to the resident individuals' characteristics.

This choice is justified by the aim of such studies, which is to accurately assess the feasibility of providing preventive, diagnostic and therapeutic services targeted to individuals who live in a specific area.

Most of these studies have utilised indices of SE deprivation that were computed for the whole nation [6, 7]. Moreover, it is noteworthy that such indices were commonly constructed in order to describe the distribution of the population with respect to SE characteristics, but not to show the specific effects of SE deprivation on deprivation-related health outcomes.

This methodological choice raises some critical problems. Firstly, these indices are often not sufficiently related to overall mortality, the main and most commonly used health indicator. Worldwide, overall mortality is related to material and social differences in the population and to inequalities in the distribution of public and private health resources [10]. Computed according to this "pure" definition of SE deprivation, the usual deprivation indices do not consider whether their constituent variables influence health status [6, 7]. They therefore risk neglecting to evaluate differences in the local allocation of resources in response to health needs. These differences can be particularly marked in countries with large differences in national and regional demographics and SE status, causing considerable disparities in health outcomes [5].

Significant examples of such situations can be found in Italy, a country where population density varies from region to region, ranging from 39 to 429 inhabitants per square km. Moreover, the various regions differ in terms of the rate of population ageing, proportion of the population that is active, birth rate, family size, and labour market characteristics, particularly from North to South [11]. In addition, the orographic characteristics of the territory in the various regions impacts on the internal distribution of goods and wealth. In brief, the economy of Northern Italy is similar to, and connected with, that of Central Europe, while the Central and Southern

https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1257

regions of Italy are penalised by their poor connection with the heart of Europe.
It is also necessary to consider how public financing is distributed. With few exceptions, funds flow from the central government to the single regional authorities, which decide how they should be allocated and determine the amount and distribution of resources devoted to socio-health policies (social support, preventive measures, etc.) [11-14].
In countries with such characteristics, all these aspects lead to health inequalities that are specific to each region, and particularly to sub-areas where population density is lower [5]. These inequalities can be accurately described and analysed only by means of indicators that are constructed at the regional level and which can take local peculiarities into account [5-7]. Such indicators are computed on the basis of health-related local demographic and SE indicators [9, 12]. These indices should be called Indices of SE and Health Inequalities (SHI), rather than Deprivation Indices, as they describe the population distribution not only in terms of mere SE inequalities, but also according to people's needs for health support.
The present study aimed to describe, discuss and validate the method and the technique for computing this kind of index, which could be applied in every nation affected by marked regional differences. The Umbria region was chosen as an example of the application of these procedures, which are derived from a previous successful attempt in another Italian region (Liguria) [8, 9, 12]. Moreover, this study assessed the ability of the Umbria regional index (USHI) to efficiently classify population subgroups in Umbria on the basis of a combination of health fragility and SE differences related to health outcomes, in comparison with the Italian National Deprivation Index (NDI) computed at the level of the Umbria region (NDI-U), which distinguishes populations only on the basis of SE status [7-9, 12].

Methods

The NDI is the benchmark for validating local indices (USHI in this study). As the NDI based on the 2011 National Census data is not yet available, we used local SE and mortality data from around 2001 (the date of the latest available NDI) in order to compute the local index (USHI), and mortality data from the period 2005-2012 to analyse the performance of the two indices.
We used 543 variables taken from the 2001 Italian Census in order to compute the two indices at the CT level; these variables describe features of individuals (age, marital status, educational level, employment, etc.), families (number of family members, single parents, average age of families, etc.) and households (ownership, over-crowding, housing conditions, services available, etc.).
The NDI considered 280 of these variables, covering five conditions which described the multidimensional concept of social and material deprivation (persons with only primary education, unemployed or searching for first employment, one-parent families and dependent children living together, rented accommodation, domestic overcrowding) [7]. The NDI was computed at the CT level as the sum of these five indicators in standardised form, grouped in population quintiles at the national level [7]. In the present study, we used a regional version of the NDI (NDI-U), categorised in quintiles of the Umbrian population.
To construct the USHI, we adopted the same method used to compute the Liguria Socio-economic and Health Inequalities Index (LSHI) [8]. Pearson's bivariate correlation ($p < 0.05$) was calculated between each of the 543 basic variables and the synthetic SE indices (employment/unemployment rates, ageing index, dependence rate, etc.) and general mortality in Umbria in 2001-2004. Significantly correlated variables were picked out and a tolerance test ($p < 0.05$) was applied to these in order to reduce collinearity [15]. From the nine variables which emerged after these steps, a principal-component analysis extracted three factors. These defined the latent structure connecting the SE variables that were able to synthetically describe the health-related SE characteristics of the population. The three factors underwent a varimax rotation, in order to render them orthogonal, and thus independent. These three independent factors were linearly combined into a single quantitative variable, the values of which were re-scaled as a percentage in order to obtain the USHI at the CT level [16] (Tab. I). Subsequently this variable was aggregated, both for the purpose of its validation and to obtain a municipality index, based on the CTs in each municipality (Tab. SI). This operation was necessary because the population of the Umbria region is small (825,796 inhabitants); therefore, a higher level of aggregation (municipality) than the CT was required in order to analyse the effects of deprivation on mortality according to the causes of death. The population of the largest municipalities (above 55,000 residents) were split into districts, on the basis of CT proximity, in order to create geographic areas with populations similar in size to those of other municipalities in the region.

Tab. I. Composition of the factors making up the USHI.

Total explained variance = 71.0%		
Factor 1= 30.7%	**Factor 2= 23.0%**	**Factor 3 = 18.3%**
% of owned houses	Youth employment rate	% of singles
% of houses with independent heating system	% of high school diplomas and university degrees	Employment rate
Number of persons in the family	Average age of 3-person families	
	% of people born in the municipality of residence	

Finally, in order to obtain a normal distribution of the population across the deprivation clusters in the final USHI classification [9, 17], a cluster discriminant analysis, based on the algorithm of Agnelli et al. [17], was applied on aggregating municipalities and districts. The level of normalization was tested at $p < 0.05$ statistical significance.

The Umbria Regional Mortality Registry was the source of the 5-year general mortality (2001-2004) data used in selecting the variables pertaining to the USHI. The same Registry also provided the data on cause-specific mortality by age-group and gender (2005-2012), which were utilised to validate the USHI and compare its performances with those of the NDI-U.

The mortality features included in the present study were: the overall mortality rate (ICD-10th: A00-Y89) and the rates of mortality due to diabetes mellitus (E10-E14), circulatory system (I00-I99), respiratory (J00-J99) and digestive (K00-K93) diseases in the period 2005-2012, by age-group (all ages, 0-64 and 65+ years old) and gender.

The Standard Mortality Ratio (SMR) of each group identified by the USHI and the NDI-U was computed against the overall regional rate, by age-group, gender and cause.

SMR variance was analysed with regard to the specific causes of death, in order to detect linear (L) or non-linear (NL) significant relationships with the deprivation groups. Significance was tested by means of the F-test ($p < 0.05$). Analyses were performed by means of SPSS 19.0 and Stata 12.0 statistical packages.

Results

Table II displays the size of the Umbrian population and the percentages of this population in each group identified by the NDI-U and USHI; it also shows trend comparisons of some synthetic SE indices (replacement, age, structural dependence, activity, employment and unemployment). The groups were labelled from 1 to 5, on the basis of decreasing SE deprivation according to the NDI-U and decreasing socio-health-economic (SHE) deprivation according to the USHI (i.e., 1 = most deprived; 5 = least deprived).

Each of the five NDI-U deprivation groups comprised approximately one-fifth of the Umbrian population, according to the NDI computing techniques. The small differences from perfect quintiles were due to the sizes of the CT populations (obviously, the CTs cannot be divided).

In each USHI deprivation group, the population size was normally distributed, being larger in the central groups and smaller in the tails.

With respect to USHI distribution, all synthetic indices showed linear (L) trends that were consistent with SHE deprivation. Positive L trends (↑, increasing on increasing deprivation) were seen with regard to replacement, ageing, structural and unemployment indices, while

Tab. II. Population size and percentage of total population (825,796 inhabitants) of SE deprivation groups identified by the NDI-U and USHI. Comparison of trends between distributions of some synthetic SES indices (ISTAT) in the NDI-U and USHI population groups.

SE deprivation groups		1	2	3	4	5	Trend
NDI-U	N° of residents (%)	156,473 (19.0%)	175,700 (21.3%)	176,965 (21.4%)	157,574 (19.1%)	159,084 (19.3%)	
	Replacement Index	147.3	132.4	141.8	150.2	151.5	n.s.
	Ageing Index	246.7	198.9	203.4	209.2	229.5	n.s.
	Structural dependence Index	59.2	57.7	58.1	57.9	61.4	n.s.
	Activity Index	63.8	64.9	64.6	64.3	64.0	n.s.
	Employment index	57.6	59.0	58.2	57.7	57.1	n.s.
	Unemployment index	9.2	8.4	9.3	9.7	10.2	$p < 0.05$ NL
SHE deprivation groups		**1**	**2**	**3**	**4**	**5**	**Trend**
USHI	N° of residents (%)	162,196 (19.6%)	176,275 (21.3%)	188,458 (22.8%)	163,401 (19.8%)	135,466 (16.4%)	
	Replacement Index	177.4	149.7	139.5	132.9	127.9	$p < 0.05$ L↑
	Ageing Index	332.2	217.3	209.6	182.4	168.6	$p < 0.05$ L↑
	Structural dependence Index	68.0	59.8	58.8	54.8	53.5	$p < 0.05$ L↑
	Activity Index	61.4	62.8	63.9	66.2	67.4	$p < 0.05$ L↓
	Employment index	54.5	55.9	57.6	59.8	61.9	$p < 0.05$ L↓
	Unemployment index	10.7	10.4	9.3	9.0	7.6	$p < 0.05$ L↑

SE group labels indicate decreasing SE deprivation from 1 = most deprived to 5 = least deprived; SHE group labels indicate decreasing SHE deprivation from 1 = most deprived to 5 = least deprived. L = linear trend; NL = non-linear trend; n.s. = non-significant trend; ↑ = positive trend (increasing with deprivation); ↓ = negative trend (decreasing with deprivation).

activity and employment indices displayed negative L trends (↓, decreasing on increasing deprivation).
In the NDI-U, no significant (NS) correlation was found, except for the unemployment index, which showed a NL relationship.
USHI overall mortality trends (Tab. III) showed L↑ trends in males and females, while NDI-U trends were NL in men and NS in women. Concerning age, the USHI trend was L↑ in the younger age-groups and in older females, but NL in older males. NDI-U age-related trends were NL among males in both age-groups and NS among females.
The distribution of the main causes of death, by SE (NDI-U) and SHE (USHI) groups, is shown in Table IV. The USHI trends in diabetes-related deaths were L↑ in women and NL in men, while the NDI-U trends were NS. By age-group, the USHI trends were L↑ only in the elderly, being NS in the young. The NDI-U trends were NS in males in both age-groups, and NL in younger women.
Regarding circulatory system diseases, USHI trends were L↓ in men and NL in women, While NDI-U trends were NL in men and NS in women. Concerning age-groups, USHI trends were L↑ in younger men, L↓ in older men and NL in both female age-groups. NDI-U displayed NL trends in males and NS in females.
Respiratory system diseases showed NL USHI trends in both sexes, while NDI-U trends were NS. Age-related USHI trends were NL in both groups of men and NS in younger women. All the age-related NDI-U trends were NS.
Finally, with regard to diseases of the digestive system, USHI trends were L↑, while NDI-U trends were NS in men and NL in women. When linked to age, USHI trends were L↑ in younger men and older women, NL in older men and NS in younger women. NDI-U trends were NL in older subjects and NS in the younger groups.

Discussion

Tables II and III show very marked differences between the two indices in terms of their relationships with the synthetic SE indicators (Tab. II) and the distribution of overall mortality across the SE groups of population (Tab. III). The NDI-U displayed only a weak correlation with mortality (the health indicator), confirming the findings at the national level [7]; moreover, correlations with the SE indicators were either non-significant or non-linear. These results confirmed those of other studies, particularly the Liguria study [12] and a national one, involving 10 other Italian regions [18, 19].
The NDI is a commonly accepted benchmark at the national level. However, if the same procedures are applied at the local level in order to obtain a local version of this index, and if the same variables and population seg-

Tab. III. 2005-2012 ove rall mortality in Umbria by gender, age and deprivation groups identified by NDI-U and USHI: Standard Mortality Ratios (SMR), cases and trend significance.

Indexes	Age groups	Indicator	MEN							WOMEN						
			1	2	3	4	5	Umbria	Trend	1	2	3	4	5	Umbria	Trend
NDI-U	All ages	SMR	97.9	96.7	98.0	103.4	99.9	99.1	P <0.05 NL	99.6	96.5	97.8	101.1	100.5	99.0	n.s.
		OBS	7245	8389	7919	7446	7764	38763		7685	8445	8202	7620	7941	39893	
	0-64 yrs	SMR	95.0	95.5	98.1	102.2	104.6	99.0	P < 0.05 NL	103.7	90.7	100.2	98.5	99.9	98.5	n.s.
		OBS	968	1082	1129	1088	1033	5300		590	566	649	579	543	2927	
	65+ yrs	SMR	98.3	96.8	98.0	103.7	99.2	99.1	P < 0.05 NL	99.3	96.9	97.5	101.4	100.5	99.1	n.s.
		OBS	6277	7307	6790	6358	6731	33463		7095	7879	7553	7041	7398	36966	
USHI	All ages	SMR	102.7	101.9	99.8	99.1	98.7	99.1	P < 0.05 L↑	103.1	101.7	99.6	99.5	97.6	99.0	P < 0.05 L↑
		OBS	7998	9011	8386	7244	6124	38763		8669	9359	8680	7364	5821	39893	
	0-64 yrs	SMR	108.8	105.4	96.6	95.0	95.6	99.0	P < 0.05 L↑	105.7	100.5	93.7	94.7	93.0	99.5	P < 0.05 L↑
		OBS	1062	1195	1143	1013	887	5300		641	623	618	551	494	2927	
	65+ yrs	SMR	99.4	101.4	94.9	99.8	100.4	99.1	P < 0.05 NL	101.3	101.8	99.9	99.9	97.6	99.1	P < 0.05 L↑
		OBS	6936	7816	7243	6231	5237	33463		8028	8736	8062	6813	5327	36966	

SE group labels indicate decreasing SE deprivation from 1 = most deprived to 5 = least deprived; SHE group labels indicate decreasing SHE deprivation from 1 = most deprived to 5 = least deprived. L = linear trend; NL = non-linear trend; n.s. = non-significant trend; ↑ = positive trend (increasing from 1 to 5 group); ↓ = negative trend (decreasing from 1 to 5 group).

mentation (quintiles) are used, its ability to distinguish population groups in terms of SE and health differences seems to be weakened.

Although the NDI-U groups were formed by quintiles, SE phenomena more frequently display a normal distribution (as do many other phenomena: e.g., many health-related indicators) [20, 21]. Thus, the USHI was constructed in accordance with a normal distribution of the population in clusters, in order to maximise the probability of relationships with SE characteristics. The validity and reliability of this methodological choice are demonstrated by the linear correlations that the synthetic SE indicators (replacement, ageing, dependence rate, activity, and employment) showed (linear correlations in USHI, but not in NDI-U).

Furthermore, only USHI trends in overall mortality almost always confirmed other reports [1-4, 22]. USHI age-trends illustrated the effects of inequalities on overall mortality, revealing that SMRs increased with SHE deprivation in both female age-groups and in younger males. The NDI-U failed to draw out this information or to identify the well-known relationship between SE deprivation and the major causes of death explored in this study (Tab. IV).

USHI trends depicted female-related advantages (e.g., greater attention to prevention) and disadvantages (e.g., greater ageing and disability) [23-25], suggesting a strong relationship with confounding factors in older men, such as deleterious habits and occupational risks.

Regarding specific causes of death (Tab. IV), the associations observed in the younger age-groups were interesting, in that the low frequency of competitive diseases made it easier to identify determinants of risk, and also SE-linked factors. Indeed, younger age-groups tend to be more receptive to campaigns for the prevention and early diagnosis of diseases. Such campaigns facilitate a timely diagnosis and are associated with more efficacious treatments and better care and outcomes, though their effects may differ across SHE clusters [14, 23, 24]. Their effects may differ in the intensity of exposure to risk factors (such as occupational exposure in older men) or to differences in implementing preventive or diagnostic/therapeutic strategies. For instance, women are known to be more likely to display beneficial behavioural patterns, such as adopting healthy dietary habits and adhering to early prevention [23, 24]. However, this predisposition is mostly culturally mediated, being greater in the less deprived than in the more deprived [25].

With regard to the main diseases, the trends which emerged from the present study mainly confirmed the findings from other studies. The more lethal diseases, for which less efficacious preventive and therapeutic options are available, showed a more homogeneous distribution of mortality among the population clusters, because, although exposure to risk factors was not similar in all individuals, care opportunities were limited in the same way for all. Conversely, when preventive and therapeutic options are available, mortality rates differ among clusters of population at different SHE deprivation levels [12, 26, 27]. Specifically, the literature indicates that ageing-linked social challenges and poor healthcare are mediated by SE differences, and that they are worse in one-person families, particularly in the elderly [26, 27].

The growing prevalence of diabetes in populations with a western life-style [28-31] has shown robust positive associations with SE deprivation in both males and females [28]. The main risk factors, i.e. overweight or obesity and inheritance of the disease from parents, suggest a common environment or gene-environment interaction and SE deprivation. These factors, however, can be partially counterbalanced by better education and the adoption of a healthier lifestyle). Moreover, diabetes is reported to increase the individual's vulnerability to airborne particles emitted by the combustion of hydrocarbons, and an inverse relationship has emerged between air pollution and nitro-glycerin-mediated reactivity in older people [29, 30]. These detrimental effects might affect the population differentially across SHE groups, as suggested by the positive trends seen in elderly persons of both sexes in Umbria.

Cardiovascular diseases are associated with lifestyle (smoking, alcohol, metabolic disorders, scant physical activity, overweight and obesity, pollution exposure) in all SE groups [29-34]. In Italy, smoking has decreased among young males, although to a lesser extent in the most deprived [35]. Among Italian women, smoking started at a later date, but spread rapidly from the most privileged to the other SE groups [35]. As yet, there are only a few signs of a decline in female smokers [36]. Umbria has the third highest smoking prevalence in Italy [36], which might partially account for the very high differences in risks between younger and older men across SHE groups and the non-linear trend in women.

Health campaigns and corrective actions on diet [38, 39] have had an effect in Italy, but SE differences still penalise the most deprived. The association between unhealthy eating and low SE status seen in the most deprived population strata in Umbria could be linked to the consumption of a traditional diet, which is rich in red meat and processed meat, even in the less deprived population strata [40].

The association between air pollution, particularly that caused by ultrafine particles, and low SE condition [41, 42] impacts on cardiovascular diseases. These particles reach cardiovascular sites, cause systemic inflammation in response to oxidative stress and promote the progression of atherosclerosis. In Umbria, this association emerged in urban areas with an industrial background (i.e., the town of Terni), while rural areas of the region appeared to be less affected.

Most deaths caused by diseases of the respiratory system are due to chronic-obstructive pulmonary diseases [43, 44], which affect the deprived more than the other groups. Although smoking is one of the main causes, significant roles are attributed to occupational exposure and air pollution. The present findings in Umbria only partially confirmed the positive association observed elsewhere in deprived people [43-46]. Lifestyle differences (rural/urban) could be partly responsible for these differences. Moreover, we recorded a few deaths attrib-

Tab. IV. 2005-2012 mortality in Umbria, by cause, gender, age and deprivation groups identified by NDI-U and USHI: Standard Mortality Ratios (SMR), cases and trend significance.

CAUSE	INDICES	AGE GROUPS	INDICATOR	MEN							WOMEN						
				1	2	3	4	5	Umbria	Trend	1	2	3	4	5	Umbria	Trend
DIABETES	NDI-U	All ages	SMR	100.0	99.1	102.2	89.8	105.7	99.5	n.s.	100.4	93.8	98.0	106.7	98.8	99.3	n.s.
			OBS	140	163	156	122	156	737		194	206	205	200	196	1001	
		0-64 yrs	SMR	130.2	75.7	121.5	93.3	79.7	100.0	n.s.	116.7	70.5	51.4	207.9	60.6	100.4	p < 0.05 NL
			OBS	20	13	21	15	12	81		6	4	3	11	3	27	
		65+ yrs	SMR	96.3	101.8	99.8	89.4	108.7	99.5	n.s.	100.0	94.4	99.3	103.7	99.7	99.3	n.s.
			OBS	120	150	135	107	144	656		188	202	202	189	193	974	
	USHI	All ages	SMR	109.3	125.8	89.9	70.3	77.4	99.5	p < 0.05 NL	117.1	101.5	90.4	81.3	86.0	99.3	p < 0.05 L↑
			OBS	166	211	150	97	113	737		255	235	204	150	157	1001	
		0-64 yrs	SMR	99.9	121.3	106.6	75.3	93.7	100.0	n.s.	143.9	123.5	50.7	57.6	132.5	100.4	n.s.
			OBS	16	21	19	12	13	81		8	7	3	3	6	27	
		65+ yrs	SMR	110.4	126.3	87.9	69.6	68.9	99.5	p < 0.05 L↑	116.4	100.9	91.4	82.0	105.2	99.3	p < 0.05 L↑
			OBS	150	190	131	85	100	656		247	228	201	147	151	974	
CIRCULATORY SYSTEM DISEASES	NDI-U	All ages	SMR	97.3	98.2	98.4	102.8	98.7	99.0	p < 0.05 NL	99.8	96.8	96.0	101.4	101.1	98.9	n.s.
			OBS	2683	3197	2949	2734	2874	14437		3488	3844	3634	3443	3626	18035	
		0-64 yrs	SMR	99.3	100.2	104.0	91.6	100.2	99.1	p < 0.05 NL	94.5	97.4	102.1	92.6	111.4	99.5	n.s.
			OBS	240	270	283	232	236	1261		75	85	92	76	85	413	
		65+ yrs	SMR	97.1	98.0	97.9	104.0	98.6	99.0	p < 0.05 NL	99.9	96.8	95.8	101.6	100.9	98.9	n.s.
			OBS	2443	2927	2666	2502	2638	13176		2070	3967	4677	4535	2373	17622	
	USHI	All ages	SMR	97.8	99.4	95.6	100.4	103.4	99.0	p < 0.05 L↓	99.6	100.6	93.7	99.7	102.4	98.9	p < 0.05 NL
			OBS	2951	3303	3143	2711	2329	14437		3951	4225	3834	3309	2716	18035	
		0-64 yrs	SMR	101.9	105.9	101.2	89.5	95.9	99.1	p < 0.05 L↑	108.4	108.0	90.3	84.3	107.7	99.5	p < 0.05 NL
			OBS	254	287	284	226	210	1261		93	94	83	68	75	413	
		65+ yrs	SMR	97.5	98.8	95.1	101.5	104.2	99.0	p < 0.05 L↓	99.4	100.4	93.8	100.0	102.3	98.9	p < 0.05 NL
			OBS	2697	3016	2859	2485	2119	13176		3858	4131	3751	3241	2641	17622	
RESPIRATORY DISEASES	NDI-U	All ages	SMR	103.5	92.7	89.5	107.9	103.3	98.9	n.s.	122.9	108.1	94.2	104.6	107.0	106.9	n.s.
			OBS	707	751	662	704	748	3572		588	501	536	517	571	2713	
		0-64 yrs	SMR	125.9	65.1	67.4	120.2	125.6	99.3	n.s.	94.0	91.6	117.5	103.8	90.9	100.0	n.s.
			OBS	33	19	20	33	32	137		14	15	20	16	13	78	
		65+ yrs	SMR	102.6	93.7	90.4	107.3	102.4	98.9	n.s.	123.8	108.7	93.4	104.7	107.4	107.1	n.s.
			OBS	674	732	642	671	716	3435		574	486	516	501	558	2635	
	USHI	All ages	SMR	96.5	103.7	89.8	101.0	106.1	98.9	p < 0.05 NL	105.3	123.6	102.0	95.7	109.2	102.9	p < 0.05 NL
			OBS	721	858	733	672	588	3572		619	626	616	467	385	2713	
		0-64 yrs	SMR	114.5	78.6	88.9	98.5	121.5	99.3	p < 0.05 NL	118.1	116.5	80.9	79.2	106.5	100.0	n.s.
			OBS	31	23	27	27	29	137		19	19	14	12	14	78	
		65+ yrs	SMR	95.8	104.6	89.9	101.1	105.4	98.9	p < 0.05 NL	105.0	123.8	102.7	96.2	109.3	101.1	p < 0.05 NL
			OBS	690	835	706	645	559	3435		600	607	602	455	371	2635	

continues

utable to pneumoconiosis, probably occupation-related, involving asbestos- and silica-processing workers [47]. This type of exposure mainly affects the most deprived groups of population [48], and indeed, this situation was observed in the Umbrian province of Terni, where a large steel-mill is located.

Finally, diseases of the digestive system are positively associated with SHE deprivation [49-51]. Indeed, cirrhosis, ulcers, diverticulitis and inflammatory bowel disease are usually associated with low SE status; this is due more to delays in diagnosis and therapy than to greater exposure to risk factors [51]. USHI trends on-

Tab. IV. *follows.*

CAUSE	INDICES	AGE GROUPS	INDICATOR	MEN							WOMEN						
				1	2	3	4	5	Umbria	Trend	1	2	3	4	5	Umbria	Trend
DIGESTIVE DISEASES	NDI-U	All ages	SMR	98.0	97.5	90.3	109.4	102.3	99.3	n.s.	85.8	100.0	103.8	111.0	95.4	99.2	p < 0.05 NL
			OBS	251	292	253	274	275	1345		226	299	297	285	258	1365	
		0-64 yrs	SMR	88.7	97.7	76.6	93.4	143.3	99.1	n.s.	110.9	127.0	36.7	94.2	124.1	97.2	n.s.
			OBS	40	49	39	44	63	235		16	20	6	14	17	73	
		65+ yrs	SMR	99.9	97.5	93.4	113.1	94.3	99.3	p < 0.05 NL	84.4	98.5	107.9	112.0	93.9	99.3	p < 0.05 NL
			OBS	211	243	214	230	212	1110		210	279	291	271	241	1292	
	USHI	All ages	SMR	125.8	118.0	89.5	87.7	82.0	99.3	p < 0.05 L↑	115.1	106.2	85.4	100.8	84.3	99.2	p < 0.05 L↑
			OBS	292	360	273	223	197	1345		341	334	263	255	172	1365	
		0-64 yrs	SMR	120.8	144.7	86.0	53.1	88.0	99.1	p < 0.05 L↑	111.0	95.4	102.1	115.6	55.2	99.2	n.s.
			OBS	56	73	45	25	36	235		17	15	17	17	7	73	
		65+ yrs	SMR	102.7	112.7	90.2	95.5	92.9	99.3	p < 0.05 NL	115.4	106.7	84.5	99.8	86.3	99.3	p < 0.05 L↑
			OBS	236	287	228	198	161	1110		324	319	246	238	165	1292	

ly partially confirmed the literature, with NL trends in males and females in all age-groups.

The above considerations seem to support the validation of USHI as an indicator of socio-economic and health-related inequalities.

A limit of USHI is that it cannot be considered a mere deprivation index. Indeed, as it is intended specifically to assess SE and health inequalities, overall mortality is one of its constituent variables. Therefore, it cannot be used to describe SE differences in a population, but only the SE differences tied to the health condition. Thus, although it is very useful for public health purposes, it cannot substitute a deprivation index for general purposes.

A second limit appears to be the local characterisation of the indices computed by means of this method, as the SHE descriptors may differ from area to area. In reality, however, given that the local indices are constructed according to the same method, they express the same conceptual definition of SHE deprivation even though they consider different SHE descriptors.

Instead, sharing the same method in order to identify SHE deprivation groups, even if they consider different SHE descriptors, they express the same conceptual definition of SHE deprivation. Therefore, similar segments of population in the different regions could be pooled, because they identify the same SHE differences and needs in people pertaining to different areas. At the European level, an analogous approach was adopted in the construction of the European Deprivation Index [52].

Conclusions

By connecting SE findings with some explanations of health conditions described in the literature, the present study confirms that the construction of regional indices of SHE inequalities allows us to formulate specific hypotheses regarding the reasons behind health outcomes in a population and, consequently, to make suggestions concerning the corrective actions to undertake.

Our aim was to provide a valid and reliable method of computing SE and health inequality-related indices at the regional level, in order to better analyse the specific elements associated with the health condition of the population.

The present findings demonstrated that the USHI better represented the association between health and inequalities, and may provide a useful guide to the allocation of regional health resources.

In conclusion, regional indices computed in the same way as the USHI could be adopted elsewhere, in order to draw up specific strategies to reduce inequalities in health, thereby contributing to the sustainability of the health system and to the evaluation of the outcomes of the policies implemented.

Acknowledgements

Funding sources: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

RL: main author; research protocol, development of the analyses and author for paragraphs "Material and Methods" and "Results".
GM: author for paragraphs "Introduction" and "Discussion", linguistic revision.
FB: data provider and data quality check, contribution to methods and analysis.
AG: quality check for data analysis and results.
FS: contribution to paragraphs "Discussion" and "Conclusion", text revision, availability of Umbria Region Cancer and Mortality Registries.
FLR: contribution to pargaragraphs "Introduction" and "Conclusion", availability of Umbria Region Cancer and Mortality Registries.
MV: author of paragraph "Conclusion", contribution to all paragraphs, coordination of the study.

References

[1] Mackenbach JP, Stirbu I, Roskam AJR, Schaap MM, Menvielle G, Leinsalu M, Kunst AE. European Union Working Group on Socioeconomic Inequalities in Health. Socioeconomic inequalities in health in 22 European countries. N Engl J Med 2008;358:2468-81. https://doi.org/10.1056/NEJMsa0707519

[2] Mackenbach JP, Kulhánová I, Bopp M, Deboosere P, Eikemo TA, Hoffmann R, Kulik MC, Leinsalu M, Martikainen P, Menvielle G, Regidor E, Wojtyniak B, Östergren O, Lundberg O, EURO-GBD-SE Consortium. Variations in the relation between education and cause-specific mortality in 19 European populations: a test of the "fundamental causes" theory of social inequalities in health. Soc Sci Med 2015;127:51-62. https://doi.org/10.1016/j.socscimed.2014.05.021

[3] Gallo V, Mackenbach JP, Ezzati M, Menvielle G, Kunst AE, Rohrmann S, Kaaks R, Teucher B, Boeing H, Bergmann MM, Tjønneland A, Dalton SO, Overvad K, Redondo ML, Agudo A, Daponte A, Arriola L, Navarro C, Gurrea AB, Khaw KT, Wareham N, Key T, Naska A, Trichopoulou A, Trichopoulos D, Masala G, Panico S, Contiero P, Tumino R, Bueno-de-Mesquita HB, Siersema PD, Peeters PP, Zackrisson S, Almquist M, Eriksson S, Hallmans G, Skeie G, Braaten T, Lund E, Illner AK, Mouw T, Riboli E, Vineis P. Social inequalities and mortality in Europe--results from a large multi-national cohort. PLoS One 2012;7:e39013. https://doi.org/10.1371/journal.pone.0039013

[4] Sommer I, Griebler U, Mahlknecht P, Thaler K, Bouskill K, Gartlehner G, Mendis S. Socioeconomic inequalities in non-communicable diseases and their risk factors: an overview of systematic reviews. BMC Public Health 2015;15:914. https://doi.org/10.1186/s12889-015-2227-y

[5] Woods LM, Rachet B, Coleman MP. Choice of geographic unit influences socioeconomic inequalities in breast cancer survival. Br J Cancer 2005;92:1279-82. https://doi.org/10.1038/sj.bjc.6602506

[6] Rey G, Jougla E, Fouillet A, Hémon D. Ecological association between a deprivation index and mortality in France over the period 1997-2001: variations with spatial scale, degree of urbanicity, age, gender and cause of death. BMC Public Health 2009;9:33-44. https://doi.org/10.1186/1471-2458-9-33

[7] Caranci N, Biggeri A, Grisotto L, Pacelli B, Spadea T, Costa G. L'indice di deprivazione italiano a livello di sezione di censimento: definizione, descrizione e associazione con la mortalità. [The Italian deprivation index at census block level: definition, description and association with general mortality]. Epidemiol Prev 2010;34:167-76.

[8] Lillini R, Quaglia A, Vercelli M. Costruzione di un indice di deprivazione per misurare lo stato di salute in Liguria. [Building of a local deprivation index to measure the health status in the Liguria region]. Epidemiol Prev 2012;36:180-7.

[9] Vercelli M, Lillini R, Quaglia A; Registro Tumori Regione Liguria. Methods to study the deprivation and its relationships with cancer incidence in a local area. Metodi per lo studio della deprivazione e le sue relazioni con l'incidenza del cancro in un'area locale. CancerStat Umbria 2014;5:621-37.

[10] OECD/EU (2016), Health at a Glance: Europe 2016 – State of Health in the EU Cycle. Paris: OECD Publishing 2016. Avalaible at: http://dx.doi.org/10.1787/9789264265592-en

[11] Costa G, Paci E, Ricciardi W (eds.). Salute e sanità a 150 anni dall'unità d'Italia: più vicini o più lontani? [Health and public health in Italy after 150 years from unification: nearer or farther?]. Epidemiol Prev 2011;35(Supplement 2):1-134.

[12] Vercelli M, Lillini R, Quaglia A and Registro Tumori Regione Liguria. Deprivation and cancer incidence in a de-industrialised and highly ageing area. [Deprivazione e incidenza di cancro in un'area ex-industriale a forte invecchiamento]. CancerStat Umbria 2014;5:638-61.

[13] Solar O, Irwin A. A conceptual framework for action on the social determinants of health. Social Determinants of Health Discussion. Paper 2 (Policy and Practice). Geneva: World Health Organization 2010.

[14] Quaglia A, Lillini R, Mamo C, Ivaldi E, Vercelli M, SEIH (Socio-Economic Indicators and Health) Working Group. Socio-economic inequalities: a review of methodological issues and the relationships with cancer survival. Crit Rev Oncol Hematol 2013;85:266-77. https://doi.org/10.1016/j.critrevonc.2012.08.007

[15] Tacq J. Multivariate Analysis Techniques in Social Research. London: Sage 1997.

[16] Corbetta P, Gasperoni G, Pisati M. Statistica per la ricerca sociale [Statistics for Social Research]. Bologna: Il Mulino 2001.

[17] Agnelli JP, Cadeiras M, Tabak EG, Turner CV, Vanden-Eijnden E.. Clustering and classification through normalizing flows in feature space. Multiscale Model Simul 2010;8:1784-802.

[18] Panatto D, Gasparini R, Amicizia D. Influenza vaccination coverage in the elderly and socio-economic inequalities in Italy. J Prev Med Hyg 2019;59(4 Suppl 2):E1-E2. https://doi.org/10.15167/2421-4248/jpmh2018.59.4s2.1198

[19] Vercelli M, Lillini R, Arata L, Zangrillo F, Bagnasco A, Sasso L, Magliani A, Gasparini R, Amicizia D, Panatto D. Analysis of influenza vaccination coverage among the elderly in Genoa (Italy) based on a deprivation index, 2009-2013. J Prev Med Hyg 2019;59(4 Suppl 2):E11-E17. https://doi.org/10.15167/2421-4248/jpmh2018.59.4s2.1171

[20] Goldthorpe JH. Sociology as a Population Science. Cambridge: Cambridge University Press 2015.

[21] Kaneko K. Life: An introduction to complex systems biology. Berlin: Springer 2006.

[22] Rogers RG, Everett BG, Onge JM, Krueger PM. Social, behavioural, and biological factors, and sex differences in mortality. Demography 2010;47:555-78.

[23] Auvinen A, Karjalainen S. Possible explanations for social class differences in cancer patient survival. In: Kogevinas M, Pearce N, Susser M, Boffetta P, eds. Social Inequalities

and Cancer. IARC Scientific Publications No. 138:377-397. Lyon: International Agency for Research on Cancer 1997.

[24] Whitaker KL, Scott SE, Wardle J. Applying symptom appraisal models to understand sociodemographic differences in responses to possible cancer symptoms: a research agenda. Br J Cancer 2015;112:S27-S34. https://doi.org/10.1038/bjc.2015.39

[25] Douglas E, Wardle J, Massat NJ, Waller J. Colposcopy attendance and deprivation: A retrospective analysis of 27,193 women in the NHS Cervical Screening Programme. Br J Cancer 2015;113:119-22. https://doi.org/10.1038/bjc.2015.176

[26] Quaglia A, Lillini R, Casella C, Giachero G, Izzotti A, Vercelli M, Liguria Region Tumour Registry. The combined effect of age and socioeconomic status on breast cancer survival. Crit Rev Oncol Hematol 2011;77):210-20. https://doi.org/10.1016/j.critrevonc.2010.02.007

[27] Manzoli L, Villari P, M Pirone G, Boccia A. Marital status and mortality in the elderly: a systematic review and meta-analysis. Soc Sci Med 2007;64:77-94. https://doi.org/10.1016/j.socscimed.2006.08.031

[28] Smith JP. Nature and causes of trends in male diabetes prevalence, undiagnosed diabetes, and the socioeconomic status health gradient. Proc Natl Acad Sci USA 2007;104:13225-31. https://doi.org/10.1073/pnas.0611234104

[29] Dubowsky SD, Suh H, Schwartz J, Coull BA, Gold DR. Diabetes, obesity, and hypertension may enhance associations between air pollution and markers of systemic inflammation. Environ Health Perspect 2006;114:992-8. https://doi.org/10.1289/ehp.8469

[30] O'Neill MS, Veves A, Zanobetti A, Sarnat JA, Gold DR, Economides PA, Horton ES, Schwartz J. Diabetes enhances vulnerability to particulate air pollution-associated impairment in vascular reactivity and endothelial function. Circulation 2005;111:2913-20. https://doi.org/10.1161/CIRCULATIONAHA.104.517110

[31] Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration. Cardiovascular disease, chronic kidney disease, and diabetes mortality burden of cardiometabolic risk factors from 1980 to 2010: a comparative risk assessment. Lancet Diabetes Endocrinol 2014;2:634-47. https://doi.org/10.1016/S2213-8587(14)70102-0

[32] Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration (BMI Mediated Effects), Lu Y, Hajifathalian K, Ezzati M, Woodward M, Rimm EB, Danaei G. Metabolic mediators of the effects of body-mass index, overweight, and obesity on coronary heart disease and stroke: a pooled analysis of 97 prospective cohorts with 1•8 million participants. Lancet 2014;383:970-83. https://doi.org/10.1016/S0140-6736(13)61836-X.

[33] Lantz PM, Golberstein E, House JS, Morenoff J. Socioeconomic and behavioral risk factors for mortality in a national 19-year prospective study of U.S. adults. Soc Sci Med 2010;70:1558-66. https://doi.org/10.1016/j.socscimed.2010.02.003

[34] Hemingway A. Determinants of coronary heart disease risk for women on a low income: literature review. J Adv Nurs 2007;60:359-67. https://doi.org/10.1111/j.1365-2648.2007.04418.x

[35] Federico B, Kunst AE, Vannoni F, Damiani G, Costa G. Trends in educational inequalities in smoking in northern, mid and southern Italy, 1980-2000. Prev Med 2004;39:919-26. https://doi.org/10.1016/j.ypmed.2004.03.029

[36] Vercelli M, Quaglia A, Lillini R. Useful indicators to interpret the cancer burden in Italy. Tumori 2013;99:425-38. https://doi.org/10.1700/1334.14808

[37] ISTAT: Health for All - Italia. Rome, June 2015. Available at: http://www.istat.it/it/archivio/14562 (Accessed on 13 November 2015).

[38] Lock K, Pomerleau J, Causer L, Altmann DR, McKee M. The global burden of disease attributable to low consumption of fruit and vegetables: implications for the global strategy on diet. Bull World Health Organ 2005;83:100-8.

[39] Rees K, Hartley L, Flowers N, Clarke A, Hooper L, Thorogood M, Stranges S. 'Mediterranean' dietary pattern for the primary prevention of cardiovascular disease. Cochrane Database Syst Rev 2013;(8):CD009825. https://doi.org/10.1002/14651858.CD009825.pub2

[40] Marra M, Migliardi A, Costa G. Disuguali a tavola, ma non troppo: le differenze sociali nell'alimentazione in Italia prima e durante la crisi [Health inequalities and nutrition in Italy during crisis times]. Epidemiol Prev 2015;39:322-31.

[41] Delfino RJ, Sioutas C, Malik S. Potential role of ultrafine particles in associations between airborne particle mass and cardiovascular health. Environ Health Perspect 2005;113:934-46. https://doi.org/10.1289/ehp.7938

[42] Zanobetti A, Schwartz J. Particulate air pollution, progression, and survival after myocardial infarction. Environ Health Perspect 2007;115:769-75. https://doi.org/10.1289/ehp.9201

[43] Wheeler A, Zanobetti A, Gold DR, Schwartz J, Stone P, Suh HH. The relationship between ambient air pollution and heart rate variability differs for individuals with heart and pulmonary disease. Environ Health Perspect 2006;114:560-6. https://doi.org/10.1289/ehp.8337

[44] Beelen R, Raaschou-Nielsen O, Stafoggia M, Andersen ZJ, Weinmayr G, Hoffmann B, Wolf K, Samoli E, Fischer P, Nieuwenhuijsen M, Vineis P, Xun WW, Katsouyanni K, Dimakopoulou K, Oudin A, Forsberg B, Modig L, Havulinna AS, Lanki T, Turunen A, Oftedal B, Nystad W, Nafstad P, De Faire U, Pedersen NL, Östenson CG, Fratiglioni L, Penell J, Korek M, Pershagen G, Eriksen KT, Overvad K, Ellermann T, Eeftens M, Peeters PH, Meliefste K, Wang M, Bueno-de-Mesquita B, Sugiri D, Krämer U, Heinrich J, de Hoogh K, Key T, Peters A, Hampel R, Concin H, Nagel G, Ineichen A, Schaffner E, Probst-Hensch N, Künzli N, Schindler C, Schikowski T, Adam M, Phuleria H, Vilier A, Clavel-Chapelon F, Declercq C, Grioni S, Krogh V, Tsai MY, Ricceri F, Sacerdote C, Galassi C, Migliore E, Ranzi A, Cesaroni G, Badaloni C, Forastiere F, Tamayo I, Amiano P, Dorronsoro M, Katsoulis M, Trichopoulou A, Brunekreef B, Hoek G. Effects of long-term exposure to air pollution on natural-cause mortality: an analysis of 22 European cohorts within the multicentre ESCAPE project. Lancet 2014;383:785-95. https://doi.org/10.1016/S0140-6736(13)62158-3

[45] Benmarhnia T, Oulhote Y, Petit C, Lapostolle A, Chauvin P, Zmirou-Navier D, Deguen S. Chronic air pollution and social deprivation as modifiers of the association between high temperature and daily mortality. Environ Health 2014;13:53. https://doi.org/ 10.1186/1476-069X-13-53

[46] Lin HH, Ezzati M, Murray M. Tobacco smoke, indoor air pollution and tuberculosis: a systematic review and meta-analysis. PLoS Med, 2007;4:e20. https://doi.org/10.1371/journal.pmed.0040020

[47] Coggon D, Harris EC, Brown T, Rice S, Palmer KT. Work-related mortality in England and Wales, 1979-2000. Occup Environ Med 2010;67:816-822. https://doi.org/10.1136/oem.2009.052670

[48] Brunt H, Barnes J, Jones SJ, Longhurst JW, Scally G, Hayes E. Air pollution, deprivation and health: understanding relationships to add value to local air quality management policy and practice in Wales, UK. J Public Health (Oxf), 2016 [Epub ahead of print].

[49] Jepsen P, Vilstrup H, Andersen PK, Sørensen HT. Socioeconomic status and survival of cirrhosis patients: a Danish nationwide cohort study. BMC Gastroenterol 2009;9:35. https://doi.org/10.1186/1471-230X-9-35.

[50] Sewell JL, Velayos FS. Systematic review: The role of race and socioeconomic factors on IBD healthcare delivery and effectiveness. Inflamm Bowel Dis 2013;19:627-43. https://doi.org/10.1002/ibd.22986

[51] Csikesz NG, Singla A, Simons JP, Tseng JF, Shah SA. The impact of socioeconomic status on presentation and treatment of diverticular disease. J Gastrointest Surg 2009;13:1993-2002. https://doi.org/10.1007/s11605-009-1031-3

[52] Guillaume E, Pornet C, Dejardin O, Launay L, Lillini R, Vercelli M, Marí-Dell'Olmo M, Fernández Fontelo A, Borrell C, Ribeiro AI, Pina MF, Mayer A, Delpierre C, Rachet B, Launoy G. Development of a cross-cultural deprivation index in five European countries. J Epidemiol Community Health 2016;70:493-9. https://doi.org/10.1136/jech-2015-205729

Received on April 16, 2019. Accepted on November 15, 2019.

Correspondence: Roberto Lillini, Department of Health Sciences, University of Genoa. Analytical Epidemiology and Health, via Antonio Pastore 1, 16132 Genova (Italy) - Tel. +39 010 3538501 - E-mail: r.lillini@campus.unimib.it

How to cite this article: Lillini R, Masanotti G, Bianconi F, Gili A, Stracci F, La Rosa F, Vercelli M. Regional indices of socio-economic and health inequalities: a tool for public health programming. J Prev Med Hyg 2019;60:E300-E310. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1257

Tab. SI. Municipalities and sub-municipalities, by SE deprivation group and USHI value.

Very high deprivation	High deprivation	Medium deprivation	Low deprivation	Very low deprivation					
Municipality/ Sub-municipality	USHI value	Municipality/ Sub-municipality	USHI value	Municipality/ Sub-municipality	USHI value	Municipality/ Sub-municipality	USHI value	Municipality/Sub-municipality	USHI value
Poggiodomo	0.02	Foligno 2	61.87	Foligno 1	68.01	Perugia 4	78.25	Avigliano Umbro	83.02
Polino	39.04	Narni	61.94	Perugia 5	68.10	Campello sul Clitunno	78.42	Cannara	83.27
Foligno 3	42.75	Arrone	62.75	Todi	68.46	Perugia 2	78.73	Montefalco	83.28
Terni 5	45.42	Nocera Umbra	62.82	Tuoro sul Trasimeno	69.22	Porano	78.86	Spello	83.66
Preci	49.21	Montefranco	63.42	Paciano	69.31	Gualdo Cattaneo	79.28	Fratta Todina	85.33
Terni 4	51.32	Otricoli	63.71	Cascia	69.50	Montecastrilli	79.64	Magione	85.87
Parrano	53.36	Guardea	63.74	Alviano	69.95	Fossato di Vico	80.09	Bevagna	85.89
Terni 3	54.95	Ficulle	64.10	Perugia 6	70.71	Piegaro	80.19	Perugia 1	86.53
Calvi dell'Umbria	55.53	Montecchio	64.35	Attigliano	71.15	Marsciano	80.32	Sigillo	86.58
Penna in Teverina	56.36	Orvieto	65.33	Gubbio	71.30	San Venanzo	80.34	Montone	87.43
Monteleone d'Orvieto	56.37	Amelia	65.83	Allerona	71.40	Città di Castello	80.61	Trevi	88.60
Vallo di Nera	56.59	Ferentillo	65.85	Sant'Anatolia di Narco	71.66	Collazzone	81.23	San Giustino	90.92
Sellano	56.70	Gualdo Tadino	66.03	Pietralunga	72.20	Valtopina	81.81	Monte Santa Maria Tiberina	92.12
Terni 2	57.65	Città della Pieve	66.14	Lisciano Niccone	72.65	Panicale	82.01	Deruta	92.94
Perugia 7	59.37	Giove	66.19	Lugnano in Teverina	73.23	Giano dell'Umbria	82.05	Torgiano	94.51
Terni 1	59.59	Castiglione del Lago	66.64	Castel Viscardo	73.38	Valfabbrica	82.16	Bettona	95.16
Stroncone	60.46	Scheggino	66.98	Fabro	73.88	Castel Ritaldi	82.63	Bastia	95.89
Monteleone di Spoleto	60.53	Norcia	67.15	Passignano sul Trasimeno	74.46	Assisi	82.69	Corciano	95.89
Montegabbione	60.58	Spoleto	67.21	Costacciaro	74.62			Citerna	99.98
Castel Giorgio	61.61	Cerreto di Spoleto	67.24	San Gemini	74.64				
		Baschi	67.59	Massa Martana	74.70				
		Acquasparta	67.88	Perugia 3	74.76				
				Umbertide	75.30				
				Scheggia e Pascelupo	76.44				
				Monte Castello di Vibio	77.40				

ORIGINAL ARTICLE

Estimates of the incidence of infection-related cancers in Italy and Italian regions in 2018

S. DE FLORA[1], S. LA MAESTRA[1], E. CROCETTI[2], L. MANGONE[3], F. BIANCONI[4], F. STRACCI[5], C. BUZZONI[6]
[1] Department of Health Sciences (DISSAL), University of Genoa, Genoa, Italy; [2] Romagna Cancer Registry, Romagna Cancer Institute (Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, IRST, IRCCS), Meldola, Forlì, Italy; [3] Epidemiology Unit, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy; [4] Independent Researcher, Perugia, Italy; [5] Department of Experimental Medicine, Section of Public Health, University of Perugia, Perugia, Italy; [6] Clinical Epidemiology Unit, Oncological Network, Prevention and Research Institute (ISPRO), Florence, Italy

Keywords

Cancer incidence • Chronic infections and cancer • Infection-related cancers • Italy • Regional distribution

Summary

Introduction. *Chronic infections and infestations represent one of the leading causes of cancer. Eleven agents have been categorized by the International Agency for Research on Cancer (IARC) in Group 1, 3 in Group 2A and 4 in Group 2B. We previously estimated that the incidence of cancers associated with infectious agents accounted for the 8.5% of new cancer cases diagnosed in Italy in 2014.*
Methods. *In the present study we evaluated the incidence of cancer in Italy and in the 20 Italian regions in 2018, based on the data of Cancer Registries, and calculated the fraction attributable to infectious agents.*
Results. *Cancers of infectious origin contributed to the overall burden of cancer in Italy with more than 27,000 yearly cases, the 92% of which was attributable to Helicobacter pylori, human papillomaviruses, and hepatitis B and C viruses. With the exception of papillomavirus-related cancers, the incidence of cancers of infectious origin was higher in males (16,000 cases) than in females (11,000 cases). There were regional and geographical variations of cancers depending on the type of cancer and on the gender. Nevertheless, the overall figures were rather similar, the infection-related cancers accounting for the 7.2, 7.6, and 7.1% of all cancers in Northern, Central, and Southern Italy, respectively.*
Conclusions. *The estimate of the incidence of cancers attributable to infectious agents in Italy in 2018 (7.3% of all cancer cases) is approximately half of the worldwide burden, which has been estimated by IARC to be the 15.4% of all cancer cases in 2012.*

Introduction

Altogether, chronic infections and infestations represent one of the leading causes of cancer worldwide. Eleven agents have been categorized by IARC (International Agency for Research on Cancer) in Group 1 (sufficient evidence of carcinogenicity to humans), 3 in Group 2A (probably carcinogenic), and 4 in Group 2B (possibly carcinogenic) [1]. Several other viruses, bacteria and protozoa have been suspected to be associated with various human cancers [2, 3].
The global burden of infection-related cancers has been estimated and periodically updated at IARC. The population attributable fraction (PAF) in the world population, in terms of incidence, was estimated to be the 15.6% in 1990 [4], 17.8% in 2002 [5], 16.1% in 2008 [6], and 15.4% in 2012 [7]. The last figure corresponds to 2 million new cancer cases of infectious origin out of 14 million cases of all cancers, with broad variations depending on the geographical region and on the developmental status. In fact, the PAFs varied from less than 5% in North America, Australia-New Zealand and some Western and Northern Europe countries to more than 50% in sub-Saharian Africa countries [7].
The PAF, indicating the proportion of cancers of infectious and parasitic origin, has been estimated in various countries based either on morbidity and/or on mortality data. Thus, mortality PAF estimates have been made in the USA in 1981 (10%) [8], in the UK both in 1998 (10-20%) [9] and 2005 (5%) [10], in France in 2000 (3.6%) [11], and in China in 2005 (29.4%) [12]. Other studies estimated incidence data or both incidence and mortality data. For instance, the 4.1% of all new cancer cases occurring in France in 2015 were attributable to infectious agents [13]. In China, infection-related cancers accounted in 2005 for 25.9 and 29.4% of the overall cancer cases and deaths, respectively [14]. In another study in China, cancers related to the main cancer-related pathogens in men and women accounted for 17.7 and 15.4% (incidence) and 20.0 and 16.9% (mortality), respectively [15]. In the Korean population, for 2007 the fractions of all cancers attributable to infection were 25.1% and 16.8% for cancer incidence in men and women, and 25.8% and 22.7% for cancer mortality in men and women, respectively [16]. The 2.9% of cancers diagnosed in Australia in 2010 were attributable to infectious agents [17].
We previously estimated the PAF of infection-related cancers in Italy, based on 2014 national incidence data [2]. That analysis demonstrated that cancers associated with 6 pathogens accounted for 31,000 out of 365,000 new yearly cases (8.5%), 42.0% of which was

attributable to *Helicobacter pylori* (Hp), 34.7% to hepatitis B virus (HBV) and hepatitis C virus (HCV), 19.8% to human papillomaviruses (HPV), 2.9% to human herpesvirus 8 (HHV8) or Kaposi's sarcoma virus (KSHV), and 0.2% to human herpesvirus 4 (HHV4) or Epstein-Barr virus (EBV). The objectives of the present study were to reassess the incidence of infection-related cancers in Italy 4 years later and to evaluate the geographical distribution of these cancers in the 20 Italian regions. To this purpose, we made estimates of the incidence and of the attributable fractions (AFs) of cancers associated with IARC Group 1 pathogens, excepting the infestations by trematodes, which are rare in Italy, and HIV-1 infections, because HIV-related immunodeficiency requires the concomitant infection with other carcinogenic infectious agents [1].

Methods

Monitored regions and coverage by cancer registries (CR)

The study covered the whole Italian territory, which includes 20 regions whose localization is shown in the map (Fig. S1). The regions were grouped according to geographic areas (North, Central or South) [18]. Northern regions include, in alphabetical order, Emilia Romagna, Friuli Venezia Giulia, Liguria (Ligury), Lombardia (Lombardy), Piemonte (Piedmont), Trentino Alto Adige (Trentino South-Tyrol), Valle d'Aosta (Aosta Valley), and Veneto, having an overall population of 27,746,158 residents as to January 2018 (Tab. SI). Central regions include Lazio (Latium), Marche (Marches), Toscana (Tuscany), and Umbria, having an overall population of 12,050,054 residents (Tab. SII). Southern and Insular regions include Abruzzo, Basilicata, Calabria, Molise, Puglia (Apulia), Sardegna (Sardinia), and Sicilia (Sicily), having an overall population of 20,697,761 residents (Tab. SIII).

The estimate of the incidence of cancers associated with chronic infections in the male and female population of whole Italy and of individual regions was based on the data available from Italian CRs, which in 2018 covered the 68% of the national population. In particular, the coverage in Northern regions was on an average 71% (Tab. SI), the coverage in Central regions was 25% (Tab. SII), and the coverage in Southern and Insular regions was 65% (Tab. SIII).

Estimates of the incidence of cancers of infectious origin

The evaluated agents covered DNA viruses, including HPV, HHV4/EBV, HHV8/KSHV, and HBV, either alone or in coinfection/superinfection with hepatitis D virus (HDV); RNA viruses, including HCV and human T-lymphotropic virus-I (HTLV-I); and bacteria, including Hp. The main cancers associated with the above pathogens are shown in Tables SI-SIII. The incidence estimates were selected from the AIOM-AIRTUM publication [19] for the available cancer sites (oropharynx, liver, uterine cervix, vulva, vagina, penis, Hodgkin's lymphoma, Kaposi's sarcoma). The remaining sites (non-cardia stomach, MALT, oral cavity, larynx, nasopharynx, Burkitt's lymphoma, adult T cell lymphoma/leukemia) were estimated by applying the previously described methodology [19], that is: a) preliminary time trend analysis; b) computation of regional incidence rates (with correction for incomplete coverage when necessary); c) projection of incidence rates to 2018; and d) application of rates to the resident population. A preliminary time trend analysis was made by selecting data from CRs with complete information on incidence during the 2003-2014 period. Cancer sites, three major age group (0-49, 50-69, 70+ years) and sex were considered. The average annual percent change (APC) of age-standardized incidence rates was estimate by a Joinpoint analysis [20]. APCs for the most recent period were considered for projections.

Regional incidence rates

Site-, sex-, age- and region-specific rates were computed in the calendar period 2010-14 (observed rate). Also site-, sex-, age- and geographic area- specific rates were computed for the same calendar period. Then regional rates were obtained as the weighted average between the observed rate and the area specific rates: Regional rate = α*Observed rate + (1-α)*Area specific rate, where α is the regional coverage. Consequently, in case the proportion of population covered by CRs is 0%, as it was the case for 3 regions (Marche, Abruzzo, and Molise), the regional rate equals the area specific rate, in case such a proportion is 100% the regional rate equals the observed rate. In Lazio and Campania the average rate was computed between observed rates and national pool data, instead of area specific ones.

Projection of incidence rates to 2018 and application of rates to the resident population

We assumed that incidence observed in the period 2010-2014 represent on an average the incidence of the year 2012 and that the time trend observed in the most recent time interval between 2003 and 2014 does not vary during the subsequent six years. Moreover, in order to take into account the random variability, when the site-, sex- and age- trend variation was not statistically significant (that is 95% confidence intervals include 0), the APC was constrained to 1. Therefore, incidence rates were projected to 2018 by multiplying the site-, sex-, age- and region- specific rates. The site-, sex-, age- and region-specific projected rates were multiplied by the region and age specific resident population in 2018, based on prevision from the National Statistical Institute [21].

Attributable fractions

The fraction of each cancer attributable to infectious agents was inferred from data available in the literature. In particular, as detailed in Table I, the data for HBV (± HDV) + HCV-related liver cancer made reference to the Italian population [22]. The data for HPV-related oro-

Tab. I. Estimates of total incident cancer cases and of incident cancer cases attributable to the main cancer-associated infectious agents in Italy in 2014 and 2018.

		Italy, 2014 [2]				Italy, 2018 [present study]			
Cancer site (ICD-10)	**Infectious agent**	**AF**	**Gender**	**Total incident cases**	**Infection-related cases**	**AF**	**Gender**	**Total incident cases**	**Infection-related cases**
Non-cardia stomach (C16.1-C16.9)	Hp	0.90 (World [6])	M F M+F	7,500 5,500 13,000	6,750 4,950 11,700	0.89 (World [7])	M F M+F	8,156 5,060 13,216	7,259 4,503 11,762
MALT (C88.4)	Hp	0.86 (World [6])	M F M+F	700 850 1,550	602 731 1,333	0.74 (World [7])	M F M+F	169 112 281	125 83 208
Total Hp-associated cancers	Hp		M F M+F	8,200 6,350 14,550	7,352 5,681 13,033		M F M+F	8,325 5,172 13,497	7,384 4,586 11,970
Liver (C22)	HBV (± HDV) +HCV	0.87 (Italy [29])	M F M+F	8,600 3,800 12,400	7,465 3,298 10,763	0.68 (Italy [22])	M F M+F	8,929 3,966 12,895	6,071 2,697 8,768
Uterine cervix (C53)	HPV	1.00 (World [6])	F	2,200	2,200	1.00 (World [23])	F	2,241	2,241
Vulva (C51)	HPV	0.40 (World [24])	F	900	364	0.25 (World [24])	F	1,266	317
Vagina (C52)	HPV	0.61 (World [24])	F	200	122	0.78 (World [23])	F	226	176
Penis (C60)	HPV	0.47 (World [24])	M	182	85	0.50 (World [23])	M	563	282
Oral cavity (C00-C08)	HPV	0.24 (World [25])	M F M+F	2,283 1,524 3,807	537 358 895	0.04 (World [7])	M F M+F	3,034 1,721 4,755	121 69 190
Oropharynx (C09-C10, C12-C14)	HPV	0.36 (World [25])	M F M+F	1,214 348 1,562	432 124 566	0.24 (South Europe [7])	M F M+F	1,552 407 1,959	372 98 470
Larynx (C32)	HPV	0.24 (World [25])	M F M+F	3,714 335 4,049	891 80 971	0.05 (World [7])	M F M+F	4,076 500 4,576	204 25 229
Total HPV-associated cancers	HPV		M F M+F	7,393 5,507 12,900	1,945 3,248 5,203		M F M+F	9,225 6,561 15,786	979 3,126 4,105
Nasopharynx (C11)	EBV (HHV4)	0.80 (Low incidence regions [6])	M F M+F	315 124 439	252 99 351	0.80 (Low incidence regions [7])	M F M+F	452 156 608	362 125 487
Burkitt's lymphoma (C83.7)	EBV (HHV4) ± Pf	0.20 (USA & Europe [6])	M F M+F	200 100 300	40 20 60	0.20 (USA & Europe [6])	M F M+F	115 44 159	23 9 32
Hodgkin's lymphoma (C81)	EBV (HHV4)			NA	NA	0.36 (Europe [7])	M F M+F	1,228 1,013 2,241	442 365 807
Total EBV-associated cancers	EBV (HHV4)		M F M+F	515 224 739	292 119 411		M F M+F	1,795 1,213 3,008	839 503 1,342
Kaposi's sarcoma (C46)	KSHV (HHV8)	1.00 (World [6])	M F M+F	600 300 900	600 300 900	1.00 (World [23])	M F M+F	646 280 926	646 280 926
Adult T cell lymphoma/ leukemia (C91.5)	HTLV1			NA	NA	1.00 (World [23])	M F M+F	12 2 14	12 2 14
All the above cancers			M F M+F	25,758 16,831 42,589	18,034 13,194 31,238		M F M+F	28,932 17,194 46,126	15,894 11,178 27,072

Hp: Helicobacter pylori; HBV: hepatitis B virus; HCV: hepatitis C virus; HDV: hepatitis D virus (Delta agent); HPV: human papillomavirus; EBV: Epstein-Barr virus; HHV4: human herpesvirus 4; PF: Plasmodium falciparum; KSKV: Kaposi's sarcoma virus; HHV8: human herpesvirus 8; HTLV: human T-lymphotropic virus; AF: attributable fraction; M: males; F: females.; NA: not available.

pharynx cancer were related to the South Europe population and those for EBV-related Hodgkin's lymphoma were related to the European population [7]. Those related to Burkitt's lymphoma were related to the USA and European populations [6]. All the other cancers were related to the world population [6, 7, 23-25]. As shown in Table I, the AFs for individual cancers ranged from a minimum of 0.04 for HPV-related oral cancer to a maximum of 1 for KSHV/HHV8-related Kaposi's sarcoma, HTLV-1-related adult T cell lymphoma/leukemia, and HPV-related uterine cervix cancer. However, it is known that lifestyle, environmental and genetic factors can affect the susceptibility to HPV-related cervical cancer [26].

Results

Comparison of the incidence of infection-related cancers in Italy in 2014 and 2018

Table I compares the incidence of infection-related cancers in Italy as estimated in 2014 [2] with the one estimated in 2018 (present study). According to the more recent estimate, there was a 13% decrease in the incidence of all cancers associated with chronic infections, which accounted for a total of 31,238 cases in 2014 and a total of 27,072 cases in 2018. Such a decrease depends both on methodological issues and on variations in the AFs adopted during that period. In particular, in the case of Hp-related cancers the incidence of non-cardia stomach was very similar in 2014 and 2018. In contrast, there was a sharp drop in Hp-related MALT cases especially because of a different methodological approach. As to the liver cancers attributable to HBV and HCV, the 1.23-fold decrease in the incidence from 2014 to 2018 parallels a 1.28-fold decrease in the adopted AFs. The estimates of HPV-related cancers of female genitals were very similar in 2014 and 2018. In contrast, there was an apparent increase in HPV-related penis cancer in 2018 because of a different methodological approach. The estimate of the incidence of HPV-related cancers of the upper aerodigestive tract was much lower in 2018 because in the meantime a considerable drop of the AFs had been proposed. Some variations also occurred for EBV-related cancers because Hodgkin's lymphoma had not been included in 2014. The estimate of KSHV (HHV8)-related Kaposi's sarcoma was almost identical in 2014 and 2018, whereas very few cases of HTLV1-related adult T cell lymphoma/leukemia had not been computed in the previous study.

Incidence of infection-related cancers in the Italian regions in 2018

Tables SI, SII and SIII report the resident male and female population in 2018 [27], the percentage of the population covered by accredited CRs, and the estimated incidence of cancers, expressed as cases/100,000 residents, attributable to infectious agents in Northern, Central and Southern Italian regions, respectively. The panels in Figure 1 display maps that show the estimated incidence of cancers of infectious origin in the male and female population of the 20 Italian regions in 2018. As to all cancers of infectious origin (Fig. 1A), there was an evident intergender difference, which among males was characterized by a general trend to a gradient from North to South, with a maximum of 67 cases/100,000 in Veneto and a minimum of 39 cases/100,000 in Sicily. A similar picture was apparent in the female population, but with some regional exceptions. In fact, the maximum incidence was in Umbria (42 cases/100,000) but with close data in a Southern region (Basilicata). On the whole, by combining the two genders, the total number of cases/100,000 residents in Northern, Central and Southern regions were 94.4, 97.2 and 85.6, respectively.
A strong difference between males and females was also evident for HBV/HCV-related liver cancer (Fig. 1B), with sharp interregional variations in the male population, where the maximum incidence values were recorded in Northwestern regions, while a more homogeneous regional distribution occurred in the female population. By combining the two genders, the number of HBV/HCV-related liver cancer cases/100,000 residents in Northern, Central and Southern regions were 31.2, 24.4 and 30.3, respectively.
The higher incidence of all HPV-related cancers in females, compared to males, is evident from the values reported in Fig. 1C. The interregional differences were not sharp, although there was a regular gradient from North to South, especially in the female population. By combining the two genders, the number of HPV- related cancer cases/100,000 residents in Northern, Central and Southern regions were 15.2, 13.8 and 11.9, respectively.
The incidence of Hp-related cancers was considerably higher in males than in females (Fig. 1D). The highest incidence values were clustered in Central Italy regions. By combining the two genders, the number of Hp-related liver cancer cases/100,000 residents in Northern, Central and Southern regions were 42.0, 52.9 and 34.3, respectively.
Table SIV reports the estimates of total incident cancer cases for the whole Italy and by region and area. By cumulating the two genders, the infection-related cancers in Northern, Central, and Southern Italy accounted in 2018 for the 7.2, 7.6, and 7.1% of all cancers, respectively. The national figure was 7.3%, corresponding to 27,381 cases of infection-related cancers out of a total of 373,300 incident cases.

Discussion

The present study estimated the incidence of the most relevant cancers associated with chronic infections over the whole national territory and, individually, in the 20 Italian regions. Drawing an up-to-date picture of the epidemiological situation is important because of the evolving estimates of PAFs and of our knowledge about the infectious origin of cancer. For instance, Hp role in gastric cancer was established in the nineties, the role

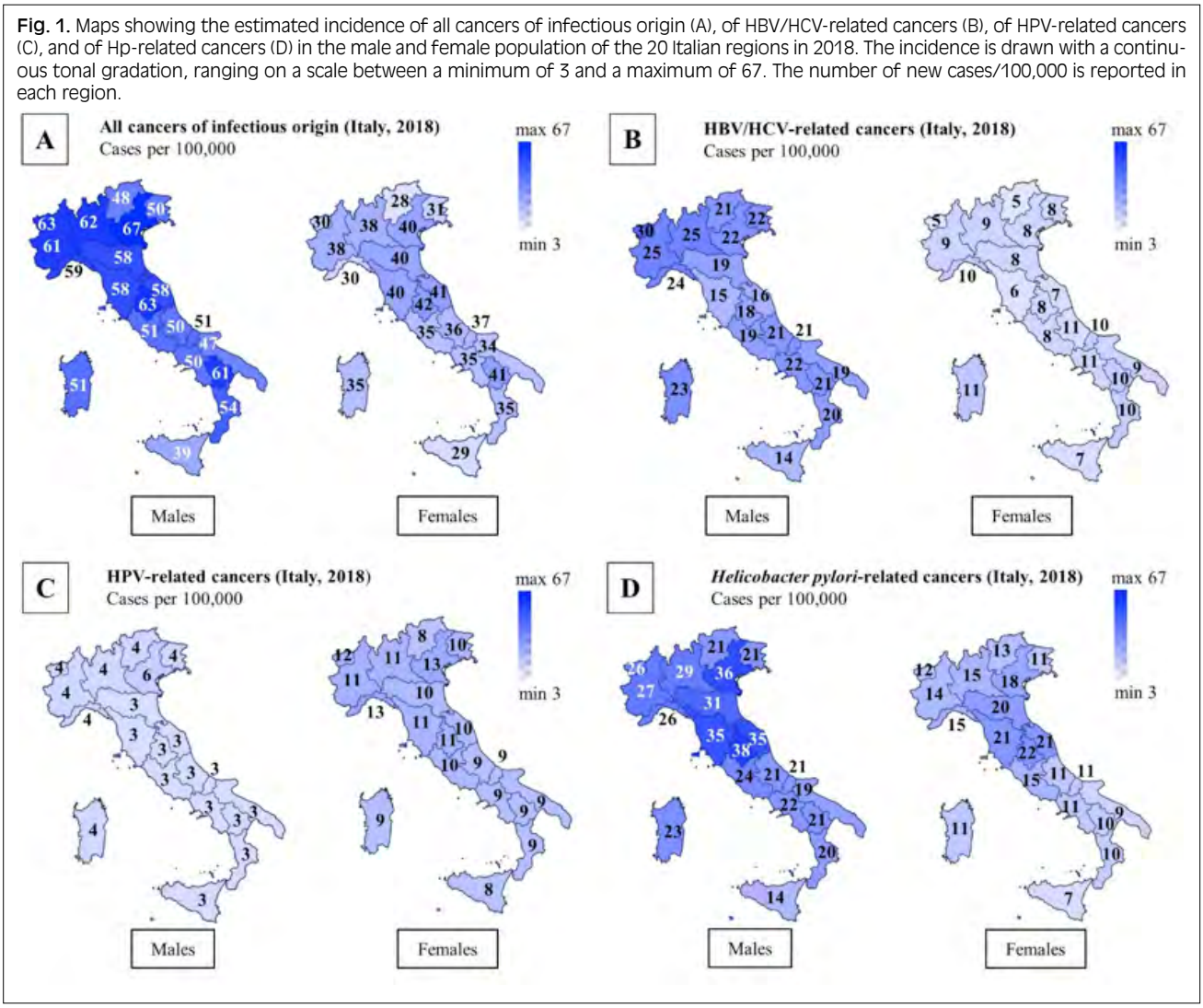


Fig. 1. Maps showing the estimated incidence of all cancers of infectious origin (A), of HBV/HCV-related cancers (B), of HPV-related cancers (C), and of Hp-related cancers (D) in the male and female population of the 20 Italian regions in 2018. The incidence is drawn with a continuous tonal gradation, ranging on a scale between a minimum of 3 and a maximum of 67. The number of new cases/100,000 is reported in each region.

of HPV in head and neck cancer has recently been assessed, and recent studies are suggesting a role for other pathogens, such as the involvement of EBV in gastric cancer development, either alone or in coinfection with Hp [28].

The overall national estimates of infection-related cancers in 2018 (present study) were slightly lower than those made in 2014 [2], with a decrease from a total of 31,238 new cases to 27,072 cases (-13%). For some cancers, such as Hp-related non-cardia stomach cancer, HPV-related uterine cervix cancer and KSHV-related Kaposi's sarcoma, the estimates were virtually overlapping in 2014 and 2018. For other cancers there were variations, mainly in the sense of a decrease, which depended either on technical issues or on the lower AFs adopted recently.

The most paradigmatic example of AF resizing is provided by liver cancer attributable to hepatotropic viruses. In the previous study, we adopted an AF of 0.87, which was based on an estimate made in Italy in 2001 [29]. Already at that time, HCV infections overwhelmed HBV infections in the causation of primary hepatocellular carcinoma in Italy [29]. Later on, the scenario has further evolved, with a progressively increasing contribution of non-viral factors and a decrease of the role of the viral etiology, which went down to 67.6% in the quinquennium 2010-2014, with the contribution of 8.6% by HBV (± HDV), 47.3% by HCV, 1.5% by HBV + HCV, and 10.5% classified as multi-etiology [22]. Indeed, as inferred from 770,000 cases of liver cancer occurring worldwide in 2012, the contribution of these hepatotropic viruses to liver cancer is sharply variable according to the geographical area, HBV causing approximately 2 of 3 liver cancers in developing countries but 1 in 4 cases in more developed countries [30]. Such a trend appears to be even more pronounced in Italy. Screening for HBV and HCV was introduced to reduce transmission among high risk groups. HCV screening has gained increasing diffusion because of the potential of antiviral drugs to eliminate the infection, which led WHO to pose the objective of eradication in 2030 [31].

Hp, HPV, HBV and HCV together accounted for the 92% of all infection-attributable cancers diagnosed both in the world in 2012 [7] and in Italy in 2018. However,

there are sharp differences in the overall contribution of the above agents to the incident cancers of infectious origin, which in the world and in Italy was 14.8% and 6.8%, respectively. In particular, Hp, HBV + HCV, and HPV were responsible on a global scale for the 5.4, 4.8, and 5.0% of all incident cancer cases [7], whereas in Italy they were responsible for the 3.2, 2.3, and 1.3% of cases, respectively. Another important difference is that the total numbers of infection-related cancers worldwide was equal in males and females, i.e. 1,100,000 cases each in 2012 [7], whereas in Italy there were in 2018 many more cases in males (16,000) than in females (11,000). This is likely to be ascribed to the fact that the most pronounced difference between the world and the Italian situation is related to HPV-related cancers, which displayed a female/male ratio of 3.2 in Italy and of 8.6 in the world [7]. Such a difference reflects the fact that in Italy there is a broad application both of primary prevention measures (anti-HPV vaccination) and of secondary prevention by means of oncological screenings (PAP test and HPV-DNA test).

There were evident variations in cancer incidence between the North, Centre and South of Italy and among the 20 regions, with some characteristic clusters of cancers according to the geographical localization. Thus, there was a general trend to a North to South downward gradient for all cancers of infectious origin, and especially to a lower incidence in Southern Italy. As to HBV/HCV-related liver cancer cancers, the maximum incidence was detected in Northwestern regions, at least in males, and the lowest incidence was in Central Italy. It should be noted that sub-regional variations may also occur. For instance, within the Campania region in 1998-2002 the incidences of liver cancer recorded by the Naples CR in the male and female populations were 34.8 and 10.2 cases/100,000 residents, respectively, whereas the corresponding values recorded in the nearby Salerno CR were 14.6 and 6.2 cases/100,000 residents, respectively [32, 33]. A moderate North to South downward gradient occurred in the incidence of HPV-related cancers. The incidence of Hp-related cancers was remarkably high in Central Italy regions, which confirms the observation that gained the definition of "gastric cancer belt" in Central Italian regions [34].

A statistically significant correlation ($r = 0.893$, $P < 0.001$) was detected between the estimated 2018 incidence data of HBV/HCV and Hp-related cancers in the male and female population of Northern, Central and Southern Italy, as detected in the present study, and the corresponding mortality data for liver and stomach cancers for the period 2010-2014, standardized on the European population [19]. It is exceedingly difficult to relate cancer incidence to the incidence of the related infections in each region, also because these cancers have a long latency time, and the information about the geographical distribution of the corresponding infections in the past is scanty. Furthermore, many of these infections are not clinically apparent and therefore they are just detectable by implementing *ad hoc* designed screening programs. For instance, HPV may be harbored in a latent state for 20 years or longer before manifesting as a precancerous lesion of the cervix [35], and the latent period between infection with hepatotropic viruses and primary hepatocellular carcinoma is in the order of decades [36].

Assessing the incidence of infection-related cancers has two intrinsic limitations. The first critical point is that we had to extrapolate incidence data to the whole population. The second one is that, for each infection-related cancer, AFs may vary depending on the regional area and may not necessarily be uniform over the whole national territory, depending on several local variables.

Conclusions

In conclusion, our estimates suggest that cancers of infectious origin contributed to the overall burden of cancer in Italy with more than 27,000 new cases, which represent the 7.3% of all incident cancer cases in 2018. This figure is comparable to the fraction of new cancer cases diagnosed in Europe in 2012 that was ascribed to infections, which accounted for the 7.2% of all cancer cases [7].

There were differences highlighting a higher incidence of these cancers in males, with the exception of HPV-related cancers. Regional variations and geographical clusters of cancer cases were evident, depending on the type of cancer and on the gender. Nevertheless, the overall figures were rather similar, the infection-related cancers accounting for the 7.2, 7.6, and 7.1% of all cancers in Northern, Central, and Southern Italy, respectively.

Acknowledgements

The study was carried out under the auspices of the Cancer Prevention/Oncological Screenings Working Group of the Italian Society of Hygiene, Preventive Medicine and Public Health (SItI).

Funding sources: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

SDF: study conception, design, and writing of the manuscript; SLM: data analysis and interpretation and statistical analysis; EC: interpretation of data and manuscript review; LM: contribution to the design of the study; FB: statistical analysis; FS: interpretation of data and manu-

script review; CB: collection of data, estimate of incidence and interpretation.

References

[1] IARC/WHO. Biological agents. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, IARC 2012;100B:1-441.

[2] De Flora S, Crocetti E, Bonanni P, Ferro A, Vitale F. Vaccines and Cancer Prevention/Screening Working Groups of the Italian Society of Hygiene, Preventive Medicine and Public Health (SItI). Incidence of infection-associated cancers in Italy and prevention strategies. Epidemiol Prev 2015;39:14-20.

[3] De Flora S, La Maestra, S. Epidemiology of cancers of infectious origin and prevention strategies. J Prev Med Hyg 2015;56:E15-20.

[4] Pisani P, Parkin D., Muñoz N, Ferlay J. Cancer and infection: estimates of the attributable fraction in 1990. Cancer Epidemiol Biomarkers Prev 1997;6:387-400.

[5] Parkin DM. The global health burden of infection-associated cancers in the year 2002. Int J Cancer. 2006;118:3030-44. https://doi.org/10.1002/ijc.21731

[6] de Martel C, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D, Plummer M. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncol 2012;13:607-15. https://doi.org/10.1016/S1470-2045(12)70137-7

[7] Plummer M, de Martel C, Vignat J, Ferlay J, Bray F, Franceschi S. Global burden of cancers attributable to infections in 2012: a synthetic analysis. Lancet Glob Healt 2016;4:e609-16. https://doi.org/10.1016/S2214-109X(16)30143-7

[8] Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst 1981;66:1191-308.

[9] Doll, R. Epidemiological evidence of the effects of behaviour and the environment on the risk of human cancer. Recent Results Cancer Res 1998;154:3-21.

[10] Doll R, Pet R. Epidemiology of cancer. In: Warrel DA, Cox TM, Firth J, eds. Oxford Textbook of Medicine. 4th edn. New York, NY: Oxford University Press 2005;3:193-218.

[11] Boffetta P, Tubiana M, Hill C, Boniol M, Aurengo A, Masse R, Valleron AJ, Monier R, de Thé G, Boyle P, Autier P. The causes of cancer in France. Ann Oncol 2009;20:550-5. https://doi.org/10.1093/annonc/mdn597

[12] Wang JB, Jiang Y, Liang H, Li P, Xiao HJ, Ji J, Xiang W, Shi JF, Fan YG, Li L, Wang D, Deng SS, Chen WQ, Wei WQ, Qiao YL, Boffetta P. Attributable causes of cancer in China. Ann Oncol 2012;23:2983-9. https://doi.org/10.1093/annonc/mds139

[13] Shield KD, Marant Micallef C, de Martel C, Heard I, Megraud F, Plummer M, Vignat J, Bray F, Soerjomataram, I. New cancer cases in France in 2015 attributable to infectious agents: a systematic review and meta-analysis. Eur J Epidemiol 2018;33:263-74. https://doi.org/10.1007/s10654-017-0334-z

[14] Xiang W, Shi JF, Li P, Wang JB, Xu LN, Wei WQ, Zhao FH, Qiao YL, Boffetta P. Estimation of cancer cases and deaths attributable to infection in China. Cancer Causes Control 2011;22:1153-61. https://doi.org/10.1007/s10552-011-9791-y

[15] Islami F, Chen W, Yu XQ, Lortet-Tieulent J, Zheng R, Flanders WD, Xia C, Thun MJ, Gapstur SM, Ezzati M, Jemal A. Cancer deaths and cases attributable to lifestyle factors and infections in China, 2013. Ann Oncol 2017;28:2567-74. https://doi.org/10.1093/annonc/mdx342

[16] Shin A, Park S, Shin HR, Park EH, Park SK, Oh JK, Lim M, Choi BY, Boniol M, Boffetta P. Population attributable fraction of infection-related cancers in Korea. Ann Oncol 2011;22:1435-42. https://doi.org/10.1093/annonc/mdq592

[17] Antonsson A, Wilson LF, Kendall BJ, Bain CJ, Whiteman DC, Neale RE. Cancers in Australia in 2010 attributable to infectious agents. Aust N Z J Public Health 2015;39:446-51. https://doi.org/10.1111/1753-6405.12445

[18] ISTAT, Demografia in cifre. Available at: www.demo.istat.it. Access on 20/07/2019.

[19] AIOM (Associazione Italiana di Oncologia Medica) / AIRTUM (Associazione Italiana dei Registri Tumori)/Fondazione Passi. The Numbers of Cancer in Italy 2018 (in Italian). Brescia, Italy: Intermedia Editore 2018, pp. 1-344.

[20] Kim HJ, Fay MP, Feuer EJ, Midthune DN. Permutation tests for join-point regression with applications to cancer rates. Statistics in Medicine 2000;19:335-51.

[21] ISTAT central Hypothesis. Available at: www.demo.istat.it. Accessed on 20/07/2019.

[22] Bucci L, Garuti F, Lenzi B, Pecorelli A, Farinati F, Giannini EG, Granito A, Ciccarese F, Rapaccini GL, Di Marco M, Caturelli E, Zoli M, Borzio F, Sacco R, Cammà C, Virdone R, Marra F, Felder M, Morisco F, Benvegnù L, Gasbarrini A, Svegliati-Baroni G, Foschi FG, Missale G, Masotto A, Nardone G, Colecchia A, Bernardi M, Trevisani F; Italian Liver Cancer (ITA.LI.CA) group. The evolutionary scenario of hepatocellular carcinoma in Italy: an update. Liver Int 2017;37:259-70. https://doi.org/10.1111/liv.13204

[23] de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. Int J Cancer 2017;141:664-70. https://doi.org/10.1002/ijc.30716

[24] De Vuyst, H, Clifford GM, Nascimento MC, Madeleine MM, Franceschi S. Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta-analysis. Int J Cancer 2009;124:1626-36. https://doi.org/10.1002/ijc.24116

[25] Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 2005;14:467-75. https://doi.org/10.1158/1055-9965.EPI-04-0551

[26] Au WW. Life style, environmental and genetic susceptibility to cervical cancer. Toxicology 2004;198:117-20. https://doi.org/10.1016/j.tox.2004.01.022.

[27] ISTAT. Available at: https://www.istat.it/it/dati-analisi-e-prodotti/contenuti-interattivi/popolazione-residente (Accessed on 20/07/2019).

[28] Singh S, Jha HC. Status of Epstein-Barr virus coinfection with Helicobacter pylori in gastric Cancer. J Oncol 2017:3456264. https://doi.org/10.1155/2017/3456264.

[29] Stroffolini T, Sagnelli E, Mele A, Almasio P. Trends of aetiological factors of hepatocellular carcinoma in Italy. Dig Liver Dis 2005;37:985-6. https://doi.org/10.1016/j.dld.2005.07.002

[30] Maucort-Boulch D, de Martel C, Franceschi S, Plummer M. Fraction and incidence of liver cancer attributable to hepatitis B and C viruses worldwide. Int J Cancer 2018;142:2471-77. https://doi.org/10.1002/ijc.31280

[31] World Health Organization. Combating hepatitis B and C to reach elimination by 2030. Advocacy brief. 2016. Available at https://www.who.int/hepatitis/publications/hep-elimination-by-2030-brief/en/

[32] Curado MP, Edwards B, Shin HR, Storm H, Ferlay J, Heanue M, Boyle P. Cancer incidence in five continents, Vol. IX. IARC Sci Publ 2007 No. 160, Lyon: IARC.

[33] Fusco M, Girardi E, Piselli P, Palombino R, Polesel J, Maione C, Scognamiglio P, Pisanti FA, Solmone M, Di Cicco P, Ippolito G, Franceschi S, Serraino D, Collaborating Study Group. Epidemiology of viral hepatitis infections in an area of southern Italy with high incidence rates of liver cancer. Eur J Cancer 2008;44:847-53. https://doi.org/10.1016/j.ejca.2008.01.025

[34] Cislaghi C, Decarli A, La Vecchia C, Laverda N, Mezzanotte G, Smans. Dati, indicatori e mappe di mortalità tumorale: Italia, 1975-1977 (in Italian). Bologna: Pitagora 1986.

[35] Watson RA. Human papillomavirus: confronting the epidemic - A urologist's perspective. Rev Urol 2005;7:135-44.

[36] de Martel C, Maucort-Boulch D, Plummer M, Franceschi S. World-wide relative contribution of hepatitis B and C viruses in hepatocellular carcinoma. Hepatology. 2015;62(4):1190-200. https://doi.10.1002/hep.27969.

Received on November 15, 2019. Accepted on November 21, 2019.

Correspondence: Silvio De Flora, Department of Health Sciences, University of Genoa, via A. Pastore 1, 161232 Genoa, Italy - E-mail: sdf@unige.it

How to cite this article: De Flora S, La Maestra S, Crocetti E, Mangone L, Bianconi F, Stracci F, Buzzoni C. Estimates of the incidence of infection-related cancers in Italy and Italian regions in 2018. J Prev Med Hyg 2019;60:E311-E326. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1434

Tab. S1. Estimated incidence (new cases/100,000 residents) of cancers attributable to infectious agents in Northern Italy regions in 2018.

		Gender	Emilia Romagna	Friuli Venezia Giulia	Liguria	Lombardia	Piemonte	Trentino Alto Adige	Valle d'Aosta	Veneto	All Northern regions
Resident population as to January 2018		M	2,162,684	589,785	743,755	4,907,685	2,123,610	525,523	61,695	2,395,801	13,510,538
		F	2,289,945	625,753	813,226	5,128,573	2,252,255	542,125	64,507	2,509,236	14,225,620
		M+F	4,452,629	1,215,538	1,556,981	10,036,258	4,375,865	1,067,648	126,202	4,905,037	27,746,158
Population covered by Cancer Registries (%)		M+F	80	100	55	92	30	100	100	53	71
Cancer site (ICD-10)	**Infectious agent**										
Non-cardia stomach (C16.1-C16.9)	Hp	M	30.6	35.8	25.9	28.2	27.1	19.7	26.0	20.2	26.7
		F	19.6	17.8	14.3	15.1	14.0	13.0	12.4	10.9	14.6
		M+F	50.3	53.6	40.2	43.3	41.1	32.7	38.4	31.1	41.3
MALT (C88.4)	Hp	M	0.4	0.0	0.4	0.5	0.4	1.1	0.0	0.4	0.4
		F	0.5	0.0	0.3	0.3	0.2	0.3	0.0	0.2	0.2
		M+F	0.9	0.0	0.7	0.8	0.6	1.4	0.0	0.6	0.6
Total Hp-associated cancers	Hp	M	31.0	35.8	26.3	28.6	27.5	20.8	26.0	20.6	27.1
		F	20.1	17.8	14.6	15.3	14.2	13.2	12.4	11.1	14.9
		M+F	51.1	53.6	40.9	43.9	41.7	34.0	38.4	31.7	42.0
Liver (C22)	HBV (± HDV) + HCV	M	18.8	22.5	24.0	24.5	24.6	20.8	29.8	22.3	23.4
		F	7.8	7.5	10.1	9.5	9.3	4.9	5.3	7.8	7.8
		M+F	26.6	30.0	34.1	34.0	33.9	25.7	35.1	30.1	31.2
Uterine cervix (C53)	HPV	M	-	-	-	-	-	-	-	-	-
		F	7.6	9.1	10.7	8.7	9.4	5.5	9.3	7.5	8.5
		M+F	7.6	9.1	10.7	8.7	9.4	5.5	9.3	7.5	8.5
Vulva (C51)	HPV	M	-	-	-	-	-	-	-	-	-
		F	1.2	1.4	1.1	1.0	1.0	0.8	0.8	1.1	1.0
		M+F	1.2	1.4	1.1	1.0	1.0	0.8	0.8	1.1	1.0
Vagina (C52)	HPV	M	-	-	-	-	-	-	-	-	-
		F	0.7	0.8	0.8	0.7	0.7	1.0	0.0	0.7	0.7
		M+F	0.7	0.8	0.8	0.7	0.7	1.0	0.0	0.7	0.7
Penis (C60)	HPV	M	0.8	0.8	1.3	0.8	1.0	0.7	0.8	0.8	0.9
		F	-	-	-	-	-	-	-	-	-
		M+F	0.8	0.8	1.3	0.8	1.0	0.7	0.8	0.8	0.9
Oral cavity (C00-C08)	HPV	M	0.4	0.6	0.4	0.4	0.5	0.5	0.5	0.5	0.5
		F	0.3	0.3	0.3	0.2	0.3	0.2	0.3	0.3	0.3
		M+F	0.7	0.9	0.7	0.6	0.7	0.7	0.8	0.8	0.8
Oropharynx (C09-C10, C12-C14)	HPV	M	1.4	3.2	1.6	1.7	1.8	2.4	2.0	1.6	1.9
		F	0.4	0.8	0.4	0.5	0.4	0.4	0.7	0.4	0.5
		M+F	1.8	4.0	2.0	2.2	2.2	2.8	2.7	2.0	2.4
Larynx (C32)	HPV	M	0.6	1.0	0.8	0.7	0.7	0.5	1.1	0.8	0.8
		F	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1
		M+F	0.7	1.1	0.9	0.8	0.8	0.6	1.3	0.9	0.9
Total HPV-associated cancers	HPV	M	3.3	5.6	4.2	3.6	3.9	4.0	4.3	3.6	4.1
		F	10.2	12.5	13.5	11.1	11.9	8.1	11.3	10.0	11.1
		M+F	13.5	18.1	17.7	14.7	15.8	12.2	15.6	13.6	15.2
Nasopharynx (C11)	EBV (HHV4)	M	1.2	0.5	1.2	1.2	1.3	0.8	1.3	0.8	1.0
		F	0.4	0.5	0.4	0.4	0.4	0.4	0.0	0.4	0.4
		M+F	1.6	1.0	1.6	1.6	1.7	1.2	1.3	1.2	1.4
Burkitt's lymphoma (C83.7)	EBV (HHV4) ± Pf	M	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1
		F	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0
		M+F	0.1	0.2	0.1	0.1	0.2	0.0	0.0	0.1	0.1
Hodgkin's lymphoma (C81)	EBV (HHV4)	M	1.6	1.3	1.8	1.4	1.4	1.2	0.0	1.6	1.3
		F	1.1	1.2	0.8	1.2	1.2	1.0	0.6	1.4	1.1
		M+F	2.7	2.5	2.6	2.6	2.6	2.2	0.6	3.0	2.4
Total EBV-associated cancers	EBV	M	2.9	2.0	3.1	2.8	2.8	1.9	1.3	2.5	2.4
		F	1.5	1.8	1.2	1.6	1.6	1.4	0.6	1.8	1.4
		M+F	4.4	3.8	4.3	4.4	4.4	3.3	1.9	4.3	3.8
Kaposi's sarcoma (C46)	KSHV (HHV8)	M	2.2	1.4	1.6	2.2	2.6	1.0	1.6	1.3	1.7
		F	0.8	0.0	1.1	0.8	0.9	0.6	0.0	0.2	0.6
		M+F	3.0	1.4	2.7	3.0	3.5	1.6	1.6	1.5	2.3

Adult T cell lymphoma/ leukemia (C91.5)	HTLV1	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		M+F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
All the above cancers		M	58.2	67.1	59.0	61.6	61.4	48.5	63.0	50.2	58.7
		F	40.4	39.6	30.3	38.3	37.9	28.3	29.5	31.0	35.7
		M+F	98.6	106.7	89.3	99.9	99.3	76.8	92.5	81.2	94.4

Hp: *Helicobacter pylori;* HBV: hepatitis B virus; HCV: hepatitis C virus; HDV: hepatitis D virus (Delta agent); HPV: human papillomavirus; EBV: Epstein-Barr virus; HHV4: human herpesvirus 4; PF: *Plasmodium falciparum*; KSKV: Kaposi's sarcoma virus; HHV8: human herpesvirus 8; HTLV: human T-lymphotropic virus; M: males; F: females.

Tab. SII. Estimated incidence (new cases/100,000 residents) of cancers attributable to infectious agents in Central Italy regions in 2018.

		Gender	Lazio	Marche	Toscana	Umbria	All Central regions
Resident population as to January 2018		M	2,848,727	743,645	1,803,203	425,547	5,821,122
		F	3, 047,966	788,108	1,933,765	459,093	6,228,932
		M+F	5,896,693	1,531,753	3,736,968	884,640	12,050,054
Population covered by Cancer Registries (%)		M+F	15	0	33	100	25
Cancer site (ICD-10)	**Infectious agent**						
Non-cardia stomach (C16.1-C16.9)	Hp	M	24.0	34.1	34.3	37.2	32.4
		F	14.4	20.9	20.9	21.9	19.6
		M+F	38.4	55.0	55.2	59.1	52.0
MALT (C88.4)	Hp	M	0.4	0.6	0.6	0.5	0.6
		F	0.3	0.4	0.3	0.3	0.3
		M+F	0.7	1.0	0.9	0.8	0.9
Total Hp-associated cancers	Hp	M	24.4	34.7	34.9	37.8	33.0
		F	14.7	21.3	21.3	22.2	19.9
		M+F	39.1	56.0	56.2	60.0	52,9
Liver (C22)	HBV (± HDV) +HCV	M	18.8	16.4	15.1	18.4	17.2
		F	7.9	6.9	6.4	7.6	7.2
		M+F	26.7	23.3	21.5	26.0	24.4
Uterine cervix (C53)	HPV	M	-	-	-	-	
		F	7.6	8.1	8.8	8.7	8.3
		M+F	7.6	8.1	8.8	8.7	8.3
Vulva (C51)	HPV	M	-	-	-	-	
		F	1.0	1.2	1.1	1.3	1.2
		M+F	1.0	1.2	1.1	1.3	1.2
Vagina (C52)	HPV	M	-	-	-	-	
		F	0.6	0.6	0.5	0.7	0.6
		M+F	0.6	0.6	0.5	0.7	0.6
Penis (C60)	HPV	M	0.9	1.2	1.1	1.3	1.1
		F	-	-	-	-	
		M+F	0.9	1.2	1.1	1.3	1.1
Oral cavity (C00-C08)	HPV	M	0.4	0.4	0.4	0.5	0.4
		F	0.2	0.2	0.2	0.2	0.2
		M+F	0.6	0.6	0.6	0.7	0.6
Oropharynx (C09-C10, C12-C14)	HPV	M	1.2	0.9	1.0	0.9	1.0
		F	0.3	0.2	0.2	0.3	0.3
		M+F	1.5	1.1	1.2	1.2	1.3
Larynx (C32)	HPV	M	0.7	0.6	0.7	0.6	0.7
		F	0.1	0.1	0.1	0.1	0.1
		M+F	0.8	0.7	0.8	0.7	0.8
Total HPV-associated cancers	HPV	M	3.1	3.2	3.2	3.3	3.2
		F	9.7	10.4	11.0	11.3	10.6
		M+F	12.8	13.6	14.2	14.6	13.8
Nasopharynx (C11)	EBV (HHV4)	M	1.2	0.9	1.0	0.8	1.0
		F	0.4	0.5	0.4	0.7	0.5
		M+F	1.6	1.4	1.4	1.5	1.5
Burkitt's lymphoma (C83.7)	EBV (HHV4) ± Pf	M	0.1	0.1	0.0	0.1	0.1
		F	0.0	0.1	0.1	0.0	0.1
		M+F	0.1	0.2	0.1	0.1	0.2
Hodgkin's lymphoma (C81)	EBV (HHV4)	M	1.6	1.8	1.9	1.7	1.7
		F	1.2	1.2	1.0	1.4	1.2
		M+F	2.8	3.0	2.9	3.1	2.9
Total EBV-associated cancers	EBV	M	2.8	2.7	2.9	2.5	2.7
		F	1.5	1.8	1.4	2.2	1.7
		M+F	4.31	4.5	4.3	4.7	4.4
Kaposi's sarcoma (C46)	KSHV (HHV8)	M	2.0	1.1	1.6	0.7	1.3
		F	0.8	0.3	0.2	0.2	0.4
		M+F	2.8	1.4	1.8	0.9	1.7
Adult T cell lymphoma/leukemia (C91.5)	HTLV1	M	0.0	0.1	0.2	0.2	0.1
		F	0.0	0.0	0.1	0.0	0.0
		M+F	0.0	0.1	0.3	0.2	0.1

All the above cancers		M	51.3	58.2	57.8	62.8	57.5
		F	34.6	40.6	40.4	43.4	39.7
		M+F	85.9	98.8	98.2	106.2	97.2

Hp: *Helicobacter pylori;* HBV: hepatitis B virus; HCV: hepatitis C virus; HDV: hepatitis D virus (Delta agent); HPV: human papillomavirus; EBV: Epstein-Barr virus; HHV4: human herpesvirus 4; PF: *Plasmodium falciparum*; KSKV: Kaposi's sarcoma virus; HHV8: human herpesvirus 8; HTLV: human T-lymphotropic virus; M: males; F: females.

Tab. SIII. Estimated incidence (new cases/100,000 residents) of cancers attributable to infectious agents in Southern Italy regions in 2018.

		Gender	Abruzzo	Basilicata	Calabria	Campania	Molise	Puglia	Sardegna	Sicilia	All Southern regions
Resident population as to January 2018		M	641,185	278,882	959,437	2,841,049	152,228	1,967,751	810,072	2,445,343	10,095,947
		F	674,011	288,236	997,250	2,985,811	156,265	838,104	2,581,646	10,601,814	
		M+F	1,315,196	567,118	1,956,687	5,826,860	308,493	4,048,242	1,648,176	5,026,929	20,697,761
Population covered by Cancer Registries (%)		M+F	0	100	64	71	0	54	42	91	65
Cancer site (ICD-10)	**Infectious agent**										
Non-cardia stomach (C16.1-C16.9)	Hp	M	20.0	30.0	24.4	19.9	20.5	17.6	17.8	16.1	20.8
		F	13.1	17.9	12.5	12.8	13.7	11.9	11.8	10.2	13.0
		M+F	33.1	47.9	36.9	32.7	34.2	29.5	29.6	26.3	33.8
MALT (C88.4)	Hp	M	04	0.00	0.2	0.3	0.5	0.5	0.7	0.3	0.4
		F	0.2	0.5	0.4	0.2	0.0	0.3	0.4	0.2	0.3
		M+F	0.6	0.5	0.6	0.5	0.5	0.8	1.1	0.5	0.7
Total Hp-associated cancers	Hp	M	20.3	30.0	24.6	20.2	21.0	18.1	18.5	16.4≤	21.1
		F	13.3	18.4	12.9	13.0	13.7	12.1	12.1	10.3	13.2
		M+F	33.6	48.4	37.5	33.2	34.7	30.2	30.6	26.7	34.3
Liver (C22)	HBV (± HDV) + HCV	M	20.8	21.5	20.4	22.2	21.0	19.4	22.5	14.3	20.3
		F	10.7	9.9	10.1	10.8	11.3	9.2	10.7	7.5	10.0
		M+F	31.5	31.4	30.5	33.0	32.3	28.6	33.2	21.8	30.3
Uterine cervix (C53)	HPV	M	-	-	-	-	-	-	-	-	-
		F	7.1	8.0	7.4	6.8	7.0	6.9	7.0	6.9	7.1
		M+F	7.1	7.98	7.42	6.80	7.04	6.87	7.04	6.86	7.14
Vulva (C51)	HPV	M	-	-	-	-	-	-	-	-	-
		F	1.0	0.9	1.1	0.9	1.1	1.1	0.8	0.9	1.0
		M+F	1.0	0.9	1.1	0.9	1.1	1.1	0.8	0.9	1.0
Vagina (C52)	HPV	M	-	-	-	-	-	-	-	-	
		F	0.5	0.3	0.2	0.5	0.5	0.5	0.4	0.3	0.4
		M+F	0.5	0.3	0.2	0.5	0.5	0.5	0.4	0.3	0.4
Penis (C60)	HPV	M	1.2	0.7	1.2	1.0	1.3	1.0	1.0	1.1	1.1
		F	-	-	-	-	-	-	-	-	-
		M+F	1.2	0.7	1.2	1.0	1.3	1.0	1.0	1.1	1.1
Oral cavity (C00-C08)	HPV	M	0.4	0.5	0.3	0.3	0.4	0.4	0.5	0.4	0.4
		F	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2
		M+F	0.6	0.6	0.5	0.5	0.6	0.6	0.6	0.6	0.6
Oropharynx (C09-C10. C12-C14)	HPV	M	0.8	1.3	0.4	0.8	0.8	0.8	1.8	0.6	0.9
		F	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2
		M+F	1.00	1.4	0.6	1.0	1.0	1.0	2.0	0.8	1.01
Larynx (C32)	HPV	M	0.8	0.5	0.7	0.8	0.8	0.6	0.8	0.6	0.7
		F	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
		M+F	0.9	0.5	0.8	0.9	0.9	0.7	0.9	0.7	0.8
Total HPV-associated cancers	HPV	M	3.2	3.0	2.6	2.9	3.3	2.8	4.1	2.6	3.0
		F	9.0	9.3	9.1	8.6	9.1	8.9	8.7	8.4	8.9
		M+F	12.2	12.3	11.7	11.5	12.4	11.7	12.8	11.0	11.9
Nasopharynx (C11)	EBV (HHV4)	M	1.6	2.3	1.5	1.2	1.6	1.3	1.2	2.0	1.6
		F	0.5	0.6	0.2	0.4	0.5	0.4	0.3	0.6	0.4
		M+F	2.1	2.9	1.7	1.6	2.1	1.7	1.5	2.6	2.0
Burkitt's lymphoma (C83.7)	EBV (HHV4) ± Pf	M	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1
		F	0.00	0.0	0.00	0.0	0.0	0.0	0.0	0.0	0.0
		M+F	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1
Hodgkin's lymphoma (C81)	EBV (HHV4)	M	1.4	1.0	1.4	1.5	1.4	1.3	1.6	1.5	1.4
		F	1.1	1.6	1.6	1.2	1.2	1.3	1.3	1.0	1.3
		M+F	2.5	2.6	3.0	2.7	2.6	2.6	2.9	2.5	2.7
Total EBV-associated cancers	EBV	M	3.1	3.4	3.0	2.7	3.0	2.8	2.9	3.5	3.0
		F	1.5	2.2	1.8	1.6	1.7	1.7	1.6	1.6	1.7
		M+F	4.6	5.6	4.8	4.3	4.7	4.5	4.5	5.1	4.7
Kaposi's sarcoma (C46)	KSHV (HHV8)	M	3.0	3.6	3.0	2.4	2.6	4.3	3.2	2.0	3.0
		F	1.3	1.0	0.8	1.1	1.3	2.2	1.4	0.7	1.2
		M+F	4.3	4.6	3.8	3.5	3.9	6.4	4.6	2.7	4.2

Adult T cell lymphoma/ leukemia (C91.5)	HTLV1	M	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
		F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		M+F	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
All the above cancers		M	50.3	61.4	53.5	50.3	50.8	47.4	51.4	38.8	50.5
		F	35.9	40.9	34.6	35.0	37.0	34.0	34.6	28.5	35.1
		M+F	86.2	102.3	88.1	85.4	87.8	81.4	86.0	67.3	85.6

Hp: Helicobacter pylori; HBV: hepatitis B virus; HCV: hepatitis C virus; HDV: hepatitis D virus (Delta agent); HPV: human papillomavirus; EBV: Epstein-Barr virus; HHV4: human herpesvirus 4; Pf: Plasmodium falciparum; KSKV: Kaposi's sarcoma virus; HHV8: human herpesvirus 8; HTLV: human T-lymphotropic virus; M: males; F: females.

Tab. SIV. Estimates of total incident cancer cases and proportion of incident cancer cases attributable to the main cancer-associated infections in Italian regions in 2018.

Region	Gender	Total incident cases[a]	Infection-related cases[b]
Northern Italy			
Emilia Romagna	M	15,550	1,270 (8.2%)
	F	15,350	943 (6.1%)
Friuli Venezia Giulia	M	4,750	399 (8.4%)
	F	4,300	253 (5.9%)
Liguria	M	6,150	441 (7.2%)
	F	5,800	336 (5.8%)
Lombardia	M	33,550	3,050 (9.1%)
	F	30,650	2,001 (6.5%)
Piemonte	M	16,300	1,216 (8.1%)
	F	14,550	870 (6.0%)
Trentino Alto Adige	M	3,100	257 (8.3%)
	F	2,800	156 (5.6%)
Valle d'Aosta	M	450	40 (8.9%)
	F	350	20 (5.7%)
Veneto	M	16,759	1,215 (7.3%)
	F	15,100	789 (5.2%)
All Northern regions	M	96,600	7,987 (8.3%)
	F	88,900	5,369 (6.0%)
Central Italy			
Lazio	M	17,150	1,472 (8.6%)
	F	16,700	1,074 (6.4%)
Marche	M	5,000	437 (8.7%)
	F	4,800	325 (6.8%)
Toscana	M	12,900	1,053 (8.2%)
	F	12,000	796 (6.6%)
Umbria	M	3,050	270 (8.9%)
	F	2,900	202 (7.0%)
All Central regions	M	38,100	3,233 (8.5%)
	F	36,400	2,397 (6.6%)
Southern Italy and Islands			
Abruzzo	M	4,200	324 (7.7%)
	F	3,800	245 (6.4%)
Basilicata	M	1,850	174 (9.4%)
	F	1,400	118 (8.4%)
Calabria	M	5,850	514 (8.8%)
	F	4,500	346 (7.7%)
Campania	M	16,350	1,420 (8.7%)
	F	13,700	1,056 (7.7%)
Molise	M	1,000	77 (7.7%)
	F	900	59 (6.6%)
Puglia	M	10,850	936 (7.9%)
	F	10,750	716 (6.7%)
Sardegna	M	5,200	417 (8.0%)
	F	4,800	292 (6.1%)
Sicilia	M	13,900	956 (6.9%)
	F	13,250	746 (5.6%)
All Southern Italy and Islands	M	60,200	4,418 (8.0%)
	F	53,100	3,577 (6.7%)
All Italian regions	M	194,900	16,038 (8.2%)
	F	178,400	11,223 (6.4%)
	M+F	373,300	27,381 (7.3%)

[a] From AIOM/AIRTUM [19]. [b] From Tables SI-SIII.

Fig. S1. Geographical distribution of the 20 Italian regions.

ORIGINAL ARTICLE

Extracellular vesicles in biological fluids. A biomarker of exposure to cigarette smoke and treatment with chemopreventive drugs

A. PULLIERO[1], L. PERGOLI[2], S. LA MAESTRA[1], R.T. MICALE[1], A. CAMOIRANO[1], V. BOLLATI[2, 3], A. IZZOTTI[1, 4], S. DE FLORA[1]

[1] Department of Health Sciences, University of Genoa, Italy; [2] EPIGET LAB, Department of Clinical Sciences and Community Health, University of Milan, Italy; [3] Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Unit of Occupational Medicine, Milan, Italy; [4] IRCCS Ospedale Policlinico San Martino, Genoa, Italy

Keywords

Extracellular vesicles • Cigarette smoke • Celecoxib • Bronchoalveolar lavage fluid • Blood serum • Urines

Summary

Extracellular vesicles (EVs) are released from cells and enter into body fluids thereby providing a toxicological mechanism of cell-cell communication. The present study aimed at assessing (a) the presence of EVs in mouse body fluids under physiological conditions, (b) the effect of exposure of mice to cigarette smoke for 8 weeks, and (c) modulation of smoke-related alterations by the nonsteroidal anti-inflammatory drug celecoxib, a selective cyclooxygenase-2 inhibitor. To this purpose, ICR (CD-1) mice were either unexposed or exposed to cigarette smoke, either treated or untreated with oral celecoxib. EVs, isolated from bronchoalveolar lavage fluid (BALF), blood serum, and urines, were analyzed by nanoparticle tracking analysis and flow cytometry. EVs baseline concentrations in BALF were remarkably high. Larger EVs were detected in urines. Smoking increased EVs concentrations but only in BALF. Celecoxib remarkably increased EVs concentrations in the blood serum of both male and female smoking mice. The concentration of EVs positive for EpCAM, a mediator of cell-cell adhesion in epithelia playing a role in tumorigenesis, was much higher in urines than in BALF, and celecoxib significantly decreased their concentration. Thus, the effects of smoke on EVs concentrations were well detectable in the extracellular environment of the respiratory tract, where they could behave as delivery carriers to target cells. Celecoxib exerted both protective mechanisms in the urinary tract and adverse systemic effects of likely hepatotoxic origin in smoke-exposed mice. Detection of EVs in body fluids may provide an early diagnostic tool and an end-point exploitable for preventive medicine strategies.

Introduction

Extracellular vesicles (EVs) are spherical structures with a lipid bilayer, which include exosomes, having a size of 30-100 nm, microvesicles (MVs), having a size of 100-700 nm, and apoptotic bodies, having a size up to 5,000 nm, which contain cell organelles and nuclear components [1, 2]. The International Society for Extracellular Vesicles proposed a series of criteria, based on current best practice, that represent the minimal characterization of EVs [3]. Exosomes are constitutively generated, stored, and released from the endosomal system, whereas MVs are released by outward budding from the bi-lipid external membrane of cells [4, 5]. Most, if not all, cell types release EVs, which then enter the body fluids [6]. Thus, EVs are endogenous delivery carriers that transport molecules to target cells and, travelling from the cells of origin to target cells, they transfer their contents after having been internalized [7]. In such a way, they provide a mechanism of cell-to-cell communication [8] and of intercellular exchange of cell components, such as nucleic acids, cytokines, lipids and proteins. Therefore, they act as signals both in cell homeostatic processes and in pathological conditions [9], also including inflammation [10] and cancer [11]. EVs can be detected in nearly all biological fluids, such as blood, urine, saliva, cerebrospinal fluid, bronchoalveolar lavage fluid (BALF), amniotic fluid, seminal plasma, and breast milk [2]. Since their content is protected from degradation by extracellular proteases and RNases, they are highly stable in storage conditions.

EVs are of particular interest in the study of lung diseases due to the high blood flow and vascular surface area of the respiratory tract. Surface proteins play a role in EVs pharmacokinetics and in particular in their distribution to the lung [12]. These biomarkers have been investigated in lung diseases, such as pulmonary hypertension [13], chronic obstructive pulmonary diseases (COPD) [14], and lung cancer [15]. Furthermore, it has been reported that a variety of environmental and lifestyle risk factors, such as air pollutants, smoking, alcohol, obesity, nutrition, physical activity, and oxidative stress, can modulate EVs trafficking [2].

The assessment of EVs modulation in biological fluids is of great importance following exposure to cigarette smoke (CS), which is the dominant risk factor for lung cancer and, in addition, has been causally associated with the induction of other cancers affecting the respiratory tract, urinary tract, digestive system, and hematopoietic system [16]. CS is also one of the main risk

factors for other chronic degenerative diseases, such as BPCO [17]. *In vitro* studies have shown the relationships between exposure to CS and release of EVs, for instance by using human macrophages [18], human mononuclear cells, depending on Ca^{2+} mobilization [19], and cultured human bronchial epithelial cells [20]. The last effect could be prevented by the antioxidant thiols glutathione (GSH) and *N*-acetyl-L-acetylcysteine (NAC) [20], which may contribute to understand the benefits of NAC as a chemopreventive agent [21]. In addition, it was shown that circulating endothelial MVs can be assumed as a measure of early lung destruction and emphysema in cigarette smokers [22], and smoking enhanced the levels of MVs in blood cells of healthy volunteers [23]. Furthermore, the analysis of human BALF showed that smoking can alter EVs profiles [24].

The present study had various goals. The first one was to evaluate in a preclinical model, under controlled experimental conditions, the physiological background concentration and size of EVs in mouse biological fluids, including BALF, blood serum, and urines, and to determine the proportion of EVs of epithelial origin. The second goal was to assess how exposure to mainstream CS (MCS), which is inhaled by active smokers as an undiluted complex mixture, can affect the concentration of EVs in these biological fluids. These experiments were carried out by using, in part, a subset of mice that had been treated in a study evaluating the release of microRNAs (miRNAs) in the same biological fluids and, additionally, in 10 organs of mice exposed to MCS [25, 26]. A further goal was to explore whether administration of a chemopreventive agent to MCS-exposed mice may be able to further modulate the concentration of EVs in biological fluids. Since chronic inflammation plays a key role at different stages of the carcinogenesis process [27] and is crucial in CS-related carcinogenesis [28, 29], we tested the nonsteroidal anti-inflammatory drug (NSAID) celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzene-1-sulfonamide, CAS 169590-42-5). Celecoxib is a selective inhibitor of cyclooxygenase-2 (COX-2), the inducible COX isoform having a pro-inflammatory function, which is expressed in response to certain stimuli such as mitogens, cytokines and growth factors [30]). In contrast, COX-1 is the housekeeping isoform, the prostaglandins derived from COX-1 being involved in the homeostatic maintenance of the gastric mucosa. Therefore, selective COX-2 inhibitors (coxibs) seem to be safer with regard to gastric damage compared to traditional NSAIDs [31]. A rationale for using celecoxib in these experiments is that, at least in synovial fibroblasts, microparticles are able to upregulate the production of prostaglandins by inducing COX-2 [11].

The results obtained showed that: a) there were baseline differences in both size and concentration of total EVs and of EVs of epithelial origin in the 3 examined biological fluids; b) exposure to MCS significantly increased EVs concentrations but only in BALF; and c) treatment of MCS-exposed mice with celecoxib further enhanced EVs concentrations in the urines and especially in the blood serum, which presumably reflects the occurrence of toxic effects related to administration of this NSAID.

Materials and methods

Mice and experimental groups

Two-month old Swiss ICR (CD-1) mice of both genders, purchased from Harlan Laboratories (San Pietro al Natisone, Udine, Italy), were housed in Makrolon™ cages on sawdust bedding and maintained on standard rodent chow (Teklad 9607, Harlan Laboratories) and tap water *ad libitum*. The animal room temperature was 23 ± 2°C, with a relative humidity of 55% and a 12 h day-night cycle.

Sixty mice were randomly assigned to 3 experimental groups, including mice kept in filtered air for 8 weeks (Group 1, sham-exposed mice), mice exposed to MCS for 8 weeks (Group 2, MCS-exposed mice), and MCS-exposed mice treated with celecoxib for the same period of time (Group 3). Each group was composed of 10 males and 10 females.

Housing and treatments of mice were in accordance with NIH, European (2010/63 UE Directive), and institutional guidelines. The issuance of the NIH Office of Laboratory Animal Welfare (OLAW) with the University of Genoa bears the identification number A5899-01 and is effective until February 28, 2021. The IACUC protocol regarding treatment of the same mice for studying modulation of miRNAs was approved by the Fox Chase Cancer Center Committee on April 13, 2015.

Exposure to MCS and treatment with celecoxib

The 40 mice belonging to Groups 2 and 3 were exposed whole-body to the MCS generated by Kentucky 2R4F reference cigarettes (University of Kentucky, Lexington, KY), having a declared content of 9.4 mg tar, 0.73 mg nicotine, and delivering 12 mg CO each. MCS was transferred to the exposure chambers by drawing 15 consecutive puffs, each of 60 ml and lasting 6 s. Each daily session involved 6 consecutive exposures, lasting 10 min each, with 1-min intervals during which a total air change was made in order to avoid excessive accumulation of MCS and toxic effects. The average concentration of total particulate matter measured in the exposure chambers was 784 mg/m^3.

The 20 MCS-exposed mice belonging to Group 3 were treated with celecoxib, starting 3 days before the first exposure to MCS. Celecoxib was supplied by the US National Cancer Institute (NCI) via MRIGlobal (Kansas City, MO). Based on a preliminary subchronic toxicity study in Swiss H mice [32], it was decided to incorporate celecoxib in the mouse diet at the dose of 1,600 g/Kg diet, which corresponded to the 80% of the maximum dose that did not produce any body loss or sufferance or alterations in the behavior of mice after 6 weeks of treatment.

Collection of body fluids

After 8 weeks, all mice were euthanized. The 2013 AVMA guidelines on euthanasia were followed using slow

introduction of CO_2 for asphyxiation of mice. Death was confirmed by absence of respiration and/or heartbeat. Cell-free biological fluids were obtained as follows. Immediately after sacrifice, BAL was performed by intubating the trachea and lavaging with 5 ml of phosphate buffered saline (PBS) per mouse. The BAL samples were centrifuged and the supernatant fluids (BALF) were pooled within each experimental group, separately for males and females. The blood was collected by heart puncture and used for preparing serum, which was pooled within each experimental group separately for males and females. The urine was collected for 8 h and pooled from the male mice belonging to each experimental group by using metabolic cages during the day preceding euthanasia of mice. The urine was centrifuged in order to remove the sediment.

Isolation of EVs from body fluids

Differential ultracentrifugation methods were used to isolate EVs from BAL, plasma and urine samples, which remain the most widely used primary isolation procedure [33]. In particular, EVs were isolated by ultracentrifugation as described previously [1]. Briefly, blood serum and BALF samples from male and female mice were centrifuged first at 1,000 x *g* for 5 min to pellet the intact cells and then at 2,000 x *g* for10 min to discard the dead cells. The supernatants were further centrifuged at 10,000 x *g* for 30 min in order to remove cell debris. EVs were isolated from the final supernatant by ultracentrifugation at 100,000 x *g* for 1 h. The EVs pellets were resuspended in a final volume of PBS corresponding to 1:100 of the original volume. Urine samples pooled from male mice were collected and centrifuged first at 1,000 x *g* for 5 min to pellet the intact cells and then at 3,000 x *g* for 10 min at 4°C to remove cell debris. The supernatants were further centrifuged at 10,000 x *g* at 4°C for 30 min to remove large membrane fragments and other debris. Finally, the supernatants were ultracentrifuged at 110,000 x *g* for 75 min at 4°C [34]. The EVs pellets were resuspended in 400 µl PBS filtered 3 times through 0.10 µm pore size membranes (EMD Millipore, Billerica, MA, USA).

Nanoparticle Tracking Analysis (NTA)

Concentrations and size of EVs were assessed by nanoparticle tracking analysis (NTA), a technique that measures the Brownian motion of vesicles suspended in fluids and displays them in real time through a charge-coupled device (CCD) camera with high sensitivity. Using a NanoSight LM10-HS system (NanoSight Ltd., Amesbury, UK), EVs were visualized by laser light scattering. Five 30-s recordings were made for each sample. The collected data were analyzed with NTA software, which provided high-resolution particle-size distribution profiles and concentration measurements of EVs.

EVs characterization

EVs were characterized by MACSQuant analyzer flow cytometer (Miltenyi Biotec, Calderara di Reno, Bologna, Italy) according to the customer protocol. 5(6)-carboxyfluorescein diacetate *N*-succinimidyl ester (CFSE) was used to discriminate the integrity of the vesicles before the specific antibody staining. CFSE is a cell permeant non-fluorescent dye. Intracellular esterases in EVs cleave the acetate groups which results in the green fluorescent molecule carboxyfluorescein that is membrane impermeant. In particular, 60 µl sample aliquots were stained with 0.02 µM CFSE at 37°C for 20 min in the dark. The CFSE-stained sample was incubated with 6 µl monoclonal antibody CD326 (EpCAM)-APC (clone: caa7-9G8) in the dark for 20 min at 4°C. The double staining with CFSE and EPCAM antibody discriminates EVs from other contaminants, such as cell membrane fragments, and allowed us to quantify the EVs from epithelial cells. Thirty µl of double stained sample were acquired on the MACSQuant Analyzer. Due to the very low amounts of blood serum remaining after Nanosight analysis, it was possible to execute the EVs characterization analysis only in urine and BALF.

Statistical analysis

Quantitative data were expressed as the mean ± SD of 5 replicate recordings. Continuous variables were tested for normality and linearity. The comparison between the EVs concentration/size curves was made by calculating the subtended areas by adding each other histogram column values recorder for each interval size. The statistical significance of the differences between groups was evaluated by ANOVA followed by Student's *t* test for unpaired data. *P* values lower than 0.05 were regarded as statistically significant. All statistical analyses were performed by using the statistical software Statview software (Abacus Concept Inc., Berkeley, CA, USA).

Results

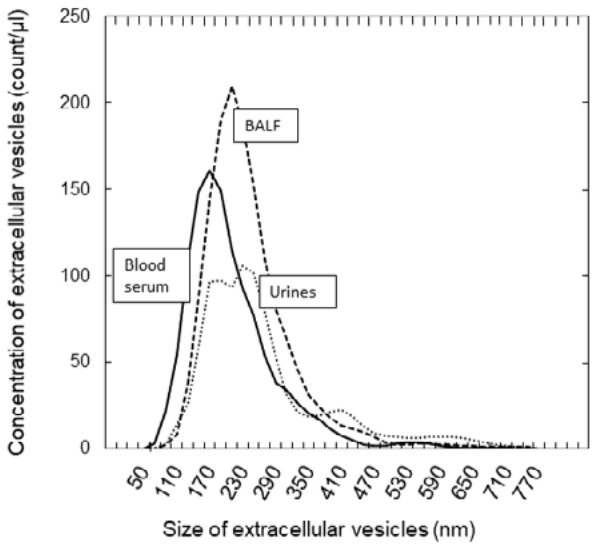
Survival and body weights

All 60 mice survived throughout duration of the experiment (8 weeks). At the beginning of the study, before starting the treatments, the body weights (means ± SE) were 38.3 ± 0.83 g in the 30 males and 28.8 ± 0.82 g in the 30 females. After 8 weeks, the body weights in males and females were 42.3 ± 1.09 g and 37.5 ± 1.16 g in Group 1 mice (sham-exposed mice), 39.4 ± 0.70 g and 31.7 ± 1.22 g in Group 2 mice (MCS-exposed mice), and 34.6 ± 1.55 g and 26.7 ± 1.16 g in Group 3 mice (MCS-exposed mice treated with celecoxib). The slight body weight loss recorded in MCS-exposed mice was statistically significant in both males ($P < 0.05$) and females ($P < 0.01$), and it was not further significantly affected by administration of dietary celecoxib.

Physiological spread of EVs into body fluids

We first evaluated comparatively the shedding of EVs into mouse BALF, blood serum, and urines under baseline conditions. To this purpose, we used sham-exposed male mice, for which all three biological fluids were available. As summarized in Figure 1, the EVs differed in the body fluids both in size and in concentration. In

Fig. 1. Concentration of EVs according to their size in the BALF (dashed line), blood serum (continuous line), and urines (dotted line) of sham-exposed male mice.



fact, the EVs curves in blood serum and BALF were unimodal, with maximum concentration peaks at a diameter of about 170 nm and 230 nm, respectively. In both cases, the curves fit a quasi-Gaussian distribution ranging between 70 and 530 nm, with a queue of larger EVs spanning until about 730 nm. Conversely, the EVs curve in the urines was multimodal, with two major peaks at 170 nm and 230 nm, and a minor peak at 430 nm, with a more abundant presence of larger EVs. As assessed by calculating the areas under the curves, the concentrations of EVs were 41,198.4/µl BALF, 23,436/µl blood serum, and 19.462.0/µl urines. The differences between blood serum and urines were not statistically significant, whereas EVs concentrations in BALF were significantly higher than those measured in each one of the other two biological fluids ($P < 0.05$ in both cases).

Concentration of EVs in body fluids as related to exposure to MCS and treatment with Celecoxib

Figure 2 shows the curves relating the size of EVs to their concentrations in the BALF and blood serum of mice of both genders and in the urines of male mice as related to exposure to MCS and treatment with celecoxib. Exposure to MCS did neither significantly affect the EVs size distribution nor their concentrations in blood serum and urines, whereas it significantly increased their concentrations in BALF, as compared with sham-exposed mice ($P < 0.001$). The oral administration of celecoxib did not further affect EVs concentrations in the BALF of MCS-exposed mice of both genders. In contrast, treatment of MCS-exposed mice with celecoxib remarkably and significantly ($P < 0.001$) increased the concentrations of EVs in the blood serum of both male and female mice as compared to either sham-exposed or MCS-exposed mice. In the urine of male MCS-exposed mice, treatment with celecoxib caused a slight but significant ($P < 0.05$) increase in the concentrations of EVs in the 150-200 nm range as compared with both sham-exposed mice and MCS-exposed mice in the absence of the COX-2 inhibitor.

Evaluation of EpCAM-positive EVs

EpCAM was characterized by flow cytometry in the BALF of both male and female mice and in the urines of male mice, as related to treatment of mice. The resulting fluorescence-activated cell sorting (FACS) scatter plots are shown in Figure 3, which relates the concentration of EpCAM-positive EVs to SSC (Side Scatter) intensity. In sham-exposed male mice, the concentration of EpCAM-positive EVs in urines was much higher than in BALF (1,249.2 *vs.* 55.1 EVs/µl, $P < 0.001$).

Following exposure to MCS, the concentration of EpCAM-positive EVs in BALF was significantly increased as compared with sham-exposed mice, both males (76.5 *vs.* 55.1 EVs/µl, $P < 0.05$) and females (102.7 *vs.* 55.4 EVs/µl, $P < 0.001$), whereas it was significantly decreased in urines (725.3 *vs.* 1,249.2 EVs/µl, $P < 0.05$).

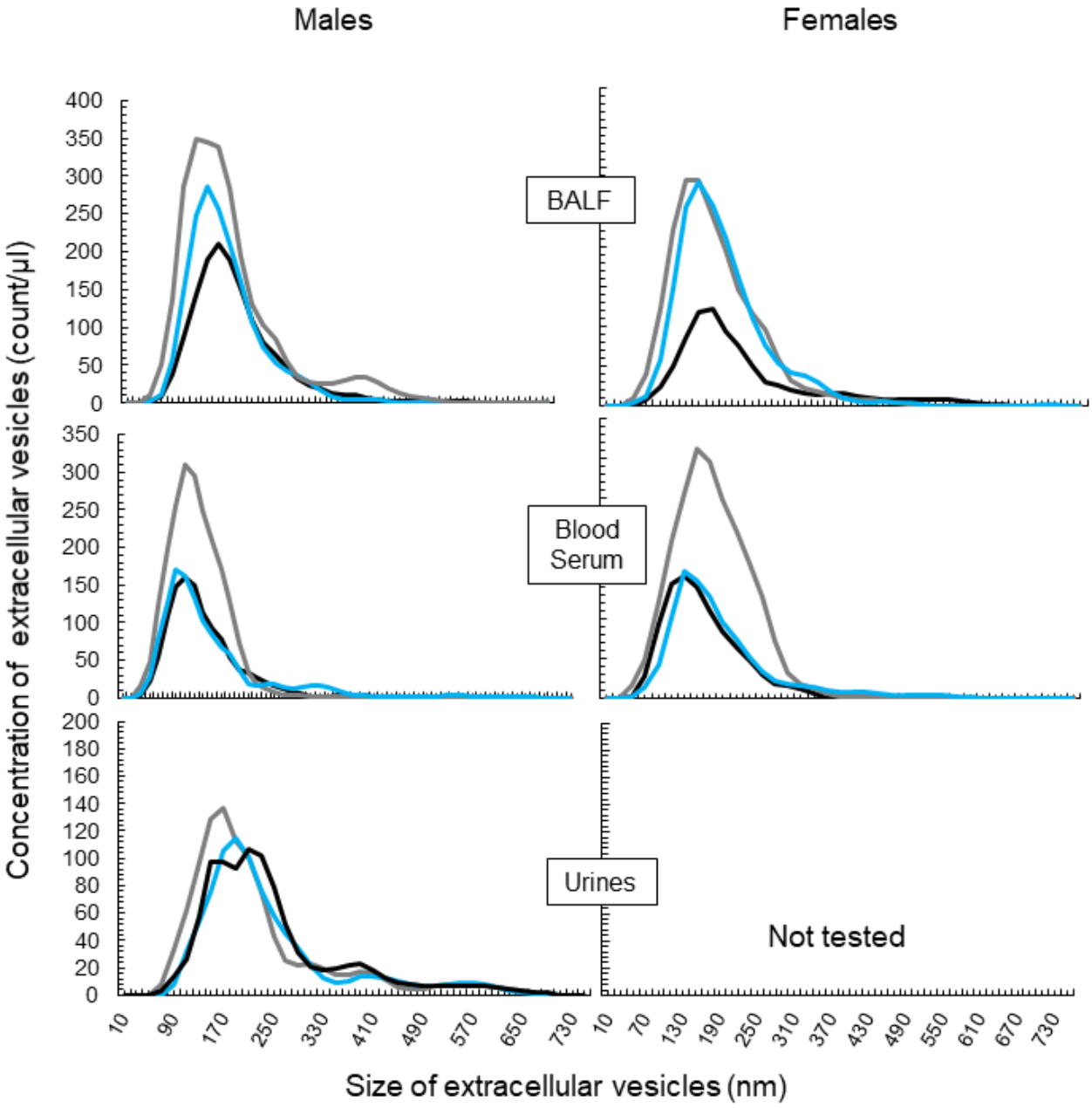
Administration of celecoxib to MCS-exposed mice did not affect the concentration of EpCAM-positive EVs in the BALF of female mice (115.2 EVs/µl) and slightly but significantly increased it in the BALF of male mice (108.3 EVs/µl, $P < 0.05$). Conversely, celecoxib significantly decreased the concentration of these EVs in the urines of male mice (478.1 EVs/µl, $P < 0.05$).

Discussion

The results obtained show that, under physiological conditions, the concentrations of EVs in the extracellular environment of the 3 examined mouse body fluids is of a similar order of magnitude, ranging between almost 20,000 particles/µl in urines and more than 40,000 particles/µl in BALF. There were some differences regarding the size of EVs, which in BALF and blood serum were mainly MVs but also contained some exosomes, whereas in urines almost all EVs were MVs and included larger vesicles having a multimodal distribution. These differences correlated with the variable proportion of EVs of epithelial origin among total EVs, as assessed by evaluating the proportion of EVs positive for EpCAM, which was much higher in the case of urines. The low proportion of EpCAM-positive EVs in BALF correlates with the finding that epithelial cells range from 0.05% to 1.5% of the total number of cells recovered in human BAL samples [35]. It is noteworthy that, besides mediating cell-cell adhesion in epithelia [36], EpCAM plays a role in tumorigenesis and metastasis of carcinomas, being expressed in most neoplastic epithelial cells [37]. In fact, normal epithelia express this cell surface glycoprotein at a variable but generally lower level than carcinomas [38].

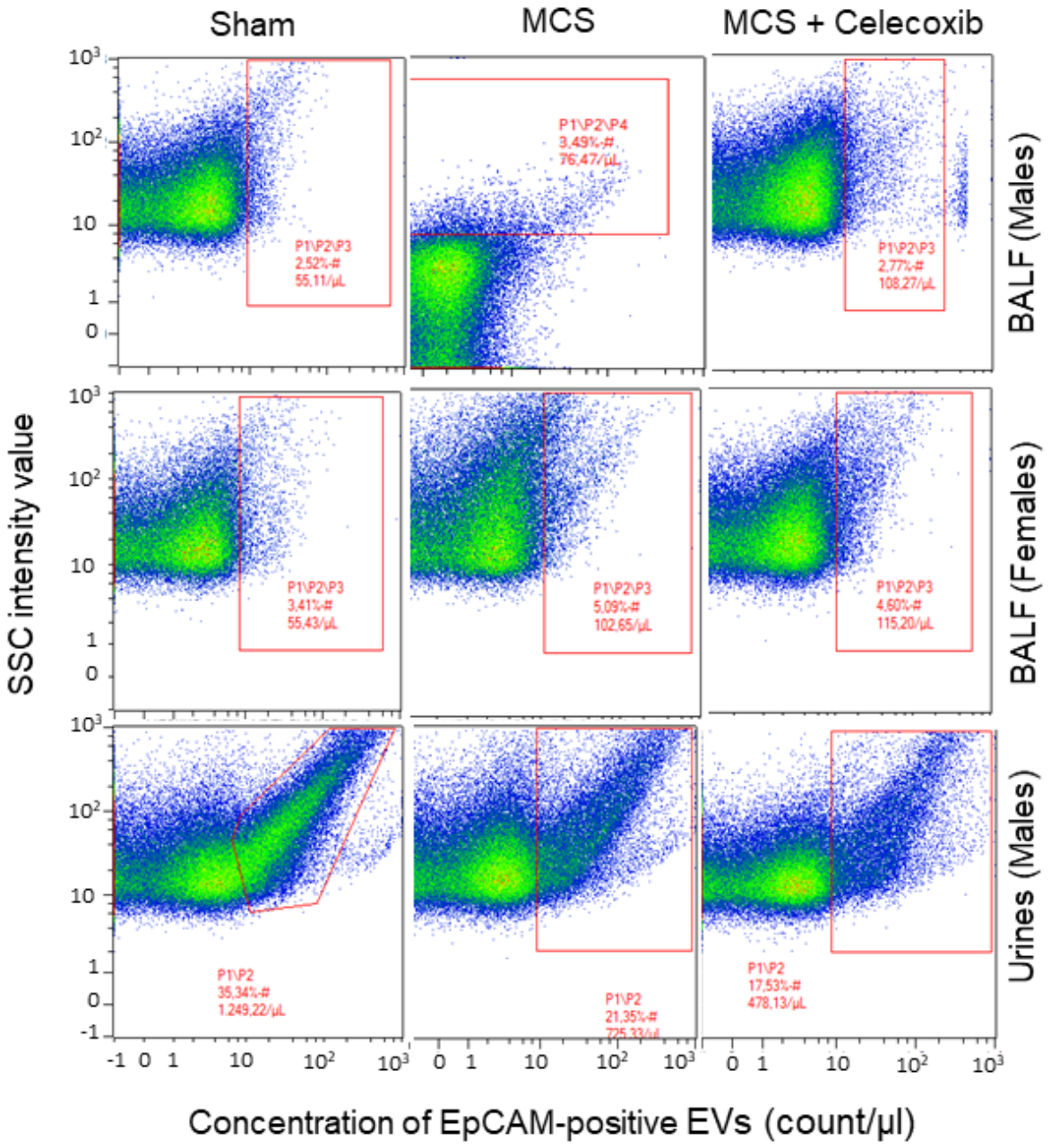
The data concerning the baseline concentrations of EVs in the examined body fluids should be related to the volumes of the same fluids. An adult mouse excretes daily

Fig. 2. Size distribution and concentration of EVs in the BALF and blood serum of mice of both genders and in the urines of male mice, either sham-exposed (black lines) or exposed to MCS (blue lines) or exposed to MCS and treated with celecoxib (gray lines).



around 0.5-1.0 ml urine and has a total blood volume of approximately 1.5 ml (https://www.nc3rs.org.uk/mouse-decision-tree-blood-sampling). More difficult are the estimates for BALF. This fluid was obtained by lavaging the lungs of each mouse with 5 ml of physiological saline and therefore it is likely that the EVs present in terminal airways were considerably diluted in the examined samples. BAL recovers the pulmonary epithelial lining fluid (ELF), which includes the surfactant and bronchial-bronchiolar secretions and, in mammals, has a relatively constant composition of about 90% lipids and 10% proteins. Surfactant lipids, which are produced by alveolar type II pneumocytes and to a lesser extent by bronchiolar non-ciliated epithelial cells, are synthesized in preformed intracytoplasmic lamellar bodies that are secreted into the aqueous subphase of ELF [39]. We have no information about the volume of ELF in mice, but in humans its volume is pretty high, having been reported to be between 37.5 and 75 ml [40]. It should be also taken into account that the EVs found in different body fluids undergo a different fate. In fact, the EVs present in urines get in contact with the mucosae of the urinary tract and thereafter are excreted from the body. Those present in the bloodstream are either eliminated through emunctory organs or transmitted to distant organs. Those found in BALF may trigger cell-to-cell communication mechanisms in bronchoalveolar cells before being removed from the lower respiratory spaces via the

Fig. 3. Analysis of EpCAM-positive extracellular vesicles in the BALF of mice of both genders and in the urines of male mice, either sham-exposed or exposed to MCS or exposed to MCS and treated with celecoxib. The x-axis reports the concentration of EpCAM-positive EVs, and the y-axis reports the SSC (Side Scatter) intensity, an indicator of granularity. The percentages and concentrations of EpCAM-positive EVs within the total EV population are reported inside the red boxes. P1 refers to all EVs, P2 to intact EVs in the 150-500 nm range, and P3 to EpCAM-positive EVs.



mucociliary escalator and being either eliminated from the body by expectoration or swallowing. Swallowed EVs are expected to get in contact with the mucosae of the GI tract, thus being either transported to the liver or ultimately eliminated with feces.

Exposure of mice to MCS resulted in a significant increase of EVs in the BALF of both male and female mice, whereas the baseline concentrations of EVs in blood serum and urines were not affected following exposure to MCS. Similar findings were observed by evaluating the miRNA profiles in 10 organs and 3 biological fluids of the same mice used in the present study [25, 26]. In fact, the miRNAs detectable in the BALF were mainly of pulmonary origin, whereas the skeletal muscle gave a striking contribution to the presence of MCS-dysregulated miRNAs in the blood serum, and the kidney was the main source of miRNAs detectable in urines. Among the examined organs, dysregulation of miRNA expression was by far most prevalent in the lung, which is consistent with the observed upregulation by MCS of proteins that defend the respiratory tract by triggering a variety of protective mechanisms, such

as antioxidant pathways, detoxification of carcinogens, DNA repair, anti-inflammatory pathways, and apoptosis. At the same time, however, MCS activates toxic and carcinogenic mechanisms, such as modulation of oncogenes and oncosuppressor genes, cell proliferation, recruitment of undifferentiated stem cells, inflammation, inhibition of intercellular communication, angiogenesis, invasion, and metastasis [41]. In addition, exposure of mice to MCS in the medium term (7.5-9 months) induces a significant increase in preneoplastic and neoplastic lesions and in other histopathological alterations, also including malignant tumors in the lung [42].

The distribution patterns of both EVs (present study) and miRNAs [25, 26] clearly reflect pharmacokinetic mechanisms. Inhaled MCS undergoes a multiorgan distribution, contains systemic toxicants, and causes cancers in about 15 human tissues [16]. Indeed, blood and urines can be used to detect some smoking-related alterations, such as increased carboxyhemoglobin levels in the blood [43] and mutagenicity of urines [44]. However, these body fluids do not appear to be suitable substrates to detect alterations of other smoking-related biomarkers, such as miRNA dysregulation [26] and shedding of EVs (present study). This is not exclusive of experimental data, but also in humans miRNA signatures in plasma do not correspond with miRNA signatures in BAL samples of lung cancer patients [45], and studies in humans have suggested that smoking alters lung EVs profiles in BALF that are expected to influence the surrounding bronchial epithelial cells [24].

Therefore, in spite of the fact that the collection of BALF is semi-invasive, the analysis of this fluid appears to be more appropriate to detect smoking-related biomarker alterations than analysis of blood serum and urine, where the alterations of pulmonary origin are confounded by contributions from other organs. In fact, BAL has widely been used in preclinical and clinical studies because this fluid contains both biochemical and cytological indicators of cellular responses to infection, cancer, or inhaled drugs or toxicants [46, 47].

A further goal of the present study was to evaluate how a putative chemopreventive agent can modulate the release of MCS-related EVs into biological fluids, which can be assumed as an indicator either of protective effects or of adverse effects. Irrespective of gender, the oral administration of celecoxib did not further affect the increase in EVs concentration in BALF caused by exposure of mice to MCS and had poor effects on the concentration of EpCAM-positive EVs in this biological fluid. This means that this selective COX-2 inhibitor failed to modulate the MCS-related release of EVs from respiratory tract cells.

On the other hand, celecoxib considerably increased the concentrations of EVs in the blood serum of mice of both genders exposed to MCS. A similar but less pronounced effect occurred in the urines of MCS-exposed male mice, which however was accompanied by a loss of EpCAM-positive EVs. Such an effect may correlate with the observed protective effects of celecoxib towards induction by MCS of preneoplastic alterations in the urinary tract of mice [32]. Moreover, celecoxib has been shown to prevent *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine-induced bladder carcinomas in rats [48]. It is noteworthy that urinary EpCAM is overexpressed in bladder tumors to such an extent that it could act as a biomarker of bladder cancer detection [49]. Thus, our results suggest that, even at an early stage, the decrease of EpCAM produced by celecoxib in urines may predict the protective effects of this drug towards MCS-induced neoplastic alterations in the urinary tract.

The increased systemic concentrations of EVs in MCS-exposed mice treated with celecoxib are likely to be related to their release from organs other than the lung, such as skeletal muscle and liver. In this light, there seems to be a relationship between upregulation of circulating EVs by celecoxib and its hepatotoxicity. In fact, the dose of celecoxib used in the present study (1,600 mg/kg diet) did not produce any apparent toxic effect in a preliminary 6-week test in smoke-free mice. However, in the long-term, this drug became toxic to MCS-exposed mice, as inferred from the decrease in survival and body weight gain as well as from some histopathological signs of hepatotoxicity in mice treated with celecoxib at the same dose [32]. It should be noted that such a dose is rather high but is comparable to the pharmacological dose in humans. In fact, a dose of 1,000 mg/kg diet of celecoxib administered to mice resulted in a plasma concentration of 1.6 μg/ml, which approximates the reported therapeutic plasma concentration of celecoxib in humans [50]. The hepatotoxicity of celecoxib observed in MCS-exposed mice is consistent with the detection of hepatocellular alterations produced by this drug in rats [51, 52]. From a mechanistic point of view, it should be taken into account that celecoxib is metabolized primarily by cytochrome P450 2C9 (CYP2C9) [53], which at the same time is involved in the metabolism of polycyclic aromatic hydrocarbons contained in CS [54]. Mediators derived from COX-2 have an important hepatoprotective function and accordingly the risk of drug-induced liver injury may be increased by COX-2 inhibition [55]. In fact, an increased clinical vigilance is required during the co-administration of celecoxib and other substrates or inhibitors of CYP2C9 [50], as it could be the case with smoking. Since drug-induced liver damage increases the number of circulating EVs [56], it may be assumed that the hepatotoxicity caused by celecoxib-induced COX-2 inhibition in MCS-exposed mice was responsible for the observed increase of EVs in blood and urines.

In conclusion, the findings of the present study shed light on the role of EVs as biomarkers of exposure to MCS, the dominant risk factor for lung cancer, other cancers, and other diseases of toxicological relevance. It has been postulated that there is immense potential for the use of EVs for biomarker detection in clinical settings [57]. Our data provide evidence that the effects of MCS on that end-point are well detectable in the extracellular environment of the lower respiratory tract, where they could behave as endogenous delivery carriers of a variety of molecules to target cells. On the other hand, the effects of MCS on EVs release into body

fluids were not discernible at a systemic level, presumably due to confounding factors such as the contribution from organs other than the lungs. The assay of a putative chemopreventive agent, the selective COX-2 inhibitor celecoxib, modulated EVs spread at a systemic level by suggesting the occurrence of both protective mechanisms in the urinary tract and adverse effects of likely hepatotoxic origin in MCS-exposed mice. It should be emphasized that these changes were detected after only 8 weeks of treatment of mice, whereas the development of MCS-related tumors and other histopathological alterations requires longer periods of time [32]. Therefore, the data regarding the spread of EVs in biological fluids may be translated to the clinical practice and taken as an early diagnostic tool and as an end-point exploitable for toxicological studies and preventive medicine strategies.

Acknowledgements

Funding sources: this work was supported by the Italian Association for Cancer Research (AIRC, grant No. IG-20699).

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

AP, AI and SDF conceived the experiments, LP, AC, AP and VB conducted the experiments, S LM and RTM collected body fluids, all authors were involved in analyzing the results, AP and SDF wrote and all authors reviewed the manuscript.

References

[1] Bonzini M, Pergoli L, Cantone L, Hoxha M, Spinazzè A, Del Buono L, Favero C, Carugn, M, Angelici L, Broggi L, Cattaneo A, Pesatori AC, Bollati V. Short-term particulate matter exposure induces extracellular vesicle release in overweight subjects. Environ Res 2017;155:228-34. https://doi.org/10.1016/j.envres.2017.02.014

[2] Neven KY, Nawrot TS, Bollati V. Extracellular vesicles: how the external and internal environment can shape cell-to-cell communication. Curr Environ Health Rep 2017;4:30-7. https://doi.org/10.1007/s40572-017-0130-7

[3] Lötvall J, Hill AF, Hochberg F, Buzás EI, Di Vizio D, Gardiner C, Gho YS, Kurochkin IV, Mathivanan S, Quesenberry P, Sahoo S, Tahara H, Wauben MH, Witwer KW, Théry C. Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. J Extracell Vesicles 2014;3:26913. https://doi.org/10.3402/jev.v3.26913

[4] Larson MC, Hillery, CA, Hogg N. Circulating membrane-derived microvesicles in redox biology. Free Radic Biol Med 2014;7:214-28. https://doi.org/10.1016/j.freeradbiomed.2014.04.017

[5] Ragni E, Banfi F, Barilani M, Cherubini A, Parazzi V, Larghi P, Dolo V, Bollati V, Lazzari L. Extracellular vesicle-shuttled mrna in mesenchymal stem cell communication. Stem Cells 2017;35:1093-105. https://doi.org/10.1002/stem.2557

[6] Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. Nat Rev Immunol 2014;14:195-208. https://doi.org/10.1038/nri3622

[7] Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L, Xiao T, Schafer J, Lee ML, Schmittgen TD, Nana-Sinkam SP, Jarjoura D, Marsh CB. Detection of microRNA expression in human peripheral blood microvesicles. PLoS One 2008;3:e3694. https://doi.org/10.1371/journal.pone.0003694. Epub 2008 Nov 11. Erratum in: PLoS One 2010;5. https://doi.org/10.1371/annotation/b15ca816-7b62-4474-a568-6b60b8959742

[8] Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol 2013;200:373-83. https://doi.org/10.1083/jcb.201211138

[9] van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol 2018;19:213-28. https://doi.org/10.1038/nrm.2017.125

[10] Prakash PS, Caldwell CC, Lentsch AB, Pritts TA, Robinson BR. Human microparticles generated during sepsis in patients with critical illness are neutrophil-derived and modulate the immune response. J Trauma Acute Care Surg 2012;73:401-6. https://doi.org/10.1097/TA.0b013e31825a776d

[11] Jüngel A, Distler O, Schulze-Horsel U, Huber LC, Ha HR, Simmen B, Kalden JR, Pisetsky DS, Gay S, Distler JH. Microparticles stimulate the synthesis of prostaglandin E(2) via induction of cyclooxygenase 2 and microsomal prostaglandin E synthase 1. Arthritis Rheum 2007;56:3564-74. https://doi.org/10.1002/art.22980

[12] Charoenviriyakul C, Takahashi Y, Morishita M, Nishikawa M, Takakura Y. Role of extracellular vesicle surface proteins in the pharmacokinetics of extracellular vesicles. Mol Pharm2018;15:1073-80. https://doi.org/10.1021/acs.molpharmaceut.7b00950

[13] Nadaud S, Poirier O, Girerd B, Blanc C, Montani D, Eyries M, Imbert-Bismut F, Pacheco A, Vigne J, Tregouet DA, Humbert M, Soubrier F. Small platelet microparticle levels are increased in pulmonary arterial hypertension. Eur J Clin Invest 2013;43:64-71. https://doi.org/10.1111/eci.12018

[14] Thomashow MA, Shimbo D, Parikh MA, Hoffman EA, Vogel-Claussen J, Hueper K, Fu J, Liu CY, Bluemke DA, Ventetuolo CE, Doyle MF, Barr RG. Endothelial microparticles in mild chronic obstructive pulmonary disease and emphysema. The Multi-Ethnic Study of Atherosclerosis Chronic Obstructive Pulmonary Disease study. Am J Respir Crit Care Med 2013;188:60-8. https://doi.org/10.1164/rccm.201209-1697OC

[15] Vykoukal J, Sun N, Aguilar-Bonavides C, Katayama H, Tanaka I, Fahrmann JF, Capello M, Fujimoto J, Aguilar M, Wistuba II, Taguchi A, Ostrin EJ, Hanash SM. Plasma-derived extracellular vesicle proteins as a source of biomarkers for lung adenocarcinoma. Oncotarget 2017;8:95466-80. https://doi.org/10.18632/oncotarget.20748

[16] International Agency for Research on Cancer. A review of human carcinogens: personal habits and indoor combustions. IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans 100, 2012 part E. IARC, Lyon, France.

[17] De Flora S, Izzotti A, D'Agostini F, La Maestra S, Micale RT, Ceccaroli C, Steele VE, Balansky R. Rationale and approaches to the prevention of smoking-related diseases: overview of recent studies on chemoprevention of smoking-induced tumors in rodent models. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2014;32:105-20. https://doi.org/10.1080/10590501.2014.907459

[18] Li CJ, Liu Y, Chen Y, Yu D, Williams KJ, Liu ML. Novel proteolytic microvesicles released from human macrophages after exposure to tobacco smoke. Am J Pathol 2013;182:1552-62. https://doi.org/10.1016/j.ajpath.2013.01.035

[19] Cordazzo C, Petrini S, Neri T, Lombardi S, Carmazzi Y, Pedrinelli R, Paggiaro P, Celi A. Rapid shedding of proinflammatory microparticles by human mononuclear cells exposed to ciga-

rette smoke is dependent on Ca2+ mobilization. Inflamm Res 2014;63:539-47. https://doi.org/10.1007/s00011-014-0723-7

[20] Benedikter BJ, Volgers C, van Eijck PH, Wouters EFM, Savelkoul PHM, Reynaert NL, Haenen GRMM, Rohde GGU, Weseler AR, Stassen FRM. Cigarette smoke extract induced exosome release is mediated by depletion of exofacial thiols and can be inhibited by thiol-antioxidants. Free Radic Biol Med 2017;108:334-44. https://doi.org/10.1016/j.freeradbiomed.2017.03.026

[21] De Flora S, Izzotti A, D'Agostini F, Balansky RM. Mechanisms of N-acetylcysteine in the prevention of DNA damage and cancer, with special reference to smoking-related end-points. Carcinogenesis 2001;22:999-1013. https://doi.org/10.1093/carcin/22.7.999

[22] Gordon C, Gudi K, Krause A, Sackrowitz R, Harvey BG, Strulovici-Barel Y, Mezey JG, Crystal RG. Circulating endothelial microparticles as a measure of early lung destruction in cigarette smokers. Am J Respir Crit Care Med 2011;184:224-32. https://doi.org/10.1164/rccm.201012-2061OC

[23] Mobarrez F, Antoniewicz L, Bosson JA, Kuhl J, Pisetsky DS, Lundbäck M. The effects of smoking on levels of endothelial progenitor cells and microparticles in the blood of healthy volunteers. PLoS One 2014;9:e90314. https://doi.org/10.1371/journal.pone.0090314

[24] Héliot A, Landkocz Y, Roy Saint-Georges F, Gosset P, Billet S, Shirali P, Courcot D, Martin PJ. Smoker extracellular vesicles influence status of human bronchial epithelial cells. Int J Hyg Environ Health 2017;220:445-54. https://doi.org/10.1016/j.ijheh.2016.12.010

[25] Izzotti A, La Maestra S, Micale RT, Pulliero A, Geretto M, Balansky R, De Flora S. Modulation of genomic and epigenetic end-points by celecoxib. Oncotarget 2018a;9:33656-81. https://doi.org/10.18632/oncotarget.26062

[26] Izzotti A, Longobardi M, La Maestra S, Micale RT, Pulliero A, Camoirano A, Geretto M, D'Agostini F, Balansky R, Miller MS, Steele VE, De Flora S. Release of microRNAs into body fluids from ten organs of mice exposed to cigarette smoke. Theranostics 2018b;8:2147-60. https://doi.org/10.7150/thno.22726

[27] Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell 2010;140:883-99. https://doi.org/10.1016/j.cell.2010.01.025

[28] Malkinson AM. Evidence that inflammation encourages pulmonary adenocarcinoma formation in mice: clinical implications. Chest 2004;125:154S-155S. https://doi.org/10.1378/chest.125.5_suppl.154s-a

[29] Takahashi H, Ogata H, Nishigaki R. Tobacco smoke promotes lung tumorigenesis by triggering IKKbeta- and JNK1-dependent inflammation. Cancer Cell 2010;17: 89-97. https://doi.org/10.1016/j.ccr.2009.12.008

[30] Mazhar D, Gillmore R, Waxman J. COX and cancer. QJM 2005;98:711-8. https://doi.org/10.1093/qjmed/hci119

[31] Wolfe F, Anderson J, Burke TA, Arguelles LM, Pettitt D. Gastroprotective therapy and risk of gastrointestinal ulcers: risk reduction by COX-2 therapy. J Rheumatol 2002;29:467-73.

[32] Balansky R, Ganchev G, Iltcheva M, Nikolov M, La Maestra S, Micale RT, D'Agostini F, Steele VE, De Flora S. Modulation by licofelone and celecoxib of experimentally induced cancer and preneoplastic lesions in mice exposed to cigarette smoke. Curr Cancer Drug Targets 2015;15:188-95. https://doi.org/10.2174/1568009615666150216170008

[33] Royo F, Zuñiga-Garcia P, Sanchez-Mosquera P, Egia A, Perez A, Loizaga A, Arceo R, Lacasa I, Rabade A, Arrieta E, Bilbao R, Unda M, Carracedo A, Falcon-Perez JM. Different EV enrichment methods suitable for clinical settings yield different subpopulations of urinary extracellular vesicles from human samples. J Extracell Vesicles 2016;15:29497. https://doi.org/10.3402/jev.v5.29497

[34] Murakami T, Oakes M, Ogura M, Tovar V, Yamamoto C, Mitsuhashi M. Development of glomerulus, tubule and collecting duct-specific mRNA assay in human urinary exosomes and microvesicles. PLoS One 2014;9:e109074. https://doi.org/10.1371/journal.pone.0109074

[35] Pollock K, Albares L, Wendt C, Hubel A. Isolation of fibroblasts and epithelial cells in bronchoalveolar lavage (BAL). Exp. Lung Re. 2013;39:146-54. https://doi.org/10.3109/01902148.2013.781720

[36] Litvinov SV, Balzar M, Winter MJ, Bakker HA, Briaire-de Bruijn IH, Prins F, Fleuren GJ, Warnaar SO. Epithelial cell adhesion molecule (Ep-CAM) modulates cell-cell interactions mediated by classic cadherins. J Cell Biol 1997;139:1337-48. https://doi.org/10.1083/jcb.139.5.1337

[37] Armstrong A, Eck SL. EpCAM: a new therapeutioc target for an old cancer antigen. Cancer Biol Ther 2003;2:320-6. https://doi.org/10.4161/cbt.2.4.451

[38] Trzpis M, McLaughlin PM, de Leij LM, Harmsen MC. Epithelial cell adhesion molecule: more than a carcinoma marker and adhesion molecule. Am J Pathol 2007;171:386-95.

[39] Slocombe R. Surfactant and its role in pulmonary disease. 1999. Available at www.the-vcrs.org/1999/CRSProceedings/SolcombePaper.DOC

[40] Effros RM. Permeability of the blood-gas barrier. In: Crystal RG, West JB, eds. The Lung: Scientific Foundations. New York: Raven Press 1991, pp. 1163-1175.

[41] De Flora S, Balansky R, D'Agostini F, Cartiglia C, Longobardi M, Steele VE, Izzotti A. Smoke-induced microRNA and related proteome alterations. Modulation by chemopreventive agents. Int J Cancer 2012;131:2763-73. https://doi.org/10.1002/ijc.27814

[42] De Flora S, Ganchev G, Iltcheva M, La Maestra S, Micale RT, Steele VE, Balansky R. Pharmacological modulation of lung carcinogenesis in smokers: preclinical and clinical evidence. Trends Pharmacol Sci 2016;37:120-42. https://doi.org/10.1016/j.tips.2015.11.003

[43] Castleden CM, Cole PV. Carboxyhaemoglobin levels of smokers and non-smokers working in the City of London. Br J Ind Med 1975;32:115-8. https://doi.org/10.1136/oem.32.2.115

[44] Camoirano A, Bagnasco M, Bennicelli C, Cartiglia C, Wang JB, Zhang BC, Zhu YR, Qian GS, Egner PA, Jacobson LP, Kensler TW, De Flora S. Oltipraz chemoprevention trial in Qidong, People's Republic of China: results of urine genotoxicity assays as related to smoking habits. Cancer Epidemiol Biomarkers Prev 2001;10:775-83.

[45] Molina-Pinelo S, Suárez R, Pastor MD, Nogal A, Márquez-Martín E, Martín-Juan J, Carnero A, Paz-Ares L. Association between the miRNA signatures in plasma and bronchoalveolar fluid in respiratory pathologies. Dis Markers 2012;32:221-30. https://doi.org/10.3233/DMA-2011-0882

[46] Hunninghake GW, Gadek JE, Kawanami O, Ferrans VJ, Crystal RG. Inflammatory and immune processes in the human lung in health and disease: evaluation by bronchoalveolar lavage. Am J Pathol 1979;97:149-206.

[47] Ballinger CA, Brand JD. Postlethwait EM. In vitro systems for studying respiratory system toxicology. In: McQueen CA, ed. Comprehensive Toxicology Oxford, UK: Elsevier Ltd 2010, pp. 243-259. https://doi.org/10.1152/ajplung.00185.2012.

[48] Sozer S, Diniz G, Lermioglu F. Effects of celecoxib in young rats: histopathological changes in tissues and alterations of oxidative stress/antioxidant defense system. Arch Pharm Res 2011;34:253-9. https://doi.org/10.1007/s12272-011-0211-3

[49] Bryan RT, Shimwell NJ, Wei W, Devall AJ, Pirrie SJ, James ND, Zeegers MP, Cheng KK, Martin A, Ward DG. Urinary EpCAM in urothelial bladder cancer patients: characterisation and evaluation of biomarker potential. Br J Cancer 2014;110:679-85. https://doi.org/10.1038/bjc.2013.744

[50] Davies NM, McLachlan AJ, Day RO, Williams KM. Clinical pharmacokinetics and pharmacodynamics of celecoxib: a selective cyclo-oxygenase-2 inhibitor. Clin Pharmaco-

kinet 2000;38:225-42. https://doi.org/10.2165/00003088-200038030-00003

[51] Liu H, Wei W, Li X. Celecoxib exacerbates hepatic fibrosis and induces hepatocellular necrosis in rats treated with porcine serum. Prostaglandins Other Lipid Mediat 2009;88:63-7. https://doi.org/10.1016/j.prostaglandins.2008.10.002

[52] Koçkaya EA, Selmanoğlu G, Kismet K. Pathological and biochemical effects of therapeutic and supratherapeutic doses of celecoxib in Wistar albino male rats. Drug Chem Toxicol 2010;33:410-4. https://doi.org/10.3109/01480540903575691

[53] Winterhalder RC, Hirsch FR, Kotantoulas GK, Kotantoulas GK, Franklin WA, Bunn PAJr. Chemoprevention of lung cancer. From biology to clinical reality. Ann Oncol 2004;15:185-96. https://doi.org/10.1093/annonc/mdh051

[54] Tranah GJ, Chan AT, Giovannucci E, Ma J, Fuchs C, Hunter DJ. Epoxide hydrolase and CYP2C9 polymorphisms, cigarette smoking, and risk of colorectal carcinoma in the Nurses' Health Study and the Physicians' Health Study. Mol Carcinog 2005;44:21-30. https://doi.org/10.1002/mc.20112

[55] Reilly TP, Brady JN, Marchick MR, Bourdi M, George JW, Radonovich MF, Pise-Masison CA, Pohl LR. A protective role for cyclooxygenase-2 in drug-induced liver injury in mice. Chem Res Toxicol 2001;14:1620-18. https://doi.org/10.1021/tx0155505

[56] Royo F, Palomo L, Mleczko J, Gonzalez E, Alonso C, Martínez I, Pérez-Cormenzana M, Castro A, Falcon-Perez JM. Metabolically active extracellular vesicles released from hepatocytes under drug-induced liver-damaging conditions modify serum metabolome and might affect different pathophysiological processes. Eur J Pharm Sci 2017;98:51-7. https://doi.org/10.1016/j.ejps.2016.10.020.

[57] Boukouris S, Mathivanan S. Exosomes in bodily fluids are a highly stable resource of disease biomarkers. Proteomics Clin 2015;9:358-67. https://doi.org/10.1002/prca.201400114

Received on May 14, 2019. Accepted on October 25, 2019.

Correspondence: Alberto Izzotti, Department of Health Sciences, University of Genoa, via A. Pastore 1, 16132 Genoa, Italy - Tel. +39 010 3538522 - Fax +39 010 3538482 - E-mail: izzotti@unige.it

How to cite this article: Pulliero A, Pergoli L, La Maestra S, Micale RT, Camoirano A, Bollati V, Izzotti A, De Flora S. Extracellular vesicles in biological fluids. A biomarker of exposure to cigarette smoke and treatment with chemopreventive drugs. J Prev Med Hyg 2019;60:E327-E336. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1284

ORIGINAL ARTICLE

Knowledge about cancer screening programmes in Sardinia

A. MEREU[1], F. CONCU[2], C. DESSÌ[3], M. GIRAU[3], M.T. IONTA[4], L. LAI[3], A. LIORI[3], M. MASALA[2], C.D. MCGILLIARD[3], P. ORIGA[1], M.F. PIAZZA[2], L. PISANU[3], B. SOGGIU[3], A. SOTGIU[1], P. CONTU[1], C. SARDU[1]
[1] Department of Medical Science and Public Health, University of Cagliari, Italy; [2] Medicine and Surgery Faculty, University of Cagliari, Italy; [3] Specialization School of Hygiene and Preventive Medicine, University of Cagliari, Italy; [4] Oncology Structure of University Hospital of Cagliari, Italy

Keywords

Knowledge • Attendance • Cancer screening • Communication programme

Summary

Background. *High level of attendance by population is considered a proof of the efficacy in the screening programmes. Public health aims to increase people's attendance to cancer screening. The study aimed at assessing the level of knowledge and awareness about screening of citizens in Cagliari, from June to July 2016.*
Methods. *Recruitment took place near the atrium of the two main shopping centres of the city. The sample included 270 adults (138 men), 18-75 years old (mean age 46 years old). The information gathered from interviews were categorized by dichotomizing answers according to the knowledge and understanding of the discussed topics. Descriptive analysis was performed. The Chi-square test was used to assess gender and educational differences.*
Results. *Results show that population's knowledge of screening is limited. Although the word "screening" is known, only half of the people who declared to have heard of this word know about the aim of screening. Colorectal cancer screening is the least known. Men and people with lower education are less informed than women and those with high education level.*
Conclusion. *In order to raise knowledge and awareness about cancer screening, special attention should be paid to communication and to the use of plain language. Future action should highlight the benefit of the screening procedure and thus contributing to spread the cancer prevention culture. Gender and socioeconomic inequalities must be taken into account when planning screening communication campaigns. General practitioner are highly trusted by people. They could play a decisive role to promote screening attendance.*

Introduction

Cancer is a leading cause of global deaths after cardiovascular diseases. Because of disabling effects, cancer is now one of the most important priorities in terms of health. The World Health Organization (WHO) has reported that almost two-thirds of all cancers could be prevented or fully cured through regular cancer screening, combined with appropriate healthy lifestyles. Furthermore WHO has recommended that national efforts to screen for cancer should be enhanced [1].
The National Health Prevention Plan [2] promotes cancer screening and offers three screening programmes completely free of charge:

- screening for cervical cancer (Pap test), for women aged between 25 and 64 years, who are recalled every three years;
- screening for breast cancer (mammography) for women aged between 50 and 69 years, who are recalled every two years;
- screening for colorectal cancer (fecal occult blood test, FOBT), for both men and women, aged between 50 and 69 years, who are recalled every two years.

The Italian Ministry of Health assigned to the Osservatorio Nazionale Screening (National Observatory of screening - ONS) the coordination, monitoring, technical and scientific support to the Italian regions for planning and conducting population based organized screening in Italy [3]. The regional administrations are accountable for organizing, managing, assuring quality of screening programmes.
The efficacy and efficiency of screening programmes are influenced by a high level of participation in order to achieve a significant impact on population health [4, 5].
The report of the ONS shows a snapshot that describes the screening attendance rate in Italy, by indicating that the coverage of the organized screening is uneven on the national territory. In 2017, in Northern regions, the attendance at organized screening for cervical cancer was 51%, 37,4% in the central regions, and 28% in the Southern regions. The attendance to organized screening compliance for breast cancer reached 63% in the Northern regions, 53% in the Central regions, and 41% in the Southern regions. In Northern regions, organized screening compliance for colorectal cancer (fecal occult blood test) is around 52%, 35% in the Central regions, and 24% in the Southern regions [6, 7].
One of the goals of the Regional prevention plan of Sardinia is to increase the attendance of target population to the three organized cancer screening programmes [8]. The plan highlights the importance of analysing and defining the information needs, as well as the importance

of providing information and communicating with citizens about cancer screening.
Until now, in Sardinia, the communication campaigns were based on two main tools:

- an invitation letter addressing the target population;
- posters and brochures, available at any time on the institutional website of the Sardinia Health System, the website is accessible both to target and general population, and it provides helpful information and explanation about cancer screening.

Both tools try to answer to the most common questions concerning cancer features and screening procedures, also providing general information for specific cancer screening: breast, cervical and colorectal screening [9].
The high level of attendance of the informed population in the organized screening programme is one of the factors making possible the effectiveness of the screenings [2]. This ambitious goal can be reached through an informed and aware participation of every person. Moreover, before the attendance, people should know the benefits and the disadvantages of the screening programmes [4, 10-12].
An effective communication programme has to take into account citizens' limited health literacy, since a positive association between inadequate health literacy and low attendance at cancer screening is widely demonstrated [13-16].
According to the WHO recommendations and the Regional prevention plan of Sardinia, the aim of the present study was to assess the level of knowledge and awareness about screening among the general population (adults subjects aged ≥ 18 years old), belonging to the Local Health Unit of Cagliari. The study was carried out between June 1st, 2016 and July 31st, 2016.

Methods

Data were collected in the city of Cagliari, between June and August 2016, by means of a questionnaire, administered by interview. Each interview took about 5 minutes to complete.

Study design

The respondents were selected through a non-probabilistic sampling. Recruitment took place in the atrium of the two main shopping centres of the city; people passing through the atrium of these centres were invited to participate in the survey and subsequently they were interviewed. Trained interviewers collected data at different times of the day, both on weekdays and weekends. People who refused to answer were less than 10%.

Questionnaire

The questionnaire included a section for the collection of socio-demographic data (age, gender, and educational level), as well as open-ended and closed questions in order to assess people's knowledge of some features concerning organized screening programmes.
Data on educational level were split in low (individuals who attended a school lesser than Senior high school) and high (individuals who attended Senior high school).
The main topics explored through open-ended questions were the following:

- knowledge of the word "screening" (open-ended question) and awareness about the goal of cancer screening (open-ended question);
- knowledge of cancers for which a screening programme has been activated (open-ended question);
- knowledge of the name of the three tests used in cancer screening programmes (3 open-ended questions) and awareness of the goal of each test (3 open-ended questions);
- information source on cancer screening programmes (open-ended question).

Appendix 1 reports the questions.
In order to analyse data, the information gathered from the open ended questions were categorized by dichotomizing answers (yes vs no) according to knowledge and understanding of discussed topics.

Statistical analysis

The sample size was 270 adults (138 men, 51%) aged 18-75 years old (mean aged 46 years old, standard deviation 16).
Descriptive analysis was performed through the estimation of the prevalence of the main variables, with 95% confidence intervals. The Chi-square test was used to assess gender and educational differences. Multivariate logistic regression analysis was performed in order to evaluate if gender, and educational level, as independent variables, are related to the awareness of the purpose of cancer screening (dependent variable).
Additionally, a multivariate logistic regression analysis was performed in order to evaluate if gender, and educational level are related to the awareness of the purpose of mammography (dependent variable).
Moreover, a multivariate logistic regression analysis was performed in order to evaluate if gender, and educational level are related to the awareness of the purpose of Pap test (dependent variable).
Again, a multivariate logistic regression analysis was performed in order to evaluate if gender, and educational level are related to the awareness of the purpose of fecal occult blood test (dependent variable).
A p-value < 0.05 was considered as statistically significant.

Results

Results have shown that the word "screening" is known by 77.4% [95% confidence interval (CI) 72.4%-82.4%] of the respondents, but among them, only 59.3% (95% CI 52.7%-66.0%) are aware that oncological screening is used for cancer early detection. People who stated to have heard about screening were asked to indicate which specific organised screening programme they had heard about. Screening for breast cancer was the most cited,

64.6% (95% CI 58.1%-71.1%), followed by screening for uterine cervical cancer, 49.8% (95% CI 43.0%-56.5%); screening for colorectal cancer was only cited by 39.2% (95% CI 32.6%-45.9%) of respondents. In addition, 32.1% (95% CI 25.7%-38.4%) of the respondents named other cancers, mentioning in particular prostate and lung ones.

Around 52.2% of the respondents, who stated to know the word "screening" knew that organised screening programme is free of charge.

In order to explore the general knowledge about the tests that are used in cancer screening programmes, participants were asked, for each of the listed tests, whether they had ever heard of them and whether they were aware of their aims. Almost all the interviewers had heard of mammography, 96.3% (95% CI 94.5%-98.8%), but only 63.6% (95% CI 57.8%-69.4%) knew that it can be used for early diagnosis. Similarly, a large proportion of the respondents knew about the Pap test, 87.1% (95% CI 83.5%91.4%), but among them, only 57.6% (95% CI 51.3%-63.9%) were aware that it can be used for early diagnosis. Around 64.2% of respondent (95% CI 58.7% -70.2%), declared having heard of the fecal blood test but among them, only 61.5% (95% CI 54.3%-68.7%) is aware that the goal of the test is early diagnosis.

Gender and education level differences in knowledge about screening are reported in Tables I and II.

Results highlight knowledge differences about the purpose of cancer screening. Women and highly-educated people appear to be more aware than other groups about purpose and tests that are used for screening, especially about mammography and Pap test. Moreover, women appear to be more aware about the specific cancer screening programmes that are available, especially about breast and cervical cancers.

As for the source of information about cancer screening programmes, general practitioners and other health professionals were indicated by 16.7% and 20.1% respectively, while mass media and friends/family were indicated by one-third of respondents (Tab. III).

More than half of the interviewed people would like to receive information on cancer screening programme from the general practitioner, 56.3% (95% CI 50.4%-62.2%).

Multivariate Logistic Regression analysis show significant associations between the awareness of the purpose of cancer screening (dependent variable) and independent variables gender and educational level: women are more aware than men [p = 0.02; odds ratio (OR) = 2.01; CI 1.13-3.60] ; individuals with high educational level are more aware than individual with low educational level (p = 0.02; OR = 2.18; CI 1.17-4.07).

Regarding awareness of the purpose of mammography, the multivariate logistic regression shows a significative effect of gender and educational level: women (p = 0.01; OR = 1.96; CI 1,19-3,25) are more aware of the purpose of mammography; individuals with high educational level (p = 0.02; OR = 1.81; CI 1.08-3.04) are more aware of the purpose of mammography.

Concerning awareness of the purpose of Pap test, the multivariate logistic regression shows a significative effect of gender and educational level: women (p < 0.001; OR = 7.15; CI 4.15-12.31) are more aware of the purpose of Pap test; individuals with high educational level (p = 0.02; OR = 1.93; CI 1.10-3.42) are more aware of the purpose of Pap test.

Considering awareness of the purpose of fecal occult blood test the multivariate logistic regression shows a significative effect of gender: women are more aware of the purpose of fecal occult blood test (p < 0.001; OR = 2.51; CI 1.52-4.15). Educational level does not result significantly associated to the awareness of the purpose of fecal occult blood test (p = 0.52; OR = 1.19; CI 0.70-2,01).

Tab. I. Knowledge and awareness about screening: differences between men and women.

	Men	Women	p value
	N (%)	N (%)	
How many people have ever heard of screening?	95 (68,8%)	114 (86,4%)	0,001
Among people having heard of screening, how many are aware of the purpose of cancer screening?	48 (50,5%)	76 (66,7%)	0,018
Among people having heard of screening, how many people did mention breast cancer screening?	49 (51,6%)	86 (75,4%)	< 0,001
Among people having heard of screening, how many mentioned cervical cancer screening?	19 (20,0%)	85 (74,6%)	< 0,001
Among people having heard of screening, how many mentioned colorectal cancer screening?	32 (33,7%)	50 (43,9%)	0,134
How many people have ever heard about mammography?	129 (93,5%)	132 (100,0%)	0,003
Among people having heard of mammography, how many are aware of the purpose of this test?	74 (57,4%)	92 (69,7%)	0,038
How many people have ever heard about the Pap test?	106 (76,8%)	130 (98,5%)	< 0,001
Among people having heard of Pap test, how many are aware of the purpose of this test?	39 (36,8%)	95 (73,1%)	< 0,001
How many people have ever heard of the fecal occult blood test?	82 (59,4%)	92 (69,7%)	0,078
Among people having heard of fecal occult blood test, how many are aware of the purpose of this test?	38 (46,3%)	60 (65,2%)	0,012

Tab. II. Knowledge and awareness on screening: differences in levels of education.

	Low education (%)	High education (%)	p value
How many people have heard of screening?	60 (62,5%)	149 (85,6%)	< 0,001
Among people having heard of screening, how many are aware of the purpose of cancer screening?	29 (48,3%)	95 (63,8%)	0,040
Among people having heard of screening, how many people did mention breast cancer screening?	33 (55,0%)	102 (68,5%)	0,070
Among people having heard about screening, how many mentioned cervical cancer screening?	26 (43,3%)	78 (52,3%)	0,238
Among people having heard about screening, how many mentioned colorectal cancer screening?	18 (30,0%)	64 (43,0%)	0,083
How many people have ever heard about mammography?	88 (91,7%)	173 (99,4%)	0,001
Among people having heard of mammography, how many are aware of the purpose of this test?	50 (56,8%)	116 (67,1%)	0,104
How many people have ever heard about the Pap test?	72 (75,0%)	174 (94,3%)	< 0,001
Among people having heard about Pap test, how many are aware of the purpose of this test?	38 (52,8%)	96 (58,5%)	0,411
How many people have ever heard of the fecal occult blood test?	55 (57,3%)	119 (68,4%)	0,068
Among people having heard of fecal occult blood test, how many are aware of the purpose of this test?	29 (52,7%)	69 (58,0%)	0,516

Tab. III. Information sources about screening (individuals gave more than one answer).

	Relative frequencies (sample 209)	95% Confidence Interval
General practitioner	35 (16.7%)	11.7%-21.8%
Other health professionals	42 (20.1%)	14.7%-25.5%
Mass media	54 (33.0%)	26.6%-39.4%
Internet	23 (11.0%)	6.8%-15.2%
Family members, friends	75 (35.9%)	29.4%-42.4%
Posters, leaflets	17 (8.1%)	4.4%-11.8%
Those who do not remember	14 (6.7%)	3.3%-10.1%

Discussion

Overall, the study shows that the general population's knowledge and understanding about organised cancer screening is rather limited. Results highlight that, although the word "screening" is widely known, only half of respondents who have heard of it are aware of the purpose of these tests (that is cancer early detection). Likewise, names for screening tests seem to be well known, individuals spontaneously mentioned mammography and Pap test; however, a large proportion of respondents seem not to know what these tests are for. Additionally, there is little knowledge about the specific type of cancers, which can be early diagnosed through organised screening programmes. The screening for colorectal cancer resulted to be the less known. Overall, men and individuals with lower education appeared to be less informed about screenings than women and individuals with high educational level. They appear the most vulnerable individuals to focus on with the aim of increasing their knowledge and their awareness about cancer screenings.

These results should be taken into account in designing local interventions, in order to increase cancer organised screening attendance. Since several factors can prevent citizens to undergo screening (i.e. individual, logistic, or cultural factors), participation should be then promoted through different strategies [11, 14-16]. Health communication can be one of them, and it could be used to promote informed choices. Scientific literature suggests the effectiveness of communication programmes address to the general population [17-19]. Awareness about cancer prevention relevance will help the general population to take into account the importance of cancer screenings during their lifetime and then it could encourage them in taking part into screening programmes when needed.

Although the words screening, mammography, and Pap tests are well known among the general population, their meaning in terms of early detection of cancer is still not understood. This aspect could be an indicator of low health literacy that should be better detected in future research.

Cancer screening campaigns should have the aim to favour and facilitate access to information in order to motivate people toward preventive health. Therefore, communication aiming at increase knowledge and awareness about screening should give special attention to language. In order to contribute to spread the cancer prevention culture, the benefit of early diagnosis of cancer should be emphasised by means of a plain language; thus the general population could became more aware of the importance of cancer prevention.

Men and people with lower education appeared to be not enough aware of the benefit of cancer screening; hence, communication campaign should be tailored to these categories, in order to tackle gender, as well as social and economic inequalities. [18, 20-22]. Likewise, it is

necessary to put particular attention to promote colorectal screening that appears the least known and with the lowest participation.
Finally, this study has indicated that general practitioners could play a decisive role in promoting screening attendance. In fact, the interviewees indicate the GP as the main source from which they would like to receive information on screening. This result suggests that patients highly trust their doctors; therefore, general practitioners could be the health professionals who could best contribute to increase people's attendance to screening programmes, by informing and encouraging patients to participate. General practitioners know their patients, they have the possibility to meet them and address discussion about the opportunity of cancer screening and clarify their doubts, they have the role of reassuring and, at the same time, alerting people about the importance of participating in cancer screening [12, 19, 24].

Acknowledgements

Funding sources: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

MA, SC, conceived and designed the study, coordinated the study activities, analysed the data, interpreted results, drafted and edited the manuscript. CP conceived the study, contributed to data interpretation and critically revised the draft of the manuscript. CF, DC, GM, LL, LA, MM, McGDC, PMF, PL, SB, SA, collected data, performed literature search and data quality control, contributed to data analysis. IMT, OP, made contributions to the conception of the research and critically revised the draft of the manuscript.
All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript

References

[1] WHO. Global report on noncommunicable diseases 2010. Available at: http://www.who.int/nmh/publications/ncd_report_full_en.pdf (Last accessed January 16, 2018).

[2] Piano nazionale di prevenzione 2014-2018. Avalilable at: http://www.salute.gov.it/imgs/C_17_pubblicazioni_2285_allegato.pdf (Last accessed September 24, 2019).

[3] Ossesvatorio Nazionale screening (ONS). Available at: https://www.osservatorionazionalescreening.it/content/chi-siamo (Last accessed September 24, 2019).

[4] Rossi PG, Camilloni L, Cogo C, Federici A, Ferroni E, Furnari G, Giordano L, Grazzini G, Iossa A, Jimenez B, Palazzi M, Palazzo F, Spadea T, Senore C, Borgia P, Guasticchi G. Methods to increase participation in cancer screening programmes. Epidemiol Prev 2012;36(suppl 1):1-104.

[5] Valle I, Tramalloni D, Bragazzi NL. Cancer prevention: state of the art and future prospects. J Prev Med Hyg 2015;56:E21-E27.

[6] Osservatorio Nazionale Screening Rapporto 2018. Available at: https://www.osservatorionazionalescreening.it/content/lo-screening-mammografico (Last accessed June 6, 2019).

[7] Osservatorio Nazionale Screening Rapporto 2018. Available at: https://www.osservatorionazionalescreening.it/content/lo-screening-cervicale (Last accessed June 6, 2019).

[8] Osservatorio Nazionale Screening Rapporto 2018. Available at: https://www.osservatorionazionalescreening.it/content/lo-screening-colorettale (Last accessed June 6, 2019).

[9] Piano Regionale di Prevenzione 2014-2018 – Sardegna – Ministero della salute. Available at: http://www.salute.gov.it/portale/temi/documenti/PNP/Sardegna_PRP.pdf (Last accessed January 12, 2018).

[10] Sardegna Salute. Gli screening oncologici. Available at: http://www.sardegnasalute.it/approfondimenti/screening/ (Last accessed January 10, 2018).

[11] Zappa M, Carozzi FM, Giordano L, Sassatelli R, Federici A. The diffusion of screening programmes in Italy, years 2011-2012. Epidemiol Prev 2015;39(3)Suppl1:1-12.

[12] Bocci G, Troiano G, Messina G, Nante N, Civitelli S. Factors that could influence women's participation in colorectal cancer screening: an Italian study. Ann Ig 2017;29:151-160. https://doi.org/10.7416/ai.2017.2142

[13] Gimeno Garcia AZ, Hernandez Alvarez Buylla N, Nicolas-Perez D, Quintero E. Public awareness of colorectal cancer screening: knowledge, attitudes, and interventions for increasing screening uptake. ISRN Oncol 2014:425787. https://doi.org/10.1155/2014/425787

[14] Tack 2: Health literacy and health behaviour: Available at: http://www.who.int/healthpromotion/conferences/7gchp/track2/en/ in WHO

[15] Davis TC, Williams MV, Marin E, Parker RM, Glass J. Health literacy and cancer communication. CA Cancer J Clin. 2002;52:134-49.

[16] Oldach BR, Katz ML. Health literacy and cancer screening: a systematic review. Patient Educ Couns 2014;94:149-57. https://doi.org/ 10.1016/j.pec.2013.10.001

[17] Morris NS, Field TS, Wagner JL, Cutrona SL, Roblin DW, Gaglio B, Williams AE, Han PJ, Costanza ME, Mazor KM. The association between health literacy and cancer-related attitudes, behaviors, and knowledge. J Health Commun 2013;18:223-41. https://doi.org/10.1080/10810730.2013.825667

[18] Unim B, Boggi R, Napoli M, Fulgenzi R, Landi A, La Torre G. Public Health. Women's satisfaction with mammography and predictors of participation in an organized breast cancer screening program: Perspectives of a Local Health Unit in Rome. Public Health 2018;155:91-4. https://doi.org/10.1016/j.puhe.2017.11.025

[19] Unim B, Boggi R, Napoli M, Fulgenzi R, Landi A, La Torre G. Predictors of mammography uptake among Italian women aged 50-69: a cross-sectional study. J Cancer Educ 2019 Jun 11 [Epub ahead of print]. https://doi.org/ 10.1007/s13187-019-01560-z

[20] De Vito C, Angeloni C, De Feo E, Marzuillo C, Lattanzi A, Ricciardi W, Villari P, Boccia S. A large cross-sectional survey investigating the knowledge of cervical cancer risk etiology and the predictors of the adherence to cervical cancer screening related to mass media campaign. Biomed Res Int 2014;2014:304602. https://doi.org/10.1155/2014/304602

[21] Davis JL1, Buchanan KL, Katz RV, Green BL. Gender differences in cancer screening beliefs, behaviors, and willingness to participate: implications for health promotion. Am J Mens Health 2012;6:211-7. https://doi.org/10.1177/1557988311425853

[22] Molina-Barceló A, Peiró-Pérez R, Vanaclocha M, Vallés G, Guaita L, Salas D. Informed participation in the Valencian Community Colorectal Cancer Screening Programme from a gender perspective. Gac Sanit 2018;32:72-6. https://doi.org/10.1016/j.gaceta.2016.07.010

[23] Smith SK, Simpson JM, Trevena LJ, McCaffery KJ. Factors associated with informed decisions and participation in bowel cancer screening among adults with lower education and literacy. Med Decis Making 2014;34:756-72. https://doi.org/10.1177/0272989X13518976

[24] Gabrielli E, Bastiampillai AJ, Pontello M, Beghi G, Ceresa P, Pirola ME, Cereda D. Observational study to evaluate the impact of internet reminders for GPs on colorectal cancer screening uptake in Northern Italy in 2013. J Prev Med Hyg 2016;57:211-5

APPENDIX 1

THE QUESTIONARE

Gender: Woman ___ Man ___

Age ___

Education level: Low education level (less of Senior high school) ___
High education level (Senior high school) ___

1. Have you ever heard of cancer screening?

2. Can you tell me what the cancer screening is?

3. What kind of cancer screening did you hear about?

4. What are your main information source about cancer screening?

5. Who would you like to contact for more information on screening?

6. Do you know the purpose of cancer screening?

7. Have you ever heard of mammography?

8. Do you know the purpose of mammography?

9. Have you ever heard of Pap test?

10. Do you know the purpose of Pap test?

11. Have you ever heard of fecal occult blood test?

12. Do you know the purpose of fecal occult blood test?

Received on November 6, 2018. Accepted on October 16, 2019.

Correspondence: Alessandra Mereu, Department of Medicine Science and Public Health, University of Cagliari, Cittadella Universitaria di Monserrato - SS554 (Bivio Sestu) - 09042 Monserrato (CA), Italy - Tel. +39 070 6753103 - E-mail: amereu@unica.it

How to cite this article: Mereu A, Concu F, Dessì C, Girau M, Ionta MT, Lai L, Liori A, Masala M, McGilliard CD, Origa P, Piazza MF, Pisanu L, Soggiu B, Sotgiu A, Contu P, Sardu C. Knowledge about cancer screening programmes in Sardinia. J Prev Med Hyg 2019;60:E337-E342. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1094

ORIGINAL ARTICLE

Multicenter screening of diabetic patients for detecting new cases of tuberculosis: an approach to intensify the case detection rate of tuberculosis in developing countries with high prevalence of diabetes

M. GADALLAH[1], W. ABDELMONIEM[2], M. FAWZY[2], A. MOKHTAR[2], A. MOHSEN[3]
[1] Department of Community Medicine, Faculty of Medicine, Ain Shams University, Egypt; [2] Ministry of Health and Population, Chest Directorate, National Tuberculosis Control Program, Cairo, Egypt; [3] Department of Community Medicine, National Research Centre, Cairo, Egypt

Keywords

Screening • Tuberculosis • Diabetes • Case detection rate

Summary

Introduction. *Tuberculosis (TB) is a major public health problem in most of developing countries. Meanwhile, the prevalence of type 2 diabetes mellitus (DM) is also increasing rapidly.*
Objectives. *To describe the feasibility of implementing screening test for tuberculosis among diabetic patients and identifying factors associated with high detection rate.*
Methods. *Study Design: Multi-center cross-sectional study. This study was implemented in the governmental healthcare settings. To diagnose TB among diabetics, we used a symptom-based questionnaire that included the symptoms of suspected TB according to the guidelines of National Tuberculosis Program in Egypt.*
Results. *Among 4283 adult diabetics, 14 TB cases were diagnosed; 9 known TB cases and 5 newly detected cases. The number needed to detect one new case of TB was 855. Male diabetics and who those suffered from liver disease experienced a significantly higher prevalence of TB and a higher detection rate of new active cases.*
Conclusions. *Screening for TB among diabetics in routine governmental healthcare services was successfully implemented. Screening DM patients in countries with a high prevalence of DM will reveal a significant number of active TB cases, which will in turn improve the case detection rate of TB.*

Introduction

Tuberculosis (TB) continues to be the leading killer among bacterial diseases worldwide. Globally, in 2017, there were an estimated 10.0 million incident cases of TB and 1.3 million TB-related deaths [1]. In the same year, diabetes mellitus (DM) affected 425 million adults and killed 4.0 million people [2]. It is projected that the number of people affected by DM will increase to 629 million by 2045, and approximately 80% of these people live in low- and middle-income countries, where TB is endemic [2]. The association between DM and TB presents a major public health problem either in the current time or near future especially in low- and middle-income countries where TB is endemic disease and the prevalence of DM is high and rising. According to a meta-analysis study, DM patients have a three-fold greater risk of contracting TB than do non-diabetics (95% confidence interval [CI]: 2.3-4.3) [3]. Another systematic review and meta-analysis done by Al-Rifai et al revealed a resilient positive association between DM and TB with a substantial variation in the effect size between different studies [4]. Other systematic reviews of bidirectional screening for TB and DM reported that the prevalence of TB among diabetics ranged between 0.38% and 14%, with median global value of 4.1% [5, 6]. DM increases the risk of developing TB as well as its complications e.g. treatment failure, relapse and death rate [7]. Accordingly, this association has a negative impact on TB control program. Screening of high risk group as diabetic patients has been part of the Stop TB strategy for many years. In 2011, the estimated incidence rate of TB in Egypt was 18 per 100,000 populations. In 2017, this figure showed improvement, as the estimated incidence rate declined to 13 per 100,000 populations [1]. The prevalence of DM among those aged 15 to 64 years increased from 15.8% in 2005 to 17.2% in 2011-12 then slightly declined to 15.5% in 2017. Accordingly, a large proportion of Egyptians will be exposed to the risk of DM, and DM patients themselves will be exposed to a high risk of acquiring TB. Moreover, patients with both TB and DM will be more likely to develop complications than TB patients without DM [8, 9]. TB screening in selected risk groups as persons with DM is considered affordable and of low cost and at the same time will improve the low case detection of TB and reduce the delay of TB diagnosis [10].
The objectives of this study are to describe the feasibility of implementing a screening program to screen DM patients for TB within the governmental health settings affiliated to the Ministry of Health and Population of Egypt and identifying factors associated with high detection rate of new TB cases.

Methods

Study design

This study was a national multicenter cross-sectional study.

Study population and sampling selection

The study population was adult DM patients aged ≥ 18 years old. All study DM patients were diagnosed by fasting blood sugar (FBS) test (≥ 126 mg/dl) and postprandial blood glucose (PPBG) test (≥ 200 mg/dl). According to the last population surveys done in Egypt, the prevalence of TB and DM differed in urban and rural areas, therefore, in this study a multistage sampling was applied to represent different geographical areas of Egypt. The country was divided geographically into 5 sectors: Greater Cairo, the Coastal zone, Upper Egypt, the Suez Canal zone, and Lower Egypt. One governorate was selected by a simple random sample from each sector except for Lower Egypt due to its high population density; in this case, two governorates were selected randomly. The selected governorates were Cairo, Alexandria, Gharbia, Daqahlia, Ismailia and Suhag. In each selected governorate, DM patients who attended outpatient clinics were recruited from all governmental hospitals from June 2012 to December 2012. A total of 4283 patients were recruited. A simple questionnaire was designed to collect data from the DM patients. These data included age, gender, residence area (urban/rural), type of treatment of DM, duration of DM (years), history of chronic diseases, history of previous TB, details of symptoms of suspected TB that used for symptom screening test.

Diagnosis of TB

All DM patients were asked about having a history of TB i.e. history of previous TB diagnosis (known TB). If the answer was yes, then those patients were excluded from the screening test but included in the study. If the answer was no, then those DM patients were screened first by a predesigned questionnaire (screening by symptoms) for detecting suspected TB. This symptom screening tool is used in Egypt and almost in all developing countries as it is cheap and affordable method for detecting suspected TB cases especially among high risk groups as diabetic patients. According to the Egyptian National TB Program, suspected TB was based on having a cough for more than 2 weeks, which may be accompanied by other respiratory symptoms (e.g., shortness of breath, chest pains, and hemoptysis) and/or constitutional symptoms (e.g., loss of appetite, weight loss, fever, night sweats, and fatigue). DM patients who showed positive symptoms suggesting suspected TB were further subjected to chest X-ray and sputum analyses (smear and culture) for pulmonary TB and histopathology and/or culture for extra-pulmonary TB. The validity of this algorithm for screening of TB was assessed by World Health Organization-Guideline Development Group [10] and reported the followings: the pooled sensitivity of using symptom screening alone was 57% and pooled specificity was 80%. While using chest radiography these percentages increased to 87% for pooled sensitivity and 89% for pooled specificity.

Sample size calculation

Sample size was calculated based on the estimated prevalence of TB among DM patients. From reviewing the literatures of similar studies in developing countries, the prevalence of TB among DM patients was approximately 2 to 7 times higher than the figure in the population or among non-diabetics. In Egypt, the estimated prevalence of TB (at the time of the study) among adult population was 28 per 100,000 adults. Accordingly, we assumed the following assumptions for calculating the sample size of the study: An estimated prevalence of 112 per 100,000 diabetic patients (4 times higher than the figure among adult population), 95% confidence level and 0.10% confidence limits. From the above assumptions, a sample of 4295 DM patients was required. The sample size was calculated using Epi Info version 7. The actual sample size in this study with complete records was 4283 DM patients.

Ethical approval

The protocol of the study was approved by the Institutional Review Board (IRB) of the faculty of medicine, Ain Shams University. All patient data were kept confidential. Informed consent was obtained from each patient included in the study after having been given a clear description of the study objectives. Patients detected by the bidirectional screening were referred to specialized clinics for further management.

Statistical analysis

All data were analyzed using SPSS version 21. Descriptive analyses with 95% confidence intervals (95% CI) were done for all study variables. The only quantitative variables in the study were age (years) and duration of diabetes (years) and both converted to categorical variables. Age was classified as two categories (< 50 years and ≥ 50 years) while the duration of diabetes was classified as less than 10 years and ≥ 10 years. The tests of significance used for qualitative variables were Chi-square test or Fisher's exact test when appropriate. Binary (simple and multiple) logistic regression models were used for identifying the predictor variables associated with the detection of new cases of TB among DM patients and to adjust for other confounding variables as age and sex. A P value of ≤ 0.05 was considered significant and all tests of significance were two tailed.

Results

In this study, 4283 DM patients were recruited from different primary healthcare centers (PHCs) and hospitals from the selected study sites. Approximately three-quarters of the DM patients aged ≥ 50 years, two-thirds

were females, 52% were from rural areas, 54.7% had DM duration of less than 10 years, approximately half of the patients were under insulin therapy, and 4.9% also suffered from liver disease. Moreover, three quarters of the diabetic patients included in the study were screened at hospitals. Hypertension was reported among 36.8% of DM patients. In this study, the prevalence of known TB was 210.1 per 100,000 population (95% CI:110.6-398.6) among DM patients, which was further examined by age, sex, residence, screening place (PHCs and hospitals), DM treatment type, DM duration and chronic disease comorbidities. The results revealed that the known prevalence of TB among DM patients was higher among those less than 50 years old, males, urban residents, those under treatment with oral hypoglycemic drugs, those with DM duration of less than 10 years, and those with liver disease. The prevalence of TB was more or less similar among those screened at PHC or hospitals (Tab. I). Screening DM patients who gave no history of TB (n = 4274) revealed that 261 diabetic patients were positive for symptom screening and referred for further assessment by chest radiography and sputum analyses. The final investigations showed five new TB cases, with a detection rate of 117.0 per 100,000 population (95% CI: 50.0-273.6). This screening detection rate was further analyzed according to patient characteristics. A higher screening detection rate of TB was reported among males, those aged ≥ 50 years, rural residents, those under oral treatment with hypoglycemic drugs, those with a DM duration ≥ 10 years, and those with liver disease (Tab. II).

Tab. I. Prevalence of known TB per 100,000 among DM patients.

	Total sample	Known TB	Prevalence of TB (95% CI)
Total	4283	9	210.1 (110.6-398.6)
Age			
< 50	1134	3	264.6 (90.0-774.9)
≥ 50	3149	6	190.5 (87.4-436.7)
Sex			
Male	1393	8	574.3 (291.3-1129)[a]
Female	2890	1	34.6 (6.1–195.7)
Residence			
Urban	2043	6	293.7 (134.7-639.3)
Rural	2240	3	133.9 (45.6-393.0)
Screening place			
PHC	954	2	209.6 (60.0-760.0)
Hospital	3329	7	210.3 (60.0-430.0)
Treatment			
Oral	2127	6	282.1 (129.4-614.1)
Insulin	2156	3	139.1 (47.4-408.9)
Duration of DM			
< 10 years	2343	7	298.8 (144.8-615.4)
≥ 10 years	1940	2	103.1 (28.3-375.1)
Chronic disease			
None	2110	2	94.8 (26.0–344.9)
Liver	211	2	947.9 (260.3–3389.0)[b]
Hypertension	1578	3	190.1 (64.7–557.4)
Others	384	2	520.8 (143.0–1879.0)

[a] p < 0.001 compared with females
[b] P = 0.044 compared with none chronic diseases

The lowest figure of CDR was among female diabetics (34.6 per 100,000; 95% CI = 6.1-195.8) while the highest value of CDR was reported among diabetic patients

Tab. II. Screened case detection rate (newly diagnosed) of TB per 100,000 among DM patients with no history of TB.

	Total no. screened for TB A	New cases of TB B	Screen detection rate (95% CI) A/B * 100,000	Number needed to screen A/B
Total	4274	5	117.0 (50.0-273.6)	855
Age				
< 50	1131	1	88.4 (15.6-499.1)	1131
≥ 50	3143	4	127.3 (49.5-326.8)	786
Sex				
Male	1385	4	288.8 (112.4-740.2)	346
Female	2889	1	34.6 (6.1-195.8)	2889
Residence				
Urban	2037	2	98.2 (26.9-357.3)	1019
Rural	2237	3	134.1 (45.6-393.5)	746
Screening place				
PHC	952	0	NA	NA
Hospital	3322	5	150.5 (60.0-350.0)	664
Treatment				
Oral	2121	3	141.4 (48.1-415.0)	707
Insulin	2153	2	92.9 (25.5-338.1)	1077
Duration of DM				
< 10 years	2336	2	85.6 (23.5-311.6)	1168
≥ 10 years	1938	3	154.8 (52.7-454.1)	646
Chronic disease				
None	2108	3	142.3 (48.4-417.6)	703
Liver	209	1	478.5 (84.5-2660.0)	209
Hypertension	1575	1	63.5 (46.2-1468.0)	1575
Others	382	0	NA	NA

NA = Not Applicable due to zero detected cases

with liver diseases (478.5 per 100,000).The number needed to screen (NNS) to detect one new case of TB among diabetics was 855. Furtherly, NNS values were calculated according to the patient characteristics. The results showed that the lowest value (NNS = 209) was found among DM patients with liver disease, followed by male patients (NNS = 346). The NNS values ranged from 209 to 2889 (Tab. II).

To study factors associated with the total prevalence (diagnosed and newly detected cases) of TB among diabetics; both bivariate and multivariate logistic regression models were applied (Tab. III). The results from the bivariate analysis showed that male DM patients and those with liver disease had a significantly higher prevalence of TB. After adjusting for age and other variables using a logistic regression model, both the male gender and the presence of liver disease remained as independent risk factors, with adjusted odds ratios (AORs) and 95% confidence intervals (CIs) of 12.57 (2.73-57.82) and 6.44 (1.45-28.72) respectively.

Discussion

This present study is considered the first national base survey in Egypt to study the feasibility of screening the comorbidity of TB and DM. Recently, there is growing evidence supporting DM as a risk factor for developing TB. There are many published reports suggesting the mechanisms of developing TB among DM patients, such as uncontrolled hyperglycemia, alveolar macrophage dysfunction, decrease in monocyte chemotaxis and neutrophil count and immune system depression that favors infection [11, 12]. However, the mechanism linking DM and TB susceptibility requires further study [13]. In this study, high prevalence of known TB (previously diagnosed elsewhere) among DM patients was found (210.1 per 100,000), which is 7.5-fold higher than the national prevalence of TB (28 per 100,000). Similar to our results, higher prevalence of TB than national figure was reported among diabetic patients in China, Ethiopia, India and Mexico [14-18]. Diabetes mellitus is considered a strong risk factor for developing TB as well as worsening the outcome of treatment and increasing the mortality rates among comorbid patients (TB with DM). Although TB is more associated with other immune suppressive diseases as HIV, yet the prevalence of HIV in Egypt is very low and the prevalence of diabetes is high and with rising prevalence. Therefore, diabetes is considered powerful significant risk factor for TB infection among Egyptian population which in turn adversely affects the global TB control [1]. Our results revealed that male patients experienced 16.6 fold higher in prevalence of TB than females. Also, diabetic patients with liver diseases showed higher significant prevalence of TB than those without chronic diseases. Other patients' characteristics were insignificantly associated with the prevalence of TB such as age, residence, type of treatment and duration of diabetes. Our results were in agreement to the report of Lin et al. [19] in China who reported higher significant increase in the prevalence of TB among male diabetics and those with liver cirrhosis

Tab. III. Factors associated with the total prevalence of TB among DM patients.

Characteristic	Total prevalence of TB (previously diagnosed and newly detected)			
	Simple logistic regression		Multivariate logistic regression	
	P value	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)
Age				
< 50				
≥ 50®	0.900	1.11 (0.35-3.55)	0.545	1.63 (0.16-12.90)
Sex				
Male				
Female®	0.001	12.55(2.80-56.14)	0.001	12.57 (2.73-57.82)
Residence				
Urban				
Rural®	0.479	1.46 (0.51-5.23)	0.701	0.80 (0.26-2.44)
Screening place				
PHC®				
Hospital	0.477	1.72 (0.39-7.71)	0.264	2.43 (0.51-11.53)
Treatment				
Oral				
Insulin®	0.273	1.83 (0.61-5.46)	0.505	1.49 (0.46-4.87)
Duration of DM				
< 10 years				
≥ 10 years®	0.471	1.49 (0.50-4.46)	0.533	1.46 (0.45-4.74)
Chronic illness				
None®	-	-	-	-
Liver	0.005	6.07 (1.44-25.59)	0.015	6.44 (1.45-28.72)
hypertension	0.813	1.07 (0.29-3.99)	0.529	1.55 (0.40-6.01)
Others	0.658	2.20 (0.43-11.40)	0.184	3.14 (0.58-16.97)

® Reference group

in addition to other factors as smoking and subjective body loss. Also, Castellanos-Joya et al in Mexico [18] reported highly significant increase in the prevalence of TB among males and those with history of TB or in contact with TB patients.

Our screening of DM patients with an unknown history of TB detected 5 new cases of TB with a case detection rate of 117 per 100,000 population. DM patients face frequent infections, which can mainly be attributed to hyperglycemia adversely affecting the immune system. The highest detection rates of new active cases of TB among diabetics were found among those with liver diseases (478.5 per 100,000 population, 95% CI = 84.5-2660.0) and males (288.8 per 100,000 population, 95% CI =112.4-740.2). The NNS of DM patients for detecting one new TB patient was 858 with a range between 209 for those with liver diseases and 2899 for female patients. A similar result (NNS = 812) was reported by Prakash et al. in South India [17] while lower values were reported as 71 and 490 in Mexico and Ghana [18, 21]. The NNS of diabetic patients to find a new case of active TB depends mainly on the prevalence of TB and DM in the community. Egypt is considered to have one of the highest prevalence rates of DM in the Middle East; and there is at least 3 million cases with DM visiting the healthcare facilities thus, the screening of DM patients in Egypt, as well as in similar countries with high DM prevalence rates, will yield a significant number of new TB cases. When the screened detected cases are added to the total number of annual notifiable TB cases it will intensify the case detection rate of TB. Therefore, screening of DM patients for TB in countries with high prevalence of DM and low rate of case detection is of great importance not only for the proper management of patients with the double burden of DM and TB but also for improving the case detection rate of TB. In this study, male diabetics and those with liver disease exhibited a significantly higher prevalence of TB and a higher detection rate of new active cases. These results were confirmed even after adjusting for age and illness duration. Most DM patients with liver disease were classified as such mainly due to hepatitis C virus infection. Egypt is considered to have the highest prevalence of hepatitis C virus infection [22]. Therefore, it is of the utmost importance when starting to treat DM patients with liver disease and TB to closely monitor their liver functions, as the first-line drugs for treating TB increase the risk of hepatitis (hepatotoxic drugs) and can lead to an increase in mortality rate.

In conclusion, the results of this study showed that screening for TB among diabetic patients is feasible and could be implemented in a governmental setting. We found a high yield of TB among DM patients, and early TB detection will improve not only the treatment outcome of this comorbidity but also the case detection rate of TB. Furthermore, the early detection of TB among DM patients will reduce the transmission of TB among DM patients. The prevalence of TB among diabetics was more prominent among males and those with liver disease. These findings support the advantages of implementing TB screening as a routine investigation during the management of diabetes, particularly in developing countries with high prevalence of DM and considered one of the strategies for addressing TB control and increasing case detection rate.

There are some limitations of this study. The study sample used for screening diabetic patients for detection of TB was restricted only to patients seen in governmental hospitals affiliated to the Ministry of Health and Population while there are other Universities and private hospitals not included in this study. This is a cross-sectional study with the inability to demonstrate a temporal relationship between exposure and outcome. However, the results of this study may throw the light on the potential risk factors associated with high detection of new TB cases among diabetic patients.

Acknowledgements

We cordially thank all the staff of the chest hospitals and diabetic clinics of the governmental health services who participated in this study for their efforts and support in collecting data.

Funding sources: this work was funded by the Global Fund, Round 6 grant number: EGY/607/G02-T.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

All authors had participated in the design, implementing the study, analysis of the results and writing all sections of the manuscript. In addition, AW, FM, AM have provided substantial contribution in data collection and field supervision. GM and MA have substantial contribution in data interpretation and data cleaning. All authors have reviewed and approved the final version.

References

[1] World Health Organization. Global tuberculosis report. 2018. Available at http://apps.who.int/medicinedocs/documents/s23553en/s23553en.pdf (Accessed 21/08/2019).

[2] International Diabetes Federation. Diabetes atlas. 8th edition.2017. Available at: https://diabetesatlas.org/resources/2017-atlas.html. (Accessed 21/08/2019).

[3] Jeon CY, Harries AD, Baker MA, Hart JE, Kapur A, Lönnroth K, Ottmani SE, Goonesekera S, Murray MB. Bi-directional screening for tuberculosis and diabetes: a systematic review. Trop Med Int Health 2010;15:1300-14. https://doi.org/10.1111/j.1365-3156.2010.02632.x

[4] Al-Rifai RH, Pearson F, Critchley JA, Abu-Raddad LJ. Association between diabetes mellitus and active tuberculosis: a systematic review and meta-analysis. PLoS One 2017;12: e0187967. https://doi.org/10.1371/journal.pone.0187967

[5] Jeon CY, Harries AD, Baker MA, Hart JE, Kapur A, Lonnroth K, Ottmani SE, Goonesekera S, Murray MB. Bi-directional

screening for tuberculosis and diabetes: a systematic review. Trop Med Int Health 2010;15:1300-14.

[6] Workneh MH, Bjune GA, Yimer SA. Prevalence and associated factors of tuberculosis and diabetes mellitus comorbidity: a systematic review. PLoS One 2017;12:e0175925. https://doi.org/10.1371/journal.pone.0175925

[7] Girardi E, Sañé Schepisi M, Goletti D, Bates M, Mwaba P, Yeboah-Manu D, Ntoumi F, Palmieri F, Maeurer M, Zumla A, Ippolito G. The global dynamics of diabetes and tuberculosis: the impact of migration and policy implications. Int J Infect Dis 2017;56:45-53. https://doi.org/10.1016/j.ijid.2017.01.018

[8] Baker MA, Lin HH, Chang HY, Murray MB. The risk of tuberculosis disease among persons with diabetes mellitus: a prospective cohort study. Clin Infect Dis 2012;54:818-25. https://doi.org/10.1093/cid/cir939

[9] Gadallah MA, Mokhtar A, Rady M, El-Moghazy E, Fawzy M, Kandil SK. Prognostic factors of treatment among patients with multidrug-resistant tuberculosis in Egypt. J Formos Med Assoc 2016;115:997-1003. https://doi.org/10.1016/j.jfma.2015.10.002

[10] World Health Organization. Systematic screening for active tuberculosis: Principles and recommendations. 2013. Available at: http://www.who.int/tb/tbscreening/en/ (Accessed 24/06/2017).

[11] Dooley KE, Chaisson RE. Tuberculosis and diabetes mellitus: convergence of two epidemics. Lancet Infect Dis 2009;9:737-46. https://doi.org/10.1016/S1473-3099(09)70282-8

[12] Yorke E, Atiase Y, Akpalu J, Sarfo-KantankaO, Boima V, Dey ID. The bidirectional relationship between tuberculosis and diabetes. Tuberc Res Treat 2017;2017:1702578. https://doi.org/10.1155/2017/1702578

[13] Harries AD, Satyanarayana S, Kumar AM, Nagaraja SB, Isaakidis P, Malhotra S, Achanta S, Naik B, Wilson N, Zachariah R, Lönnroth K, Kapur A. Epidemiology and interaction of diabetes mellitus and tuberculosis and challenges for care: a review. Public Health Action 2013;3(Suppl 1):S3-9. https://doi.org/10.5588/pha.13.0024

[14] Wang H-T, Zhang J, Ji L-C,You S-H, Dai W, Wang Z-Y. Frequency of tuberculosis among diabetic patients in the People's Republic of China. Ther Clin Risk Mang 2014;10:45-9. https://doi.org/10.2147/TCRM.S3887.

[15] Feleke Y, Abdulkadir J, Aderaye G. Prevalence and clinical features of tuberculosis in Ethiopian diabetic patients. East Afr Med J 1999;76:3614.

[16] Lin Y, Innes A, Xu L, Li L, Chen J, Hou J, Mi F, Kang W, Harries AD. Screening of patients with diabetes mellitus for tuberculosis in community health settings in Chine. Trop Med Int Health 2015;20:1073-80. https://doi.org/10.1111/tmi.12519

[17] India Diabetes Mellitus - Tuberculosis Study Group. Screening of patients with diabetes mellitus for tuberculosis in India. Trop Med Int Health 2013;18:646-54. https://doi.org/10.1111/tmi.12084

[18] Castellanos-Joya M, Delgado-Sánchez G, Ferreyra-Reyes L, Cruz-Hervert P, Ferreira-Guerrero E, Ortiz-Solís G, Jiménez MI, Salazar LL, Montero-Campos R, Mongua-Rodríguez N, Baez-Saldaña R, Bobadilla-del-Valle M, González-Roldán JF, Ponce-de-León A, Sifuentes-Osornio J, García-García L. Results of the implementation of a pilot model for the bidirectional screening and joint management of patients with pulmonary tuberculosis and diabetes mellitus in Mexico. PLoS One 2014;9:e106961. https://doi.org/10.1371/journal.pone.0106961

[19] Lin YH, Chen CP, Chen PY, Huang JC, Ho C, Weng HH, Tsai YH, Peng YS. Screening for pulmonary tuberculosis in type 2 diabetes elderly: a cross-sectional study in a community hospital. BMC Public Health 2015;15:3. https://doi.org/10.1186/1471-2458-15-3

[20] Prakash BC, Ravish KS, Prabhakar B. Tuberculosis-diabetes mellitus bidirectional screening at a tertiary care center, South India. Public Health Action 2013;3:S18-S22. https://doi.org/10.5588/pha.13.0032

[21] Ohene SA, Bonsu F, Hanson-Nortey NN, Toonstra A, Sackey A, Lonnroth K, Uplekar M, Danso S, Mensah G, Afutu F, Klatser P, Bakker M. Provider initiated tuberculosis case finding in outpatient departments of health care facilities in Ghana: yield by screening strategy and target group. BMC Infect Dis 2017;17:739. doi: 10.1186/s12879-017-2843-5.

[22] Kandeel A, Genedy M, El-Refai S, Funk A, Fontanet A, Talaat M. The prevalence of hepatitis C virus infection in Egypt 2015: implications for future policy on prevention and treatment. Liver Int 2017;37:45-53. https://doi.org/10.1111/liv.13186

Received on January 7, 2018. Accepted on August 28, 2019.

Correspondence: Mohsen Gadallah, Department of Community Medicine, Faculty of Medicine, Ain Shams University, Ramsis Street, Abbassyia Square, Cairo-Egypt, 11566 - E-mail: mohsengadallah@gmail.com

How to cite this article: Gadallah M, Abdelmoniem W, Fawzy M, Mokhtar A, Mohsen A. Multicenter screening of diabetic patients for detecting new cases of tuberculosis: an approach to intensify the case detection rate of tuberculosis in developing countries with high prevalence of diabetes. J Prev Med Hyg 2019;60:E343-E348. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.883

ORIGINAL ARTICLE

The reliability of self-reporting chronic diseases: how reliable is the result of population-based cohort studies

F. NAJAFI[1], M. MORADINAZAR[1], B. HAMZEH[1], S. REZAEIAN[2, 3]
[1] Research Center for Environmental Determinants of Health (RCEDH), Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran; [2] Infectious Diseases Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran; [3] Clinical Research Development Center, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran

Keywords

Self-reported • Chronic diseases • Reliability • Test-retest

Summary

Objectives. *To evaluate the reliability of self-reporting chronic diseases in the baseline data of the Ravansar Non-Communicable Diseases (RaNCD) cohort study in Kermanshah province, western Iran.*
Methods. *The study was conducted in RaNCD cohort study. To assess the reliability of self-report of chronic disease, a random sample of 202 participants were asked about some of chronic conditions 30-35 days (mean = 32) after recruitment.*
Results. *A range of kappa agreement between 39.52-100%, which the lower statistics was for hypertension and hepatitis and the higher one for cancer, cardiac ischemic, and diabetes.*
Conclusion. *The self-report of chronic diseases was relatively reliable. Therefore self-reporting data for some conditions can be used in situations where the validity is acceptable.*

Introduction

There are several epidemiological studies on chronic conditions in both developed and developing countries that are based on self-reported information [1-4]. Although such studies provide valuable information about common frequency measures with lower cost compared to those in which researchers use clinical and paraclinical criteria, there are still strong argument against reliability of self-report data.
Chronic diseases are increasing in terms of number and rates in both developed and developing countries and are responsible for about 70% of mortality in Iran [5]. About 45% of the mortality and 85% of total burden of chronic disease occur in the population aged under 70 years [6]. To tackle this important public health issue, the deputy of research and technology in ministry of health in Iran with cooperation of 17 universities decided to conduct the Prospective Epidemiological Research Studies in Iran (PERSIAN) [7]. Ravansar Non-Communicable Diseases (RaNCD) study is one the 17 cohort studies in Kurdish people of Kermanshah province, west of Iran. Most of the information related to chronic diseases in the PERSIAN cohort is often self-reported data.
While previous studies showed the importance of the self-reported data, there are no general agreement on reliability of such data in different cultures and for different chronic diseases [8-10]. In fact, reliability of self-reported chronic morbidity is related to different socio-demographic variables as well as type of chronic diseases. Similar to reliability, validity of self report is different for chronic conditions. While for diabetes, stroke, hypertension, asthma, and cancer there are some reports on 90% specificity [11-13], for other chronic diseases such as hypo-hyperthyroidism and arthritis is lower [2, 14]. There are some reports on association between single item self-rated health and overall mortality [15, 16].
RaNCD is the only cohort from PERSIAN in Kurdish people and there is no published data on reliability of self-reported of chronic morbidity in PERSIAN. Hence, our study aimed to evaluate the reliability of self-reporting chronic diseases in a sample from RaNCD study in Kermanshah province, western Iran.

Methods

Study population

In order to investigate the reliability of self-report of chronic morbidity, a cross-sectional study conducted in the RaNCD study in 2017. Ravansar, located in west of Kermanshah province is one of 17 centers of the PERSIAN Cohort study [17]. Ravansar is a district with both urban and rural areas, close to the Iraqi border, with a total population of around 50,000. The majority of residents have Iranian Kurdish ethnicity.
There are two substudies in Ravansar: adult PERSIAN and PERSIAN Youth Cohort. While Youth cohort is mainly focus on mental and psychological disorders among those aged 15-35 years old, in adult PERSIAN, researcher investigate about non-communicable diseases and their determinants in people aged > 35 years [17, 18]. For the purpose of this study, we used a random sample of 202 participants from adult cohort.

Data collection and quality control

The original design and sample size for the RaNCD was recruitment of 10,000 people living in Ravansar in Kermanshah province. However, for the purpose of this study and in order to assess the reliability of self-report of chronic morbidities, a random sample of 202 participants were reinvited to attend our center in Ravansar after 30-35 days (mean: 32 days) of their recruitment. According to the related guidline, a maximum sample size with 95% confidence level, power 80%, and ICC of 0.2 was calculated as 152 subjects [19]. Using the same questionnaire, center and staff, we tried to provide the same situation similar to their first attendance during their recruitment. The inclusion criteria for the RaNCD were the age range of 35-65 years, inhabitant in Ravansar county for one year or more and providing oral and written informed consent for participant in the cohort study. There was no any inclusion and exclusion criteria for present study.

Chronic disease detection

Chronic diseases are recorded based on self-report of participants, history of past and present treatment and medication plus physical exam by trained staff in all PERSIAN cohort sites (Fig. 1). In order to asses the reliability of self-report of chronic morbidities, we reinvited a sample of 202 participants and the whole process repeated 30-35 days later.

All stages of the study design and implementation were approved by Ethical committee of Kermanshah University of Medical Sciences (kums.res.1394.315).

Statistical analysis and calculations

Reliability of self-report was assessed using kappa (K) statistics for each chronic disease. In addition, reliability was assessed using two-way random effects intraclass correlations coefficient (ICCs) and total agreement. All analyses were carried out at 95% confidence interval using STATA software version 14.1 (Stata Corp, College Station, TX, USA).

Results

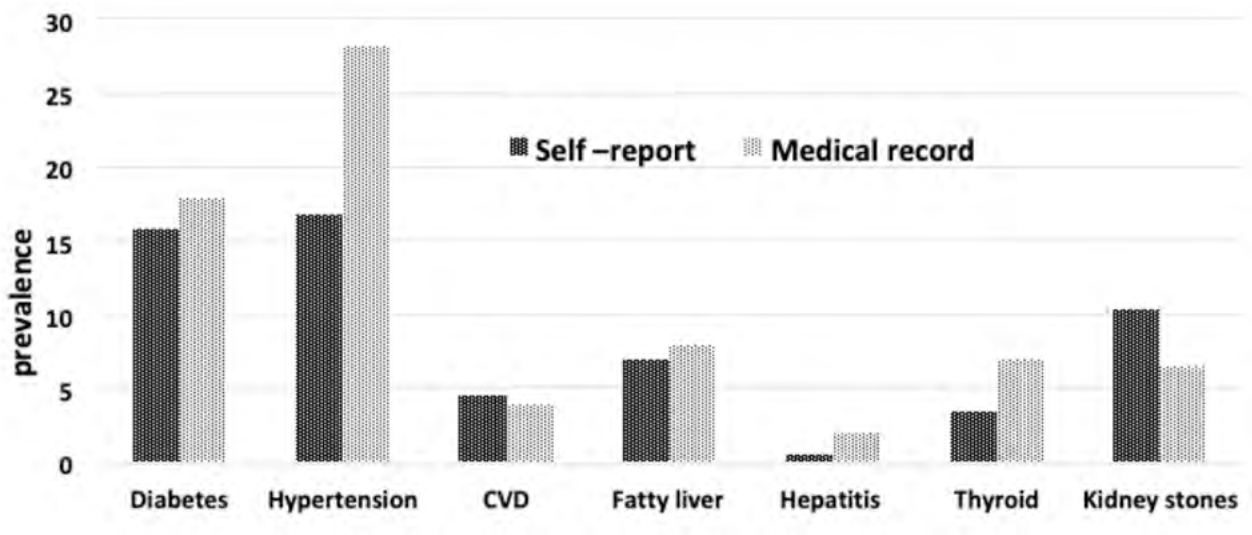
Table I presents the characteristics of the study sample by gender. A sample of 202 subjects was selected. Majority (64.18%) of them were aged more than 46 years (Tab. I). From total, 59.9% were illiterate (73.6% of women and 26.4% of men).

The test-retest reliability with range of ICC between 90.59% and 100% was found for different type of chronic diseases. There was an ICC of 100% for cancer and hepatitis. The agreement for self-reported hypertension was 78.50%, while it was ≥ 89.97% for all other self-reported chronic diseases. The reported kappa was between 39.52%-100% with the lowest value for hypertension and hepatitis and much higher values for cancer, ischemic heart disease, and diabetes. While self-report of chronic morbidity had a moderate sensitivity (range between 25%-100%), the reported specificity ranged between 93.1%-100% (Tab. II).

Discussion

Self-reporting health-related morbidities such as chronic diseases are frequently used in the epidemiological studies [20-23]. Both quality and reliability of these data are important to estimate the health indicies (such as incidence and prevalence) of chronic diseases and their determinants. In this study, for the first time, the reliability of self-reporting chronic diseases was evaluated in a small sample of RaNCD study in western Iran.

Fig. 1. The proportion of self-reported and medical records of chronic diseases in RaNCD, Kermanshah, western Iran.



Tab. I. The characteristics of the study sample by sex in RaNCD, Kermanshah, western Iran.

Variables		Total N (%)	Female n (%)	Male n (%)	P value
Age group (years)	35-45	72 (35.82)	44 (59.72)	29 (40.28)	0.75
	46-65	129 (64.18)	74 (57.36)	55 (42.64)	
Marital status	Single	31 (15.35)	27 (87.10)	4 (12.90)	< 0.001
	Married	171 (84.65)	91 (53.22)	80 (46.78)	
Level of education (years)	Illiterate	121 (59.90)	74 (73.55)	32 (26.45)	< 0.001
	1-5	54 (26.73)	23 (42.59)	31 (57.41)	
	> 6	27 (13.37)	6 (22.22)	21 (77.78)	

We found a good test-retest reliability with range of ICC between 90.59 and 100. Reliability was better for hepatitis, cancer, thyroid disease, and ischemic heart disease than for diabetes. We also observed a wide range of reliability score with calculating Kappa statistic (range 39.52-100). These differences exemplify the fact that the score of Kappa takes into account the possibility of the agreement occurring by chance. While in our study the Kappa score for diabetes (82.33%), ischemic heart disease (86.76) and cancer (100%) were high, for other condition, such as hepatitis (39.52%) and hypertension (39.96%) were low. A study to examine test-retest reliability of self-reported diabetes among 33,919 participants showed an acceptable Kappa score of 0.65 for both type 1 and type 2 diabetes diagnoses [2]. It should be noted that with such a low value for kappa among those who reported suffering from hypertension, there are some reports on undertreatment of such people. There is a report from China that only 25%-50% of hypertensive subjects who are aware of their disease receive appropriate medical treatment [24]. In fact, because of low public awareness regarding the chronic condition, the burden of diseases such as diabetes mellitus and hypertension are assumed as iceberg and most of the patients don't know about their disease status. Moreover, the most of these patients don't receive treatment [6]. Although, there are numerous reliability studies on different aspects of diabetes and hypertension diseases [2, 4, 6, 25], we found no similar study conducted on other chronic diseases for more comparisons. A study to determine the validity and reliability of self-reported stillbirth data in Australia [26] has concluded that self-reported data are important to resolve inconsistencies in administrative datasets.

To our knowledge, this is the first study on assessment of reliability of self-report of chronic morbidities in PERSIAN cohort. The ICC and the kappa values in our study may not be comparable to other studies in other countries or even in other provinces in Iran. The reliability indices is closely related to factors such as culture, public awareness, socioeconomic factors, and lifestyle, as well as type of chronic condition, severity and even prevalence in population. Another factor may be the time interval between test and retest studies. In the present study, the average time of retest was 32 days after the recurement whereas a time interval of seven years was used in the study by Sheikh MA et al (2016) [2]. In a test–retest reliability of diabetes diagnosis with time interval of 30 days the kappa agreement was found to be 0.48 [27]. However, our results can be considered as a reference for other PERSIAN studies with some consideration.

In conclusion, with increase in public awareness regarding the chronic conditions, the self-report of chronic morbidities can provide a highly reliable feature for estimation of burden of such morbidities among participants aged 35-65 years in RaNCD. Although these measures vary among different chronic diseases, these scores provide an important information on self-reported questions and can be useful for increasing precision in the PERSIAN cohort studies.

Acknowledgements

We respect and thank Professor Reza Malekzadeh director of the PERSIAN cohort and Dr. Hossein Poustchi executive director of the PERSIAN cohort for helping us to conduct this study in Ravansar.

Tab. II. Reliability of self-reported chronic diseases in RaNCD, Kermanshah, western Iran.

	Internal consistency (ICC)	Total agreement (%) (95%CI)	Kappa (%) (95%CI)
Diabetes	90.59	95.02 (91.08-97.60)	82.33 (69.0-90.25)
Hypertension	94.06	78.50 (72.32-83.75)	39.96 (25.45-57.15)
Ischemic heart disease	96.53	89.97 (85.31-95.25)	86.76 (74.1-97.25)
Fatty liver	93.07	96.04 (92.35-98.27)	71.20 (49.2-85.0)
Hepatitis B	100	98.51 (95.72-99.69)	39.52 (6.7-78.0)
Cancer	100	100 (98.18-100)	100 (24.11-100)
Thyroid	97.52	96.52 (92.69-98.59)	65.04 (38.5-82.3)
Kidney stones	91.58	96.04 (93.26-98.89)	74.44 (30.30-95.37)

The authors would also like to thank the Clinical Research Development Center of Imam Reza Hospital for their wise advices.
Funding sources: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

FN and BH: study design and carried out the implementation; MM and SR: statistical analysis and writing the manuscript; All authors discussed the results and contributed to the final manuscript.

References

[1] Rezaeian S, Esmailnasab N. Social Determinants of Health Associated with Self-Reported HIV Testing among Women. Iran J Public Health 2013;42:436-42.

[2] Sheikh MA, Lund E, Braaten T. Test-retest reliability of self-reported diabetes diagnosis in the Norwegian Women and Cancer Study: A population-based longitudinal study (n =33,919). SAGE Open Med2016;4:2050312115622857. https://doi.org/10.1177/2050312115622857

[3] Streed CG, Jr., McCarthy EP, Haas JS. Association between gender minority status and self-reported physical and mental health in the United States. JAMA Intern Med 2017;177:1210-2. https://doi.org/10.1001/jamainternmed.2017.1460

[4] Hinkle SN, Rawal S, Zhu Y, Grewal J, Albert PS, Zhang C. Validation of self-reported diagnosis of gestational diabetes at 6-weeks postpartum. Epidemiology 2017;28:747-52. https://doi.org/10.1097/ede.0000000000000695

[5] Azizi F, Ghanbarian A, Momenan AA, Hadaegh F, Mirmiran P, Hedayati M, Mehrabi Y, Zahedi-Asl S. Prevention of non-communicable disease in a population in nutrition transition: Tehran Lipid and Glucose Study phase II. Trials 2009;10:5.

[6] Okello S, Nasasira B, Muiru AN, Muyingo A. Validity and reliability of a self-reported measure of antihypertensive medication adherence in Uganda. PLoS One 2016;11:e0158499. https://doi.org/10.1371/journal.pone.0158499

[7] Malekzadeh R 2014. Accessed at Ministry of Health and Medical Education (MOHME) at http://persiancohort.com/2017

[8] Brady TJ, Murphy L, O'Colmain BJ, Beauchesne D, Daniels B, Greenberg M, House M, Chervin D. A meta-analysis of health status, health behaviors, and health care utilization outcomes of the Chronic Disease Self-Management Program. Prev Chronic Dis 2013;10:120112. https://doi.org/10.5888/pcd10.120112

[9] Vargas CM, Burt VL, Gillum RF, Pamuk ER. Validity of self-reported hypertension in the National Health and Nutrition Examination Survey III, 1988–1991. Prev Med 1997;26:678-85.

[10] Chow SKY, Wong FK. The reliability and validity of the Chinese version of the Short-form Chronic Disease Self-Efficacy Scales for older adults. J Clin Nurs 2014;23:1095-104. https://doi.org/10.1111/jocn.12298

[11] Engstad T, Bonaa KH, Viitanen M. Validity of self-reported stroke: The Tromso Study. Stroke 2000;31:1602-7.

[12] Rauscher GH, Johnson TP, Cho YI, Walk JA. Accuracy of self-reported cancer-screening histories: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2008;17:748-57. https://doi.org/10.1158/1055-9965

[13] Pan A, Teng GG, Yuan JM, Koh WP. Bidirectional association between self-reported hypertension and gout: The Singapore Chinese Health Study. PLoS One 2015;10:e0141749. https://doi.org/10.1371/journal.pone.0141749

[14] Huerta JM, Tormo MJ, Egea-Caparros JM, Ortola-Devesa JB, Navarro C. Accuracy of self-reported diabetes, hypertension and hyperlipidemia in the adult Spanish population. DINO study findings. Rev Esp Cardiol 2009;62:143-52.

[15] Nery Guimarães JM, Chor D, Werneck GL, Carvalho MS, Coeli CM, Lopes CS, Faerstein E. Association between self-rated health and mortality: 10 years follow-up to the Pró-Saúde cohort study. BMC Public Health 2012;12:676. https://doi.org/10.1186/1471-2458-12-676

[16] Sargent-Cox KA, Anstey KJ, Luszcz MA. The choice of self-rated health measures matter when predicting mortality: evidence from 10 years follow-up of the Australian longitudinal study of ageing. BMC Geriatr 2010;10:18. https://doi.org/10.1186/1471-2318-10-18

[17] Poustchi H, Eghtesad S, Kamangar F, Etemadi A, Keshtkar AA, Hekmatdoost A, Mohammadi Z, Mahmoudi Z, Shayanrad A, Roozafzai F, Sheikh M, Jalaeikhoo A, Somi MH, Mansour-Ghanaei F, Najafi F, Bahramali E, Mehrparvar A, Ansari-Moghaddam A, Enayati AA, Esmaeili Nadimi A, Rezaianzadeh A, Saki N, Alipour F, Kelishadi R, Rahimi-Movaghar A, Aminisani N, Boffetta P, Malekzadeh R. Prospective epidemiological research studies in Iran (the PERSIAN Cohort Study): rationale, objectives, and design. Am J Epidemiol 2018;187:647-55. https://doi.org/10.1093/aje/kwx314

[18] Eghtesad S, Mohammadi Z, Shayanrad A, Faramarzi E, Joukar F, Hamzeh B, Farjam M, Zare Sakhvidi MJ, Miri-Monjar M, Moosazadeh M, Hakimi H, Rahimi Kazerooni S, Cheraghian B, Ahmadi A, Nejatizadeh A, Mohebbi I, Pourfarzi F, Roozafzai F, Motamed-Gorji N, Montazeri SA, Masoudi S, Amin-Esmaeili M, Danaie N, Mirhafez SR, Hashemi H, Poustchi H, Malekzadeh R. The PERSIAN Cohort: Providing the evidence needed for healthcare reform. Arch Iran Med 2017;20:691-5.

[19] Bujanga MA, Baharum N. A simplified guide to determination of sample size requirements for estimating the value of intraclass correlation coefficient: a review. Arch Orofac Sci 2017;12(1-11).

[20] Keith NR, Clark DO, Stump TE, Miller DK, Callahan CM. Validity and reliability of the Self-Reported Physical Fitness (SRFit) survey. J Phys Act Health 2014;11:853-9. https://doi.org/10.1123/jpah.2012-0264

[21] Coster MC, Bremander A, Rosengren BE, Magnusson H, Carlsson A, Karlsson MK. Validity, reliability, and responsiveness of the Self-reported Foot and Ankle Score (SEFAS) in forefoot, hindfoot, and ankle disorders. Acta Orthop 2014;85:187-94. https://doi.org/10.3109/17453674.2014.889979

[22] Lee WY, Ahn J, Kim JH, Hong YP, Hong SK, Kim YT, Lee SH, Morisky DE. Reliability and validity of a self-reported measure of medication adherence in patients with type 2 diabetes mellitus in Korea. J Int Med Res 2013;41:1098-110. https://doi.org/10.1177/0300060513484433

[23] Leatherdale ST, Laxer RE. Reliability and validity of the weight status and dietary intake measures in the COMPASS questionnaire: are the self-reported measures of body mass index (BMI) and Canada's food guide servings robust? Int J Behav Nutr Phys Act 2013;10:42. https://doi.org/10.1186/1479-5868-10-42

[24] Feng XL, Pang M, Beard J. Health system strengthening and hypertension awareness, treatment and control: data from the China Health and Retirement Longitudinal Study. Bull World Health Organ 2014;92:29-41. https://doi.org/10.2471/blt.13.124495

[25] Wang Y, Lee J, Toh MP, Tang WE, Ko Y. Validity and reliability of a self-reported measure of medication adherence

in patients with Type 2 diabetes mellitus in Singapore. Diabet Med 2012;29:e338-44. https://doi.org/10.1111/j.1464-5491.2012.03733.x

[26] Hure AJ, Chojenta CL, Powers JR, Byles JE, Loxton D. Validity and reliability of stillbirth data using linked self-reported and administrative datasets. J Epidemiol 2015;25:30-7. https://doi.org/10.2188/jea.JE20140032

[27] Kargman DE, Sacco RL, Boden-Albala B, Paik MC, Hauser WA, Shea S. Validity of telephone interview data for vascular disease risk factors in a racially mixed urban community: the Northern Manhattan Stroke Study. Neuroepidemiology 1999;18:174-84. https://doi.org/10.1159/000026209

Received on November 26, 2018. Accepted on June 17, 2019.

Correspondence: Shahab Rezaeian. Dept. of Epidemiology, School of Health, Kermanshah University of Medical Sciences, 6719851351 Kermanshah, Iran -Tel. +98-8337102026 - E-mail: shahab.rezayan@gmail.com

How to cite this article: Najafi F, Moradinazar M, Hamzeh B, Rezaeian S. The reliability of self-reporting chronic diseases: how reliable is the result of population-based cohort studies. J Prev Med Hyg 2019;60:E349-E353. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1118

ORIGINAL ARTICLE

Early and current physical activity: cross-sectional associations with overweight among adults

D.H.C. COLEDAM
Federal Institute of Education, Science and Technology of São Paulo, Boituva, São Paulo, Brazil

Keywords

Adolescence • Body Mass Index • Child • Health • Motor activity

Summary

Introduction. *The health benefits of physical activity in all ages are widely known, however the effects of early physical activity on future health are not yet fully understood. The aim of this study was to analyze the cross-sectional associations between previous and current physical activity with overweight among adults.*
Methods. *A probabilistic sample of 534 teachers was included in the study. Independent variables were physical activity in childhood, adolescence, and current, and clustering of the variables, all analyzed using a self-report questionnaire. The dependent variable was overweight, estimated by the body mass index, assessed using self-report measures of weight and height. Covariates were sex, age, skin color, income, sedentary behavior, medication use for weight control, and nutritionist counseling. Poisson regression was adopted to estimate Prevalence Ratios (PR) in the multivariate analysis.*
Results. *Physical activity at ages 6-10 (PR = 1.03 to 1.13), 12-14 (PR = 0.96 to 0.98), and 15-17 (PR = 0.76 to 0.90) years was not associated with overweight. Participants who do not meet the recommendation of current physical activity have a higher likelihood of being overweight (PR = 1.55 to 2.17) and the magnitude of the association increased when analyzing those who were not physically active through all periods analyzed (PR = 3.69 to 4.69).*
Conclusion. *Performing physical activity only in early life does not seem to promote health benefits in the sample analyzed. Although current physical activity is associated with the outcome, the promotion of both early and current physical activity seems to be a better strategy to prevent overweight among adults.*

Introduction

Overweight and obesity is a worldwide epidemic and the prevalence is approximately 40%, although this varies according to the region of the world [1]. The health consequences are widely known and include cardiovascular, metabolic, and musculoskeletal diseases, some types of cancer, depression, anxiety, body dissatisfaction, low self-esteem, a negative self-concept, and poor quality of life [2]. The etiology of overweight is complex and multifactorial and involves environmental, behavioral, biological, and contextual factors that result in a positive energy balance [1-3].
Among behavioral factors, physical activity is an important aspect in the emergence of overweight, independent of age [4-6]. Observational data demonstrated that physical activity is associated with a lower prevalence of overweight and obesity among adults [7, 8]. Results of experimental studies corroborate observational data and indicate that an increase in physical activity reduces weight, the body mass index, and body fat among adults [9]. Any amount of physical activity is encouraged, however, it is recommended that adults perform at least 150 min a week of aerobic physical activity at moderate to vigorous intensity to receive health benefits [4, 5], whereas a higher amount of physical activity ≅ 300 min provides additional benefits. In addition to aerobic activity, two or more sessions of muscle-strengthening activities of moderate or higher intensity involving major muscle groups are recommended [4, 5].
Although physical activity is recommended to prevent overweight and obesity through life, the effects of physical activity during childhood and adolescence on weight status in adulthood are not yet well understood. Physical activity guidelines do not describe any effects of physical activity in childhood and adolescence on subsequent ages [4, 6, 10]. Information is available describing that early physical activity promotes a lower prevalence of chronic diseases [11-13], carotid intima-media thickness [14], a better metabolic profile [15], and bone health [16-18]. One study showed that extracurricular physical activities reduce the risk of being overweight in adulthood [19]; however data were not adjusted for current physical activity in adult age. Another study showed a negative association between sports practice early in life and body fat [15], but the sample was not classified according to weight status. Besides the information available, the role of physical activity in childhood and adolescence on overweight in adulthood is unclear, as well as whether continuity of physical activity can provide better effects when compared to physical activity in only one period of life.
Investigation of the long-term effect of physical activity during early age on subsequent overweight is relevant since it is a priority in health promotion, and the prevalence has increased over the years independently of age,

https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1265

sex, or socioeconomic status [20]. Furthermore, overweight is determinant in the etiology of non-communicable diseases as well as some other health disorders [2]. Considering the scarcity of information related to this topic, the aim of this study was to analyze the cross-sectional associations between physical activity during childhood, adolescence, and current, as well as the clustering of these variables with overweight among adults.

Methods

A cross-sectional study with a probabilistic sample of elementary teachers from public schools from Londrina, Paraná, Brazil was carried out in 2014. Information about the characteristics of the city can be found elsewhere [21]. The present study is part of a larger project aiming to investigate the sociodemographic, work condition, and health risk predictors of health service use, medication consumption, absenteeism, and presenteeism. A study was conducted with a representative sample of teachers from 63 schools according to each region of the city (north, south, east, west, and center). All measures were self-reported and assessed using a standardized questionnaire. The study was approved by the Ethics Committee for Research involving human beings of the State University of Londrina, process 118/2014. The guidelines of Resolution Nº 466/2012 of the Brazilian National Health Council were followed.

The minimum sample size was estimated using the following parameters: N = 2.500, a 50% outcome prevalence, 5% sample error, confidence interval of 95%, and design effect of 1.5, using the software OpenEpi 3.0. A minimum of 500 teachers was required to compose the sample of the study. All schools from the urban region (N = 74) were invited to participate in the study and 63 agreed. The proportion of participating schools in relation to the total was 85%. The following eligibility criteria were adopted: a) having been a teacher in municipal schools for at least 1 year and be working in an elementary school; b) not being retired or on medical leave during data collection; c) not having been work relocated (i.e., teacher working as a secretary or in administration). These eligibility criteria were applied as they are derived from the main project described above.

Procedures

Before data collection all participants signed an informed written consent containing the objectives, procedures, risks, and benefits of the study, as well as the researchers' contact details. The project was authorized by the Municipal Education Department who provided permission to conduct the study. All schools that agreed to participate in the study were visited to present the study proposal and obtain authorization from the principals. The purpose and procedures of the study were presented to teachers and data collection was scheduled randomly in the schools where the teachers were enrolled. The participants completed the self-report questionnaire and for those who were absent on the scheduled date, another data collection was performed. All the procedures were carried out in the school where each teacher worked and performed by the coordinator of the project.

Variables

The dependent variable was overweight and the independent variables were physical activity between 6-10, 11-14, and 15-17 years old, as well as current, and the clustering of physical activity in all ages analyzed. Sex, age, skin color, income, sedentary behavior, medication use for weight control, and nutritionist counseling were the covariates.

Overweight was assessed by the body mass index (weight/heigth^2). Weight and height were self-reported and the cut-off adopted for overweight classification was ≥ 25kg/m^2 [22]. The validity of self-report measures of weight and height in adults for classification purposes in epidemiological studies has been described elsewhere [23, 24]. In addition, we conducted a pilot study to analyze the validity of measures (self-report and measured weight and height) with 50 teachers. There was high agreement between data for overweight (Kappa index = 0.864), with relative agreement in 93.5% of cases. The difference in overweight prevalence between predicted and observed values was 2.2%, with a mean difference of 0.16 (-0.32 to 0.64) kg for weight and 0.02 (-0.27 to 0.12) m for height, and there were no statistical differences (P > 0.05) between self-report and measured values. Intraclass correlation coefficients were as follows: Weight = 0.98 (0.97-0.99), height = 0.91 (0.83-0.94), body mass index = 0.96 (0.93-0.98).

Current physical activity was assessed with the Brazilian version of the International Physical Activity Questionnaire, details of which can be found elsewhere [25]. The procedures described by Hallal et al. [26] were followed. The domains recreation, sports, exercise, and leisure-time physical activity were used for aerobic activity and the assessment of strength exercise practice was performed through the question: "How many days a week do you perform muscle strengthening exercises?" with response options "0, 1, 2, 3, and ≥ 4". The current recommendation of 150 minutes/week of moderate to vigorous physical activity and muscle-strengthening activities on 2 or more days a week was adopted [4, 5]. Physical activity in childhood and adolescence was assessed using the question: "When you were between 6 and 10 years old, how many days a week did you practice physical activity outside of school (supervised or not)" with answer options "none; 1-2 days; 3-4 days; > 5 days". The same question was performed for 11-14 and 15-17 years of age. Participants that reported "3-4 days" or "> 5 days" were considered active. Any type of physical activity commonly performed by children and adolescents in leisure time was considered, such as sports practice, physical exercises, gymnastics, dance, running, walking, swimming, riding a bike, active games, and outdoor play.

Age and skin color were assessed by an open question. Similarly, sedentary behavior was estimated by hours spent watching TV and using a PC: "On a normal day

during leisure time, how much time do you watch TV", and the same question was asked for PC. Income was estimated according to the values proposed by the questionnaire of the Brazilian Association of Polling Companies [27]. Medication use for weight control was estimated by the question: "Do you regularly use (continuously) any type of medication? Indicate only medicines used under medical prescription during the last six months." A list of categories of medicine was displayed, including weight control. The response options were "Yes or No". The question "How many times in the last 12 months have you attended consultations with a nutritionist" was used to assess nutritionist counseling.

Due to the lack of instruments, some variables were assessed by questions that were developed to achieve the aims of the present study (previous physical activity, muscle strength exercise, medication use for weight control, and nutritionist counseling). Some steps were adopted for the development of questions: Proposal of the questions, assessment of the content validity by a panel of experts, and modification of the questions according to suggestions from the experts. Furthermore, a pilot study with 50 elementary teachers was conducted to assess comprehension and the reproducibility of the questions within seven days test-retest. The cut-off for the inclusion of variables in this study were (one-way intraclass coefficient > 0.5 for continuous variables and kappa index > 0.40 for categorical variables).

Descriptive statistics are presented as mean, confidence interval of 95%, and absolute and relative frequency. The comparison of self-report versus measured weight and height was performed by the T-test for independent samples and the Kappa statistic was used to test the agreement between overweight classifications. Reproducibility of measures was performed by the Intraclass Correlation Coefficient for continuous variables and Kappa statistic for categorical variables. The bivariate association between independent variables with overweight was performed by the Chi-Squared test and variables that presented $P < 0.20$ were inserted in multivariate models. Multivariate analysis was conducted using Poisson Regression to estimate Prevalence Ratios (PR) and confidence intervals of 95% (CI95%) considering strata, weight, and primary sample units using the package "survey" of the software STATA 13.0. Statistical significance was set at $P < 0.05$.

Results

Of the 595 participants, 61 teachers were excluded from the study due to incomplete information on the questionnaire. The final sample was composed of 534 teachers, stratified according to region of the city. The respective proportion of teachers in the population and sample were similar according to regions of the city: north (32.8 and 30.8%), south (20.0 and 17.9%), east (20.3 and 18.3%), west (22.1 and 28.0%), and center (4.8 and 4.9%).

The sample was composed of a higher proportion of female teachers, between 30 and 49 years old, middle and high income, white color, with sedentary behavior for less than 2h, not users of medication for weight control, with no nutritionist counseling, and not physically active in adolescence; not currently active or active in childhood, adolescence, and adulthood physical activity were clustered. The prevalence of overweight was 40.4% (Tab. I).

Table II describes the bivariate association between physical activity at 6-10, 12-14, and 15-17 years old, as well as current physical activity, and the clustering

Tab. I. Characteristics of the sample (n = 534).

Sample characteristics	n (%)
Sex	
Male	25 (4.7)
Female	509 (95.3)
Age	
22 - 29	70 (13.1)
30 - 39	169 (31.6)
40 - 49	210 (39.3)
> 50	85 (15.9)
Income	
Low	83 (15.5)
Middle	235 (44.0)
High	216 (40.4)
Skin color	
White	424 (79.4)
Other	110 (20.6)
Sedentary behavior	
< 2 h	304 (56.9)
≥ 2 h	230 (43.1)
Overweight	
No	318 (59.6)
Yes	216 (40.4)
Medication use for weight control	
No	519 (97.2)
Yes	15 (2.8)
Nutritionist counseling	
No	461 (86.3)
Yes	73 (13.7)
Physically active between 6-10 years old	
No	268 (50.2)
Yes	266 (49.8)
Physically active between 12-14 years old	
No	291 (54.5)
Yes	243 (45.5)
Physically active between 15-17 years old	
No	373 (69.9)
Yes	161 (30.1)
Current Physical activity *	
No	439 (82.2)
Yes	95 (17.8)
Physically active between 6-17 years old and currently active	
No	482 (90.3)
Yes	52 (9.7)

* 150 min of moderate to vigorous aerobic physical activity plus strength activities two times/week.

Tab. II. Association between previous and current physical activity and overweight among elementary teachers.

	Overweight	
Variables	**% (CI 95%)**	**Crude PR (CI 95%)**
Physically active between 6-10 years old	P = 0.181	
Yes	46.2 (40.1-52.4)	Reference
No	40.4 (34.6-46.3)	0.86 (0.66-1.12)
Physically active between 12-14 years old	P = 0.069	
Yes	47.6 (41.2-54.0)	Reference
No	39.6 (34.1-45.4)	0.82 (0.63-1.08)
Physically active between 15-17 years old	P = 0.033	
Yes	50.3 (42.5-58.0)	Reference
No	40.2 (35.2-45.3)	0.79 (0.61-1.05)
Current physical activity	P < 0.001	
Yes	23.9 (16.3-33.5)	Reference
No	47.4 (42.7-52.1)	**1.98 (1.27-3.08)**
Physically active between 6-17 years old and current physical activity	P = 0.002	
Yes	16.1 (7.0-32.6)	Reference
No	44.9 (40.5-49.4)	**2.78 (1.15-6.76)**

% (CI95%): Relative frequency and confidence interval of 95% for prevalence; PR (CI95%): Prevalence Ratio and confidence intervals of 95%.
Bold denotes statistical significance at P < 0.05.

of physical activity of all categories with overweight. A higher prevalence of overweight was found in teachers who do not perform physical activity currently compared to active teachers (47.4 vs 23.9 %, PR = 1.98) and in those who were not classified as active in childhood, adolescence, and currently (44.9 vs 16.1 %, PR = 2.78), P < 0.05.

The multivariate association between independent variables and overweight is presented in Table III. In all models analyzed, teachers who are currently physically inactive (PR = 1.55 to 2.17) and those who were physically inactive between 6-17 years of age and remain inactive currently (PR = 3.69 to 4.69) presented a higher likelihood of being overweight compared to those who

Tab. III. Multivariate association between previous and current physical activity and overweight among elementary teachers.

Variables	**Adjusted PR[a] (CI95%)**	**Adjusted PR[b] (CI95%)**	**Adjusted PR[c] (CI95%)**
Physically active between 6-10 years old			
Yes	Reference	Reference	Reference
No	1.03 (0.76-1.40)	1.12 (0.77-1.62)	1.13 (0.79-1.63)
Physically active between 12-14 years old			
Yes	Reference	Reference	Reference
No	0.98 (0.73-1.31)	0.96 (0.66-1.40)	0.96 (0.66-1.39)
Physically active between 15-17 years old			
Yes	Reference	Reference	Reference
No	0.90 (0.68-1.19)	0.83 (0.61-1.13)	0.76 (0.52-1.10)
Current physical activity			
Yes	Reference	Reference	Reference
No	**2.09 (1.43-3.08)**	**2.17 (1.48-3.19)**	**1.55 (1.04-2.31)**
Physically active between 6-17 years old and current physical activity			
Yes	Reference	Reference	Reference
No	**4.69 (1.81-12.17)**	-	**3.69 (1.36-10.02)**

PR (CI95%): Prevalence Ratio and confidence intervals of 95%; Bold denotes statistical significance at P < 0.05;
[a] Multivariate analysis adjusted for sex, age, skin color and income;
[b] Multivariate analysis adjusted for sex, age, skin color, income, sedentary behavior, medication use for weight control, nutritionist counseling, current and previous physical activity;
[c] Variables inserted in model [b] plus the clustering of current and previous physical activity.

are currently active or those who were active in all periods analyzed respectively. Being inactive in any period of childhood or adolescence was not associated with overweight (PR = 0.76 to 1.13).

Discussion

Previous studies have demonstrated that physical activity during early life is associated with a variety of health indicators such as a lower prevalence of chronic diseases [11-13], lower carotid intima-media thickness [14], better metabolic profile [15], bone health [16-18], overweight [19], and body fat [15]. Two hypotheses can explain the long-term benefits of early physical activity. First, the classical model advocates that the practice of physical activity during childhood leads to lower body fatness and higher physical activity in adulthood and consequently prevents future health disorders. Second, in the alternative model the same mechanisms as the classical models occur, however modifications in DNA methylation also occur, which result in future protection for cardiovascular disease and diabetes [12].

In the present study there was no association between previous physical activity and overweight in adulthood, while teachers who reported not being currently active presented a higher likelihood of overweight. These results are not in line with previous studies that described positive benefits of physical activity during childhood on adult health [11-18], including overweight and body fat [15, 19]. On the other hand, the results corroborate those describing the positive effect of current physical activity on overweight [7, 8]. The results suggest that the positive effect of physical activity on body mass and body fat occurs at the period when physical activity is performed, and for this reason, adults who currently practice physical activity have more favorable benefits on overweight compared to those who only performed earlier physical activity. This can be seen through the recommendation of regular physical activity on the prevention of weight regain after initial weight loss [4]. When analyzing the participants who reported physical activity in all periods analyzed, the magnitude of association was more than twofold higher compared to those who were only currently active (PR = 1.55 vs 3.69). This suggests that exposure to higher energy expenditure in all stages of life results in less body fat accumulation in adult life. This finding reinforces in part both classical and alternative models that explain the mechanisms by which long-term benefits of physical activity in childhood occur [12] and indicates that although current physical activity promotes a positive effect on adult health, the benefits are more pronounced if physical activity is performed in more than one period of life.

The association between clustering of physical activity during childhood, adolescence, and current with overweight described in this study has relevant public health implications, however only 9.7% of the participants presented this characteristic (Tab. I). This demonstrates the complexity of physical activity maintenance, which is affected by multilevel individual, interpersonal, environmental, regional, and global factors [28]. Although there is tracking of physical activity through childhood into adulthood, longer periods of monitoring result in lower stability of physical activity [29] and a decline can be evidenced over time [30]. The same tendency was found in the present study, since the prevalence of participants with three or more days of physical activity in a week at ages 6-10, 11-14, and 15-17 years were 49.8, 45.5, and 30.1%, while 17.8% of the sample met the guideline for current physical activity. This information suggests the need for support for physical activity in various stages of life to achieve higher health benefits as described in the present study.

The following points provide the implications of the present results. First, although there is growing interest regarding the effects of early physical activity on adult health, literature about this topic is still scarce. The results improve knowledge about the benefits of physical activity during childhood and adolescence on overweight among adults and reinforce the need for physical activity promotion in both early and adult life. Although this is a cross-sectional study, the analysis showed that physical activity in more than one period of life can prevent overweight to a greater extent when compared to physical activity performed only in adult ages. This finding is relevant since overweight is determinant in the emergence of a variety of health disorders [1-3] and the prevalence is still increasing from a global perspective [20]. Considering the body of evidence in the literature and in the present study, it is suggested that future guidelines for physical activity include the long-term effects of physical activity performed in childhood and adolescence. Likewise, future studies should investigate this topic using longitudinal designs to confirm findings from cross-sectional studies.

Some limitations should be described to better understand the results of the present study. Regarding the sample, it was composed of elementary school teachers and a higher proportion of females compared to males is a common characteristic of this profession. It is widely described that males present higher physical activity compared to females [31], however the relationship between physical activity and overweight is present in both sexes [32] and is not sex dependent. In the present study, a multivariate analysis adjusted for sex was used to control this limitation. Physical activity during work should also be considered when investigating overweight in a sample of workers. The health effects of leisure time physical activity can be masked in individuals who perform high physical activity during their job due to higher energy expenditure resulting from labor tasks. The sample of the present study was composed of elementary teachers that commonly present high demands in their job, but not related to high energy expenditure. Furthermore, the prevalence of both overweight and physical activity of the sample studied was similar to the general population [1, 31], which enables generalization of the present results.

Another limitation is that the instruments used to assess physical activity were self-reported and there is an inherent bias of recall when using questionnaires. In addition, isolated questions were elaborated for the present study to estimate physical activity in childhood and adolescence. Unfortunately, there is no questionnaire to assess previous physical activity and isolated questions are often used [11, 12, 14-16, 19]. To minimize this limitation, the construct validity and reproducibility of the questions were tested. The period for which physical activity was assessed should also be discussed; childhood, adolescence, and current physical activity. However, the sample is heterogeneous regarding age (22 to 66 years), which together with the cross-sectional study design, prevents accurate estimation of physical activity between the beginning of adult life and the current period, as well as understanding of the influence of the disruption of an active life in the adult period on the associations. The consequence of this limitation for the present results was that the magnitudes of the associations were probably attenuated. The absence of measures of the body mass index during childhood and adolescence is another limitation that should be considered, since it is a predictor of adult overweight [33]. Although the cross-sectional design can be considered a limitation of the present study, it is an alternative to longitudinal designs which are expensive and require decades of follow-up. The present study is based on the conceptual model of the relationship between physical activity and overweight, a phenomenon widely described in both observational and experimental studies [7-9], which reduces the probability of bias, common when investigating variables that are not yet well understood.

Conclusion

In summary, self-reported physical activity during childhood and adolescence was not associated with overweight in adulthood. Individuals who were not currently engaged in physical activity presented a higher likelihood of being overweight, while not being physically active in childhood, adolescence, and currently increased the magnitude of the association. Although current physical activity is associated with the outcome analyzed, promoting both early and current physical activity seems to be a better strategy to prevent overweight among adults. However, the absence of information regarding physical activity in all periods of adult life prevents understanding of how discontinuity can influence the associations between physical activity and overweight, and, thus, this should be considered in future studies.

Acknowledgements

The author would like to thank the Municipal Department of Education of Londrina, Paraná, Brazil, as well as the principals and teachers who agreed to participate voluntarily in this study. The author appreciates the useful suggestions of the anonymous reviewers.

Funding sources: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The author declares no conflict of interest.

Authors' contributions

DHCC conceived and coordinated the project, worked on the study design, data collection, analysis and interpretation, drafting, critical review and final approval of the manuscript.

References

[1] World Health Organization. Obesity and Overweight factsheet from the WHO. 2018. Available from: https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight accessed march 21st, 2019

[2] Williams EP, Mesidor M, Winters K, Dubbert PM, Wyatt SB. Overweight and obesity: prevalence, consequences, and causes of a growing public health problem. Curr Obes Rep 2015;4:363-70. https://doi.org/10.1007/s13679-015-0169-4

[3] Kadouh HC, Acosta A. Current paradigms in the etiology of obesity. Tech Gastrointest Endosc 2017;19:2-11. https://doi.org/10.1016/j.tgie.2016.12.001

[4] Piercy KL, Troiano RP, Ballard RM, Carlson SA, Fulton JE, Galuska DA, George SM, Olson RD. The physical activity guidelines for Americans. JAMA 2018;320:2020-28. https://doi.org/10.1001/jama.2018.14854

[5] World Health Organization. Global recommendations on physical activity for health. Geneva: WHO 2010.

[6] Poitras VJ, Gray CE, Borghese MM, Carson V, Chaput JP, Janssen I, Katzmarzyk PT, Pate RR, Connor Gorber S, Kho ME, Sampson M, Tremblay MS. Systematic review of the relationships between objectively measured physical activity and health indicators in school-aged children and youth. Appl Physiol Nutr Metab 2016;41:S197-S239. https://doi.org/10.1139/apnm-2015-0663

[7] Wanner M, Martin BW, Autenrieth CS, Schaffner E, Meier F, Brombach C, Stolz D, Bauman A, Rochat T, Schindler C, Kriemler S, Probst-Hensch N. Associations between domains of physical activity, sitting time, and different measures of overweight and obesity. Prev Med Rep 2016;3:177-84. https://doi.org/10.1016/j.pmedr.2016.01.007

[8] Hansen BH, Holme I, Anderssen SA, Kolle E. Patterns of objectively measured physical activity in normal weight, overweight, and obese individuals (20-85 years): a cross-sectional study. PLoS One 2013;8:e53044. https://doi.org/10.1371/journal.pone.0053044

[9] Oja P, Kelly P, Murtagh EM, Murphy MH, Foster C, Titze S. Effects of frequency, intensity, duration and volume of walking interventions on CVD risk factors: a systematic review and meta-regression analysis of randomised controlled trials among inactive healthy adults. Br J Sports Med 2018;52:69-775. https://doi.org/ 0.1136/bjsports-2017-098558

[10] Janssen I, LeBlanc AG. Systematic review of the health benefits of physical activity and fitness in school-aged children and youth. Int J Behav Nutr Phys Act 2010;7:40. https://doi.org/10.1186/1479-5868-7-40

[11] Fernandes RA, Zanesco A. Early physical activity promotes

lower prevalence of chronic diseases in adulthood. Hypertens Res 2010;33:926-31. https://doi.org/10.1038/hr.2010.106

[12] Fernandes RA, Coelho-e-Silva MJ, Lima, MCL, Cayres SU, Codogno JS. Possible underestimation by sports medicine of the effects of early physical exercise practice on the prevention of diseases in adulthood. Curr Diabetes Rev 2015;11:201-5. https://doi.org/10.2174/1573399811666150401104515

[13] Fernandes RA, Zanesco A. Early sport practice is related to lower prevalence of cardiovascular and metabolic outcomes in adults independently of overweight and current physical activity. Medicina 2015;51:336-42. https://doi.org/10.1016/j.medici.2015.10.003

[14] Lima, MCS, Barbosa MF, Diniz TA, Codogno JS, Freitas Júnior IF, Fernandes RA. Early and current physical activity: relationship with intima-media thickness and metabolic variables in adulthood. Braz J Phys Ther.2014;18(5):462-69. doi: 10.1590/bjpt-rbf.2014.0040.

[15] Lima MCS, Cayres SU, Machado-Rodrigues A, Coelho-e-Silva MJ, Kemper H, C, Fernandes RA. Early sport practice promotes better metabolic profile independently of current physical activity. Medicina Sportiva 2014;18:172-8. https://doi.org/10.5604/17342260.1133108

[16] Mantovani AM, de Lima MCS, Gobbo LA, Ronque ERV, Romanzini M, Turi-Lynch BC, Codogno JS, Fernandes RA. Adults engaged in sports in early life have higher bone mass than their inactive peers. J Phys Act Health 2018;15:516-22. https://doi.org/10.1123/jpah.2017-0366

[17] Nilsson M, Ohlsson C, Mellström D, Lorentzon M. Previous sport activity during childhood and adolescence is associated with increased cortical bone size in young adult men. J Bone Miner Res 2009;24:125-3. https://doi.org/10.1359/jbmr.080909

[18] Nilsson M, Sundh D, Ohlsson C, Karlsson M, Mellström D, Lorentzon M. Exercise during growth and young adulthood is independently associated with cortical bone size and strength in old Swedish men. J Bone Miner Res 2014;29:1795-804. https://doi.org/10.1002/jbmr.2212

[19] Menschik D, Ahmed S, Alexander MH, Blum RW. Adolescent physical activities as predictors of young adult weight. Arch Pediatr Adolesc Med 2008;162:29-33. https://doi.org/10.1001/archpediatrics.2007.14

[20] Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014;384:766-81. https://doi.org/10.1016/S0140-6736(14)60460-8

[21] Instituto Brasileiro de Geografia e Estatística (IBGE). IBGE cidades, Londrina. Available in https://cidades.ibge.gov.br/brasil/pr/londrina/panorama accessed November 14th, 2018

[22] World Health Organization. Obesity: preventing and managing the global epidemic: report of a WHO consultation. Geneva: WHO. 2000. Available from: https://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/ accessed July 18th, 2019

[23] Moreira NF, Luz VG, Moreira CC, Pereira RA, Sichieri R, Ferreira MG, Muraro AP, Rodrigues PRM. Self-reported weight and height are valid measures to determine weight status: results from the Brazilian National Health Survey (PNS 2013). Cad Saude Publica 2018;34:e00063917. https://doi.org/10.1590/0102-311X00063917

[24] Olfert MD, Barr ML, Charlier CM, Famodu OA, Zhou W, Mathews AE, Byrd-Bredbenner C, Colby SE. Self-Reported vs. measured height, weight, and BMI in young adults. Int J Environ Res Public Health 2018;15:2216. https://doi.org/10.3390/ijerph15102216.

[25] Matsudo S, Araújo T, Matsudo V, Andrade D, Andrade E, Oliveira LC, Braggion G. Questionário internacional de atividade física (IPAQ): estudo de validade e reprodutibilidade no Brasil. Rev Bras Ativ Fís Saúde 2001;6:5-18. https://doi.org/10.12820/rbafs.v.6n2p5-18

[26] Hallal PC, Gomez LF, Parra DC, Lobelo F, Mosquera J, Florindo AA, et al. Lessons learned after 10 years of IPAQ use in Brazil and Colombia. J Phys Act Health 2010;7(s2):S259-64. https://doi.org/10.1123/jpah.7.s2.s259

[27] Associação Brasileira de Empresas de Pesquisa. Critério de classificação econômica Brasil 2012. Available in: http://www.abep.org/criterio-brasil acessed March 22nd, 2019

[28] Bauman AE, Reis RS, Sallis JF, Wells JC, Loos RJ, Martin BW, Lancet Physical Activity Series Working Group. Correlates of physical activity: why are some people physically active and others not? Lancet 2012;380:258-71. https://doi.org/10.1016/S0140-6736(12)60735-1

[29] Telama R, Yang X, Leskinen E, Kankaanpaa A, Hirvensalo M, Tammelin T, Raitakari OT. Tracking of physical activity from early childhood through youth into adulthood. Med Sci Sports Exerc 2014;46:955-62. https://doi.org/10.1249/MSS.0000000000000181

[30] Farooq MA, Parkinson KN, Adamson AJ, Pearce MS, Reilly JK, Hughes AR, Janssen X, Basterfield L, Reilly JJ. Timing of the decline in physical activity in childhood and adolescence: Gateshead Millennium Cohort Study. Br J Sports Med 2018;52:1002-6. https://doi.org/10.1136/bjsports-2016-096933

[31] Mielke GI, Hallal PC, Rodrigues GBA, Szwarcwald CL, Santos FV, Malta DC. Prática de atividade física e hábito de assistir à televisão entre adultos no Brasil: Pesquisa Nacional de Saúde 2013. Epidemiol Serv Saude 2015;24:277-86. https://doi.org/10.5123/S1679-49742015000200010

[32] Tudor-Locke C, Brashear MM, Johnson WD, Katzmarzyk PT. Accelerometer profiles of physical activity and inactivity in normal weight, overweight, and obese US men and women. Int J Behav Nutr Phys Act 2010;7:60.https://doi.org/10.1186/1479-5868-7-60

[33] Simmonds M, Llewellyn A, Owen CG, Woolacott N. Predicting adult obesity from childhood obesity: a systematic review and meta-analysis. Obes Rev 2016;17:95-107. https://doi.org/10.1111/obr.12334

Received on April 30, 2019. Accepted on July 12, 2019.

Correspondence: Diogo Henrique Constantino Coledam, Federal Institute of Education, Science and Technology of São Paulo, 100 Zélia de Lima Rosa av. Boituva, São Paulo, Brazil - Tel. +55 15 33638610 - E-mail: diogohcc@yahoo.com.br

How to cite this article: Coledam DHC. Early and current physical activity: cross-sectional associations with overweight among adults. J Prev Med Hyg 2019;60:E354-E360. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1265

ORIGINAL ARTICLE

Smoking and alcoholism among adult population and its association with outlet density in a hilly area of North India

Y.S. RAUTELA[1], B.V. REDDY [2], A.K. SINGH[3], A. GUPTA[4]
[1] Veer Chandra Singh Garhwali Government Medical Sciences and Research Institute, Uttarakhand, India; [2] Department of Community Medicine, NRI Academy of Medical Sciences Andhra Pradesh, India; [3] Department of Community Medicine, Veer Chandra Singh Garhwali Government Medical Sciences and Research Institute, Uttarakhand, India; [4] Department of Community and Family Medicine, All India Institute of Medical Sciences Mangalagiri, Andhra Pradesh, India

Keywords

Smoking • Alcohol • Outlet • Environment • Neighborhood

Summary

Background. *The rising burden of non-communicable diseases is a threat to India. The behavioural risk factors having largest contribution to NCDs. Neighbourhood retailing of tobacco products, and alcohol are important risk factors. The objectives of the present study were to determine the prevalence of smoking and alcoholism among adults of Srikot, Uttarakhand, and to determine its relationship with tobacco, and alcohol retail outlet density.*
Materials and methods. *The study design was a community based cross sectional study, which was done in Srikot, Uttarakhand among adults aged above 20 years, selected by systematic sampling. A semi-structured questionnaire was used. The outlet density was measured mapped using android application.*
Results. *A total of 155 were enrolled in the study of which 61.3% were females. A total of 11.6% of the study participants were smokers. On multivariate analysismale gender, and alcohol use was significantly associated with increased risk of smoking ($p < 0.05$). A total of 16.8% of the study participants were alcohol user. On multivariate analysis it was found male gender, and smoking was significantly associated with increased risk of alcohol use ($p < 0.05$). On mapping the study area, it was found that total tobacco selling outlets were 40. There was no alcohol-selling outlet in the study area.*
Conclusion. *The community is in an early stage of urbanization as evident from the burden of smoking, and alcohol use. This burden of smoking might be worsened by outlet density of tobacco seller.*

Introduction

The rising burden of non-communicable diseases (NCDs) is a threat to India [1]. Non-communicable diseases (NCDs) contribute to around 5.87 million deaths that account for 60% of all deaths in India [2]. Hence 1 in 4 Indians risks dying from an NCD before they reach the age of 70. The main NCDs in India are cardiovascular diseases, hypertension, diabetes mellitus, chronic obstructive pulmonary diseases, and cancer [3].
The behavioural risk factors having largest contribution NCDs morbidity and mortality are tobacco use, harmful use of alcohol, unhealthy diet, and physical inactivity [3]. Tobacco use is the one of the primary causes of preventable death. The Global Adult Tobacco Survey study indicates that more than one third of Indian adults use tobacco [4]. India has over 275 million tobacco users, with 164 million users of only smokeless tobacco, 69 million exclusive smokers, and 42 million users of both smoking and smokeless tobacco [5]. The prevalence of smoking was significantly higher among rural residents as compared to urban residents in Chandigarh (17.7 vs. 13.6 %, $P < 0.001$) as reported by ICMR study conducted in 2008-10. The prevalence of smoking among residents of rural Jharkhand, Maharashtra and Tamil Nadu was 13.7%, 10.5% and 21% respectively. The prevalence of smoking among residents of urban Jharkhand, Maharashtra and Tamil Nadu was 14.5%, 11.1% and 20.1% respectively [6].
In present world, Alcohol use is a major public health concern. Each year harmful use of alcohol causes approximately 3.3 million deaths worldwide. Nearly 5.1% of the global burden of disease is attributable to alcohol consumption [7]. According to World Health Organization, in India 3.5% males > 15 years and < 0.1% females > 15 years age consumed at least 60 grams or more of pure alcohol on at least one occasion in the past 30 days, heavy episodic drinking, in 2010 [8]. A cross-sectional survey was done among males aged 15-64 years in rural Wardha, India. The prevalence of alcohol intake was 22.7% [9]. The prevalence of alcoholism was significantly higher among rural residents as compared to urban residents in Jharkhand (33.6% vs. 18.1%, $P < 0.001$) as reported by a study conducted in 2008-10. The prevalence of alcoholism among residents of rural Chandigarh, Maharashtra and Tamil Nadu was 17%, 11.8% and 22.2% respectively. The prevalence of smoking among residents of urban Chandigarh, Maharashtra and Tamil Nadu was 16.4%, 13.3% and 21.4% respectively [6].
Various aspects of the local environment are important factors in explaining health and health-related behaviours. Neighbourhood retailing of tobacco products,

and alcohol, had been implicated in affecting prevalence rates of smoking, and alcoholism, respectively [10, 11]. High density of such retailers may increase the ease with which individuals can access these products. The smoking behavior may be reduced by limiting retail tobacco outlet density [12]. Moreover, the rural areas are rapidly undergoing urbanization. Much of this urbanization leads to large social, economic, and environmental transformations. This further leads to lifestyles characterized by unhealthy nutrition, reduced physical activity and tobacco consumption. Thereby increasing the risk and burden of non-communicable diseases [13]. The aim of the present study was to determine the prevalence of smoking and alcoholism among adult population of Srikot, Uttarakhand and its association with outlet density.

Materials and methods

This study was a community based cross sectional study, carried out in Srikot about 5 km from Srinagar towards Joshimath, Uttarakhand. It was conducted in May to July 2016. Study participants were the adult male and female aged above 20 years. The sample size was calculated based on the prevalence of smoking in Indian population. For the calculation of the sample size the prevalence of smoking was taken to be 17.7% among adult population of rural Chandigarh, north India [6]. For logistic issues we took a precision of 6%. The final sample size came to be 155 after taking 6% precision and 95% confidence level.

Based on the previous studies [14-16], we developed interview schedule as relevant to the objectives of the present study. The interview schedule was finalized after incorporating changes based on the pretesting. This questionnaire contained questions relevant to socio-demographic data, smoking, tobacco use and alcohol behavior. These questions were asked in the local language. The density of tobacco, and alcohol outlet was measured in Srikot by walking. House to house survey was done. Systematic sampling in the community of Srikot identified eligible households. Every nth house was chosen after a random starting point between 1 and 10. The youngest eligible person in a house was considered for the study. Informed written consent was taken from study participants. The study participants were interviewed according to the interview schedule. The survey was conducted until the final sample size was achieved. The detailing of the geographic location and count of outlets were carried out by walking in the community. The coordinates of tobacco/alcohol outlets were picked up using free android app, Map it. The coordinate was recorded while standing at the entrance of the outlet. The appropriate items were purchased from the outlet to mark them as tobacco/alcohol outlets.

Current smoker/tobacco use/alcoholic was a person who smoked/consumed tobacco/alcohol respectively at any point within the past thirty days. Former smoker/tobacco use/alcoholic was a person who smoked/consumed tobacco/alcohol respectively at any point before the past thirty days. Never smoker/tobacco use/alcoholic was a person who had never smoked/ consumed tobacco/alcohol respectively at any point in the past. Tobacco/alcohol outlet was any settlement selling tobacco/alcohol respectively [15, 17].

Data were entered in Microsoft excel spreadsheet and analysed with SPSS version 17.0 (Chicago, IL, USA). Descriptive statistics was conducted for the percentage, mean, and standard deviation (SD); inferential statistics was conducted using test of significance to measure associations between outcome and explanatory variables. P value less than 0.05 was considered significant. Ethical clearance was received from institute Ethics committee.

Results

In the present study, 38.7 percent were males and 61.3 percent were females. The majority (63.2%) of the study participants belonged to age group 21-40 years. The mean age of the study participants was 38.3 years (SD 13.6).Out of the 155, study participants, more than two third (68.3%) of males studied graduation or higher. On the other hand only 46.3 percent of females had studied graduation or higher. Overall, almost ten percent of the study participants studied primary class or less.Among males, 41.7% were office workers, having a private or government job. On the contrary among females three fourth (75.7%) were homemaker. The mean individual income and family income of the study participant was INR 10856.1 (SD 18117.2) and INR 28409.0 (SD 19950.1) respectively. Nearly 73.5% of the study participants had nuclear family. The mean per capitamonthly income was INR 7440.9 (SD 6261.0). Using revised modified BG Prasad socioeconomic classification scale, January 2014, it was found 47.7% of the study participants belonged upper class (INR > 5357) and 31.6% to upper middle class (INR 2652-5356) (Tab. I).

A total of 11.6% of the study participants were smokers and 7.7% were smokeless tobacco user. Among smokers, 8.4% were current smokers and 3.2% were former smoker. Nearly 3.2% were both smoker and smokeless tobacco user. Approximately 20.6% stated one or more family member as tobacco smoker (Tab. II). Table shows the pattern of smoking among the smokers with respect to frequency and quantity (Tab. III). Male gender, and alcohol use was significantly associated with increased risk of smoking ($p < 0.05$) (Tab. IV). A total of 16.8 % of the study participants were alcohol user. Over 61% of the alcohol user reported daily consumption. The mean number of drinks consumed in atypical day was 1.95 (SD 0.87). Approximately 19.4% stated one or more family member as tobacco smoker. 23.1% of study participants reported failed attempt to stop alcohol use. The 7.7% of the study participants reported failure to perform normal activity due to alcohol use. Over 73% had knowledge of alcohol use is hazardous to health (Tab. V). On multivariate adjustment it was found male gender and smoking was significantly associated with increased risk of

Tab. I. Socio-demographic distribution of the study participants (n = 155).

Variable		N (%)
Gender	Male	60 (38.7)
	Female	95(61.3)
Age	20-40	98 (63.2)
	40-60	44 (28.4)
	> 60	13 (8.4)
Occupation	Homemaker	72 (46.5)
	Teaching	17 (10.9)
	Not working/students	27 (17.4)
	Business	9 (5.8)
	Office worker/Job/Clerk	30 (19.4)
Education	Primary school or less	15 (9.7)
	Middle school	8 (5.2)
	Higher secondary or secondary school	47 (30.3)
	Graduation or higher	85 (54.8)
Monthly individual income	Mean (SD)	10856.1 (18117.2)
Monthly family income	Mean(SD)	28409.0 (19950.1)
Family size	Mean(SD)	4.4 (w1.6)
Socioeconomic status	Lower	7 (4.5)
	Lower middle	9 (5.8)
	Middle	16 (10.3)
	Upper middle	49 (31.6)
	Upper	74 (47.8)

Tab. II. The prevalence of smoking/tobacco use among the study participants (n = 155).

Variable	n (%)	95%CI
Total no. of tobacco smoker	18 (1.6)	7 to 17.7
Total no. of current smoker	13 (8.4)	4.5 to 13.9
Total no of former smoker	5 (3.2)	1.1 to 7.3
Total no. of smokeless tobacco user	12 (7.7)	4.1 to 13.1
Total no. of current smokeless tobacco user	7 (4.5)	1.8 to 9.1
Total no. of former smokeless tobacco user	5 (3.2)	1.1 to 7.3
Total no. of both tobacco smoker and smokeless tobacco user	5 (3.2)	1.1 to 7.3
Total no. of tobacco user	25 (16.1)	10.7 to 22.8
Reported one or more family member as tobacco smoker	32 (20.6)	14.5 to 27.9
Reported one or more family member as smokeless tobacco	19 (12.3)	7.5 to 18.5

Tab. III. The pattern of smoking/tobacco use among tobacco user.

Variable	Value
Frequency of smoking (n = 18) [n(%)]	
Daily	11(61.1)
Less than daily	7(38.9)
Mean number of cigarette/Bidi smoked in a day (SD)	14(11.5)
Frequency of use of smokeless tobacco (n = 12) [n(%)]	
Daily	6(50.0)
Less than daily	6(50.0)
Mean number of packets of tobacco use in a day (SD)	1.14(0.6)
Knowledge of tobacco use is hazardous to health present (n = 25) [n (%)]	23(92.0)

alcohol use while consumption of junk food was protective ($p < 0.05$) (Tab. VI).Total tobacco selling outlets were 40. There was no outlet selling tobacco only. There was no alcohol outlet in the study area. The number of tobacco outlet per study participant was 0.3. There was no alcohol outlet in the study area.

Discussion

To the best of our knowledge, this study is the first of its type to assess tobacco and alcohol outlet density relationship with prevalence of smoking and alcoholism among adults in a rural community in India. In addition, there is

Tab. IV. Distribution of various risk factors with smoking among study participants.

Variable		Smoking n (%) n = 18	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Age in years	< 60	13 (72.2)	0.2 (0.06-0.7)	0.017	0.3 (0.04-2.1)	0.219
	≥ 60	6 (27.8)				
Sex	Male	17 (94.4)	37.1 (4.7-288.3)	0.000	12.6 (1.2-128.4)	0.03
	Female	1 (5.6)				
Literacy status	≤ Secondary	11 (61.1)	2.1 (0.8-5.7)	0.208	-	-
	≥ Graduation	7 (38.9)				
Occupation	Unemployed/ Student/ Housewife	5 (27.8)	0.2 (0.1-0.5)	.001	0.7 (0.1-4.1)	0.683
	Employed	13 (72.2)				
Socioeconomic status	≤ Middle class	3 (16.7)	0.7 (0.2-2.7)	1.000	-	-
	≥ Upper class	15 (83.3)				
Type of family	Nuclear	14 (77.8)	1.3 (0.4-4.2)	0.782	-	-
	Extended	4 (22.2)				
Consumption of junk food	Present	13 (72.2)	0.1 (0.0-0.5)	.006	0.2 (0.02-1.9)	0.181
Obesity	Present	12 (66.7)	1.7 (0.6-4.8)	0.450	-	-
Smokeless tobacco use	Present	5 (27.8)	25.9 (4.5-147.3)	.000	9.6 (0.9-102.8)	0.062
Alcohol use	Present	13 (72.2)	24.8 (7.6-80.6)	.000	5.4 (1.2-24.2)	0.029

Tab. V. The prevalence and pattern of alcohol use among the study participants.

Variable	n (%)
Number of study participants ever used alcohol (n = 155)	26 (16.8)[95% CI 11.3 to 23.6]
Mean number of drinks in a typical day (SD)	1.95 (0.87)
Frequency of alcohol use in a week (n = 26)	
1 day	16 (61.5)
2-4 day	5 (19.2)
> 4 day	5 (19.2)
Reported failed attempt to stop alcohol use (n =26)	6 (23.1)
Reported failed to perform normal activity due to alcohol use (n = 26)	2 (7.7)
Knowledge of alcohol use is hazardous to health present (n = 26)	19 (73.1)
Reported one or more family member alcohol use (n = 155)	30 (19.4) [95% CI 13.5 to 26.4]

a lack of published literature about presence of tobacco and alcohol outlet density the rural community of India.The prevalence of smoking in present study was 11.6% [confidence interval (CI): 7.0% to 17.7%] and 72.2% of these were current smokers. The present results were comparable to ICMR INDIDAB study. They found the prevalence of smoking among residents of rural Jharkhand, Maharashtra and Chandigarh was 13.7%, 10.5% and 17.7% respectively [6]. The present study prevalence of smoking was also similar to study from rural Kerala (14.8%) [18], and rural Chennai (14.3%) [19]. The prevalence of smoking was lower than that reported from tribes of central India (18.9%) [20], Nigeria (25%) [21] and Madagascar (19%) [22]. This might be due to difference in study tool and study setting.

The prevalence of current smoking in present study was 8.4% (CI: 4.5% to 13.9%). This was similar to studies from rural Wardha (14.2%) [9]. However, a study from rural Uttarakhand stated the prevalence of current smoking was 28.5% [4]. This difference could be due to small sample size in the present study and a single site study area. Among males 28.3% and among females 1.1% was tobacco smokers. Among males, 21.6% were current tobacco smokers. This was lower than those found by studies in rural Andhra Pradesh [23], rural Uttarakhand [4], rural Rajasthan [24], primarily due to difference in sample size and study population. It was found in the present study that the risk of smoking significantly increases with male gender, smokeless tobacco uses and alcohol use. There was no association with literacy status or occupation. On contrary a study from urban slums of Chandigarh found the prevalence of tobacco use was high among low education status [5, 24, 25] and employed people [5].

The prevalence of alcohol use in present study was 16.8% (CI: 11.3% to 23.6%). The results of the present study was analogous with studies from rural Wardha (22.7%) [9], rural Chandigarh (17%), rural Maharashtra (11.8%), rural Tamil Nadu (22.2%) [6], tribes of central India (23.0%) [20], Mumbai (18.8%) [26], rural Rajast-

Tab. VI. Distribution of various risk factors with alcohol use of study participants (n = 155).

Variable		Alcoholism n (%) n = 26	Unadjusted OR (95% CI)	p-value	Adjusted OR (95%CI)	p-value
Age in years	< 60	21 (80.8)	0.4 (0.1-1.1)	0.136	0.9 (0.1-5.8)	0.874
	≥ 60	5 (19.2)				
Sex	Male	23 (88.5)	19.1 (5.4-67.4)	0.000	5.9 (1.2-28.9)	0.027
	Female	3 (11.5)				
Literacy status	≤ Secondary	13 (50.0)	1.3 (0.5-2.9)	0.668	-	-
	≥ Graduation	13 (50.0)				
Occupation	Unemployed/ Student/ Housewife	6 (23.1)	0.1 (0.04-0.3)	0.000	0.2 (0.1-1.0)	0.057
	Employed	20 (76.9)				
Socioeconomic status	≤ Middle class	4 (15.4)	0.7 (0.2-2.1)	0.600	-	-
	≥ Upper class	22 (84.6)				
Type of Family	Nuclear	19 (73.1)	1 (0.4-2.5)	1.000	-	-
	Extended	7 (26.9)				
Consumption of Junk food	Present	20 (76.9)	0.2 (0.04-0.6)	0.006	0.1 (0.02-0.9)	0.04
Obesity	Present	14 (53.8)	0.9 (0.4-2.2)	1.000	-	-
Smokeless Tobacco use	Present	5 (19.2)	15.1 (2.8-83.1)	.002	3.7 (0.5-29.7)	0.212
Smoking	Present	13 (50.0)	24.8 (7.6-80.6)	0.000	5.3 (1.3-21.5)	0.020

han (24.7%) [27]. Nevertheless, the prevalence of alcohol use in present study was lower compared to another study from rural Tamil Nadu (35.7%) [28], (62.4%) [29], West Bengal (65.8%) [30] and rural Goa (39.5%) [31]. This might be because the later study included only male participants. It was found 38.3% of men and 3.2% of women had consumed alcohol in the past 1 month. This was higher compared to a study from Madhya Pradesh [32]. Main reason for this could be the later study included both urban and rural population. In addition, there was difference in the operational definitions. An overall 23.1% of alcohol user in present study stated they consume more than 4 days a week. This was similar to study form Mumbai, which mentioned of all current alcoholic 32.8%, drank on at least 6 days per week [26]. The alcohol use in the present study was significantly associated with male gender, employment, non-consumption of junk food and associated smoking. Studies urban slums of north India [33], Vellore [34] and south India reported a likely, statistically significant association of alcohol use and occupation [35]. The study from Vellore also mentioned smoking is associated with alcohol use [34]. The disease burden clearly reflects that the community is in early stages of urbanization.

The density tobacco outlet in present study, per study participant was 0.3 respectively. There was no outlet selling only tobacco. This is high or low tobacco outlet density is difficult to understand, as there is no similar study from India or a like countries. A study conducted in US, which revealed less than half of study subjects lived within ½ mile of a tobacco outlet. They stated higher tobacco outlet density near home was associated with risk of smoking [36]. Another study from Rhode Island, Australia, and Scotland mentioned association exits between high odds of smoking and high tobacco outlet density [37-39]. A study revealed the prevalence of current smoking was higher at schools in neighborhoods with the more than five tobacco outlets [38].

There was no alcohol outlet in the study area. This is because of implemented laws and regulation of government of Uttarakhand, India. However, prevalence of alcohol use was similar to many previous studies. This could be due to the presence of the alcohol outlet in nearby city. In addition, previous studies stated alcohol outlet density [8, 40] were directly related to greater drinking frequencies. Another study found the greatest variation in drinking were for an upper limit of 70 alcohol outlets per square mile [40]. The study has its limitations as well. We were not able to map the smoker and alcohol user's location for logistic reasons. A better understanding of practices especially that focuses on urbanization of rural population one needs a qualitative study, which was beyond the scope of this study.

Conclusion

The high burden of smoking is influenced by prevalent tobacco selling outlet density in the environment. The tobacco outlet environment may be a critical factor in promoting and sustaining tobacco use. This suggests greater outlet densities may have affected smoking behaviours, purchases from shops or lower cigarette prices. The community is in an early stage of urbanization as evident from the burden of smoking, and alcohol use. The present study suggested the importance of environmental when studying alcohol consumption. Despite the strict policies for alcohol outlets, supply varies significantly across space for off-licences, the burden of alcoholism is still high. Knowledge of the environment

could help policy makers better to determine the density of alcohol outlet burden in the community to combat the burden of alcoholism. This quest for research on prevention approaches for neighbourhoods at risk.

Acknowledgements

Our sincere thanks to the community who participated in the study.
Funding sources: Indian Council of Medical Research, Short Term Studentship (STS 2016-06758).

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

AG conceived the study, YSR collected data, VSR analyzed the data, YSR and AG wrote the manuscript draft. AKS, VSR, AG and YSR reviewed the manuscript. AG approved the final draft.

References

[1] India: first to adapt the Global Monitoring Framework on non-communicable diseases (NCDs). Available from http://www.who.int/features/2015/ncd-india/en/ (Last accessed on January 12, 2016).

[2] NCDs and development. Available from http://www.who.int/features/2015/ncd-india/en/ (Last accessed on January 10, 2016).

[3] Burden of NCDs and their risk factors in India. Available from http://www.searo.who.int/india/topics/noncommunicable_diseases/ncd_situation_global_report_ncds_2014.pdf (Last accessed on January 10, 2016).

[4] Grills NJ, Singh R, Singh R, Martin BC. Tobacco usage in uttarakhand: a dangerous combination of high prevalence, widespread ignorance, and resistance to quitting. Biomed Res Int 2015;2015:132120. https://doi.org/10.1155/2015/132120

[5] Barik A, Rai RK, Gorain A, Majumdar S, Chowdhury A. Socioeconomic disparities in tobacco consumption in rural India: evidence from a health and demographic surveillance system. Perspect Public Health 2016;136:278-87. https://doi.org/10.1177/1757913915609947

[6] Pradeepa R, Anjana RM, Joshi SR, Bhansali A, Deepa M, Joshi PP, Dhandania VK, Madhu SV, Rao PV, Geetha L, Subashini R, Unnikrishnan R, Shukla DK, Kaur T, Mohan V, Das AK. Prevalence of generalized and abdominal obesity in urban and rural India- the ICMR - INDIAB Study (Phase-I) [ICMR - INDIAB-3] Indian J Med Res 2015;142:139-50. https://doi.org/10.4103/0971-5916.164234

[7] Geneva: World Health Organization, 2014. World Health Organization. Global Status Report on Alcohol and Health. Available from:http://www.who.int/substance_abuse/publications/global_alcohol_report/en/ (Last accessed on August 1, 2016).

[8] Freisthler B, Wolf JP. Testing a social mechanism: does alcohol outlet density moderate the relationship between levels of alcohol use and child physical abuse? Violence Vict 2016;31:1080-99. https://doi.org/10.1891/0886-6708

[9] Kumar R. Anthropometric and behavioral risk factor for non-communicable diseases: a cluster survey from rural Wardha. Indian J Public Health 2015;59:61-4. https://doi.org/10.4103/0019-557X.152868

[10] Shortt NK, Tisch C, Pearce J Richardson EA, Mitchell R.The density of tobacco retailers in home and school environments and relationship with adolescent smoking behaviours in Scotland. Tob Control 2016;25:75-82. https://doi.org/10.1136/tobaccocontrol-2013-051473

[11] Berke EM, Tanski SE, Demidenko E, Alford-Teaster J, Shi X, Sargent JD. Alcohol retail density and demographic predictors of health disparities: a geographic analysis. Am J Public Health 2010;100:1967-71. https://doi.org/10.2105/AJPH.2009.170464

[12] Cantrell J, Anesetti-Rothermel A, Pearson JL, Xiao H, Vallone D, Kirchner TR. The impact of the tobacco retail outlet environment on adult cessation and differences by neighborhood poverty. Addiction 2015;110:152-61. https://doi.org/10.1111/add.12718

[13] Allender S, Lacey B, Webster P, Rayner M, Deepa M, Scarborough P, Arambepola C, Datta M, Mohan V. Level of urbanization and noncommunicable disease risk factors in Tamil Nadu, India. Bull World Health Organ 2010;88:297-304. https://doi.org/10.2471/BLT.09.065847

[14] Tobacco Questionnaire Survey. World Health Organization. Available from http://www.who.int/tobacco/surveillance/en_tfi_tqs.pdf (Last accessed January 15, 2016).

[15] Klesges RC, Ebbert JO, Morgan GD, Sherrill-Mittleman D, Asfar T, Talcott WG, Debon M. Impact of differing definitions of dual tobacco use: implications for studying dual use and a call for operational definitions. Nicotine Tob Res 2011;13:523-31. https://doi.org/10.1093/ntr/ntr032

[16] Alcohol Use Questionnaire. Available from https://www.cafcass.gov.uk/media/215145/alcohol_use_tool.pdf (Last accessed January 15, 2016).

[17] Substance abuse. World Health Organization. Available from http://www.who.int/substance_abuse/publications/en/india.pdf (Last accessed January 14, 2016)

[18] Sathish T, Kannan S, Sarma PS, Thankappan KR. Incidence of tobacco use among adults (15-64 years) in rural Kerala. Asia Pac J Public Health 2015;27:NP626-9. https://doi.org/10.1177/1010539513485787

[19] Chockalingam K, Vedhachalam C, Rangasamy S, Sekar G, Adinarayanan S, Swaminathan S, Menon PA. Prevalence of tobacco use in urban, semi urban and rural areas in and around Chennai City, India. PLoS One. 2013;8:e76005 https://doi.org/10.1371/journal.pone.0076005

[20] Jonas JB, Nangia V, Rietschel M, Paul T, Behere P, Panda-Jonas S. Prevalence of depression, suicidal ideation, alcohol intake and nicotine consumption in rural Central India. The Central India Eye and Medical Study. PLoS One 2014;9:e113550 https://doi.org/10.1371/journal.pone.0113550

[21] Troost JP, Barondess DA, Storr CL, Wells JE, Obaid Al-Hamzawi A, Andrade LH, Bromet E, Bruffaerts R, Florescu S, de Girolamo G, de Graaf R, Gureje O, Haro JM, Hu C, Huang Y, Karam AN, Kessler RC, Lepine JP, Matschinger H, Medina-Mora ME, O'Neill S, Posada-Villa J, Sagar R, Takeshima T, Tomov T, Williams DR, Anthony JC. An updated global picture of cigarette smoking persistence among adults. J Epidemiol Glob Health 2012;2:135-44. https://doi.org/10.1016/j.jegh.2012.06.003

[22] Veeranki SP, Mamudu HM, John RM, Ouma AE. Prevalence and correlates of tobacco use among school-going adolescents in Madagascar. J Epidemiol Glob Health 2015;5:239-47. https://doi.org/10.1016/j.jegh.2014.12.005

[23] Corsi DJ, Subramanian SV, Lear SA, Teo KK, Boyle MH, Raju PK, Joshi R, Neal B, Chow CK. Tobacco use, smoking quit rates, and socioeconomic patterning among men and women: a cross-sectional survey in rural Andhra Pradesh, India. Eur J Prev Cardiol 2014;21:1308-18. https://doi.org/10.1177/2047487313491356

[24] Gupta R, Gupta VP, Bhagat N, Rastogi P, Sarna M, Prakash

H, Deedwania PC. Obesity is major determinant of coronary risk factors in India: Jaipur Heart Watch studies. Indian Heart J 2008;60:26-33.

[25] Kathirvel S, Thakur JS, Sharma S. Women and tobacco: a cross sectional study from North India. Indian J Cancer 2014;51(Suppl 1):S78-82. https://doi.org/10.4103/0019-509X.147478

[26] Gupta PC, Saxena S, Pednekar MS, Maulik PK. Alcohol consumption among middle-aged and elderly men: a community study from western India. Alcohol Alcohol 2003;38:327-31. https://doi.org/10.1093/alcalc/agg077

[27] Sundaram KR, Mohan D, Advani GB, Sharma HK, Bajaj JS. Alcohol abuse in a rural community in India. Part I: Epidemiological study. Drug Alcohol Depend 1984;14:27-36. https://doi.org/10.1016/0376-8716(84)90016-4

[28] Dutta R, Gnanasekaran S, Suchithra S, Srilalitha V, Sujitha R, Sivaranjani SS, Subitha S, Dcruze L. A Population based study on alcoholism among adult males in a rural area, Tamil Nadu, India. J Clin Diagn Res 2014;8:JC01-3. https://doi.org/10.7860/JCDR/2014/6308.4411

[29] Kaur P, Rao SR, Radhakrishnan E, Ramachandran R, Venkatachalam R, Gupte MD. High prevalence of tobacco use, alcohol use and overweight in a rural population in Tamil Nadu, India J Postgrad Med 2011;57:9-15. https://doi.org/10.4103/0022-3859.74284

[30] Ghosh S, Samanta A, Mukherjee S. Patterns of alcohol consumption among male adults at a slum in Kolkata, India J Health Popul Nutr 2012;30:73-81. https://doi.org/10.3329/jhpn.v30i1.11279

[31] Pillai A, Nayak MB, Greenfield TK, Bond JC, Nadkarni A, Patel V. Patterns of alcohol use, their correlates, and impact in male drinkers: a population-based survey from Goa, India. Soc Psychiatry Psychiatr Epidemiol 2013;48:275-82. https://doi.org/10.1007/s00127-012-0538-1

[32] Rathod SD, Nadkarni A, Bhana A, Shidhaye R. Epidemiological features of alcohol use in rural India: a population-based cross-sectional study. BMJ Open 2015;5:e009802. https://doi.org/10.1136/bmjopen-2015-009802

[33] Katyal R, Bansal R, Agrawal V, Goel K, Chaudhary V. Cross-sectional study to acknowledge the independent association of the socio-demographic determinants of alcohol use in an urban slum of North India. Int J Prev Med 2014;5:749-57.

[34] Kim S, Rifkin S, John SM, Jacob KS. Nature, prevalence and risk factors of alcohol use in an urban slum of Southern India. Natl Med J India 2013;26:203-9.

[35] Vignesh BT, Singh AK, Mohan SK, Murthy S, Joshi A. Association between socio-demographics and alcohol dependence among individuals living in an Indian setting. Glob J Health Sci 2014;6:16-26. https://doi.org/10.5539/gjhs.v6n3p16

[36] Schleicher NC, Johnson TO, Fortmann SP, Henriksen L.Tobacco outlet density near home and school: Associations with smoking and norms among US teens. Prev Med 2016;91:287293. https://doi.org/10.1016/j.ypmed.2016.08.027

[37] Tucker-Seeley RD, Bezold CP, James P, Miller M, Wallington SF. Retail Pharmacy Policy To End The Sale Of Tobacco Products: What Is The Impact On Disparity In Neighborhood Density Of Tobacco Outlets? Cancer Epidemiol Biomarkers Prev 2016;25:1305-10. https://doi.org/10.1158/1055-9965.EPI-15-1234

[38] Henriksen L, Feighery EC, Schleicher NC, Cowling DW, Kline RS, Fortmann SP. Is adolescent smoking related to the density and proximity of tobacco outlets and retail cigarette advertising near schools? Prev Med 2008;47:210-4. https://doi.org/10.1016/j.ypmed.2008.04.008

[39] Scully M, McCarthy M, Zacher M, Warne C, Wakefield M, White V. Density of tobacco retail outlets near schools and smoking behaviour among secondary school students. Aust N Z J Public Health 2013;37:574-8. https://doi.org/10.1111/1753-6405.12147

[40] Ahern J, Colson KE, Margerson-Zilko C, Hubbard A, Galea S. Predicting the population health impacts of community interventions: the case of alcohol outlets and binge drinking. Am J Public Health 2016;106:1938-43. https://doi.org/10.2105/AJPH.2016.303425

Received on July 1, 2019. Accepted on November 20, 2019.

Correspondence: Arti Gupta, Department of Community and Family Medicine, All India Institute of Medical Sciences Mangalagiri, Andhra Pradesh, India - Tel. +91 9013902976 - Email: guptaarti2003@gmail.com

How to cite this article: Rautela YS, Reddy BV, Singh AK, Gupta A. Smoking and alcoholism among adult population and its association with outlet density in a hilly area of North India. J Prev Med Hyg 2019;60:E361-E367. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1341

ORIGINAL ARTICLE

Self-reported recurrent pain and medicine use among 15-year-olds: results from the HBSC Italian study

F. CENTAURI[1], A. PAMMOLLI[2], R. SIMI[2], P. DALMASSO[3], P. BERCHIALLA[4], A. BORRACCINO[3], L. CHARRIER[3], M. LENZI[5], A. VIENO[5], P. LEMMA[3], F. CAVALLO[3], G. LAZZERI[1, 2]

[1] Hospital Direction, Azienda Ospedaliera Universitaria Senese; [2] Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy; [3] Department of Public Health and Paediatrics, University of Torino, Italy; [4] Department of Clinical and Biological Sciences, University of Torino, Italy; [5] Department of Developmental and Social Psychology, University of Padova, Italy

Keywords

Medicine use • Adolescents • Recurrent complaints

Summary

Background. *The prevalence of adolescent pain varies considerably across epidemiological studies, and little information is available on pain-related behaviours among adolescents, including medicine use. The aims of this study were: [1] to examine the prevalence of recurrent pain among 15-year-old adolescents in Italy; [2] to investigate the association between recurrent pain and medicine use among boys and girls; and [3] to evaluate the consistency of these associations across Regions.*

Methods. *The World Health Organization (WHO) collaborative International Health Behaviour in School-aged Children 2013/2014 study collected self-reported data on pain and medicine use from 13611 15-year-old adolescents in 21 Italian Regions. We used multi-level multivariate logistic regression, stratified by gender, to analyse the association between recurrent pain and medicine use for headache, stomachache, nervousness and difficulties in getting to sleep.*

Results. *On average, across all Regions, almost 45% of adolescents reported recurrent headache, more than 30% reported recurrent backache and approximately 30% reported recurrent stomachache. Although the prevalence of both pain and medicine use was much higher among girls, the association between pain and medicine use was similarly strong in adolescents of both genders. Adolescents with recurrent pain proved more likely to use medicines also for non-corresponding pain, nervousness and difficulties in getting to sleep. The association between recurrent pain and medicine use was consistent across Regions despite large inter-regional differences in the prevalence of both phenomena.*

Conclusions. *Recurrent pain in adolescence is common nationwide. Adolescents with recurrent pain are more likely to use medicines in general. Recurrent pain and medicine use should be addressed by adolescent health policies.*

Introduction

The World Health Organization (WHO) defines adolescence as the age between 10 and 19 years, which constitutes a key period of human growth [1]. Numerous studies have shown that about 10-50% of adolescents report suffering from recurrent pains. Recurrent pain is defined as pain occurring at least once a week within the time frame of three or six months. The use of medicines to treat common complaints, such as headache and stomachache, is widespread and increasing among adolescents [2-6]. Overall, the use of medicines and the prevalence of recurrent complaints are much higher among girls than boys [7-9]. Studies have demonstrated an increasing use of medicines for headache and stomachache among girls, and an association between the use of medicines and the frequency of the corresponding complaints [2, 3]. Adolescents who suffer from recurrent pains take medicines more frequently, and it has been hypothesized that medicine use during adolescence continues into adulthood [10]. In general, the use of pharmaceutical drugs seems to be facilitated by their easy accessibility and availability, and by changes in pharmaceutical regulations and the attitudes of parents, who are the primary source of medicines [2, 6, 7]. As adolescents generally have scant knowledge of the use of pharmaceutical drugs, it seems important, from the public health standpoint, to instruct these young people in the appropriate use of such medicines and to conduct studies in target groups in order to analyse the phenomenon of medicine use in greater depth [2, 8, 10]. Although trends in the use of medicines have been investigated by several in-country studies, the results are difficult to compare owing to differences in study populations, data collection and measurements. The International Health Behaviour in School-aged Children (HBSC) study is a single tool for the collection of data on medicine use among adolescents; as it is based on a unified methodology that involves all Italian Regions, it enables direct comparison of the data collected.

The aims of the present study were: to determine the prevalence of recurrent pains, such as headache, stomachache and backache, among 15-year-old adolescents in 21 Italian Regions; to evaluate any correlation between recurrent pains and medicine use among males and females; and to assess the possible similarities and differences among the Italian Regions considered.

https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1382

Materials and methods

Study population

The data utilised were taken from the HBSC Italian study, a cross-sectional study promoted by the WHO at the international level in order to document health-related behaviours among school-age adolescents. Details of the method and the investigative tools used to gather the data have been reported elsewhere [11-13].

Specifically, we used the data from 21 Italian Regions; these were collected from a representative sample of adolescents aged 11, 13 and 15 years in each Region from 2013 to 2014. In order to obtain a random sample of school classes, we implemented the strategy of cluster sampling. Data were collected by means of an internationally validated questionnaire, which was self-administered (in the presence of qualified personnel) in schools, in conformity with the national protocol [11]. This questionnaire is subject to ongoing development and validation by all the researchers in the countries participating in the international HBSC project. Parental consent to participation in the study is mandatory. The study protocol, which was approved by the Ethics Committee of the University of Turin, provided for the use of an "opt-out" consent form, meaning that a child would be included by default unless his or her parents chose to opt out by explicitly refusing consent. All the data were collected anonymously, so that the individual participants could not be identified. For what concerns the present article, we selected only 15-year-old adolescents (n = 13611), 6907 girls (50.7%) and 6704 boys (49.3%), in order to observe the behaviour of only those subjects who are more independent in their use of medicines (Tab. I).

Tab. I. Subdivision of the sample by sex and region.

Italian Regions	Boys	Girls	Total
Abruzzo	262	269	531
Basilicata	209	210	419
Bolzano	170	255	425
Calabria	317	316	633
Campania	313	287	600
Emilia Romagna	340	317	657
Friuli Venezia Giulia	391	354	745
Lazio	241	213	454
Liguria	374	420	794
Lombardia	462	444	906
Marche	319	424	743
Molise	282	277	559
Piemonte	311	306	617
Puglia	285	293	578
Sardinia	204	206	410
Sicilia	299	308	607
Toscana	356	398	754
Trento	299	323	622
Umbria	427	363	790
Val d'Aosta	141	150	291
Veneto	702	774	291
Total	6704	6907	13611

Variables

From the HBSC questionnaire, we used the questions concerning the following complaints: headache, stomachache, difficulties in getting to sleep and nervousness, which were measured on a scale ranging from "almost every day" to "rarely or never". Each symptom was dichotomized as "recurrent", if it occurred at least weekly, and "not recurrent" otherwise. The percentages of non-response to the questions were: headache, 0.31%; stomachache, backache and nervousness, 0.35%; and difficulty in getting to sleep, 0.38%.

In addition, the question *"Have you taken any medicines or drugs for the following complaints in the last month?"* was used to assess the recurrence of the use of medicines for headache, stomachache, difficulty in sleeping and nervousness. The possible response options were: *no*, *yes once,* and *yes more than once*. In this case, too, the answers were dichotomised into *yes* and *no*. The percentages of non-response were: 1.04% to the question regarding medicines for headache; 1.18% for stomachache; 1.13% for nervousness, and 1.16% for sleeping difficulties.

Statistical analysis

The prevalence of the various complaints and of the use of medicines among males and females, subdivided by Region, was evaluated by means of the chi-square test. In all statistical analyses, we applied a significance value of 0.05 and 95% confidence intervals. Logistical regression was used to evaluate the association among the various complaints, on considering boys and girls separately. The odds ratios (OR) and the confidence intervals were then calculated in order to evaluate the association. The association between complaints and medicine use was evaluated by means of multilevel logistical regression, in which the clusters were identified by the Italian Regions. Subsequently, we calculated the ORs and the pertinent confidence intervals in order to evaluate the association, the Median OR (MOR) as a measure of heterogeneity in order to assess the random effect of the Regions, and the Intra-Class Correlation (ICC), which enabled us to assess both the variance within the clusters and the variance among the clusters. The ICC thus enables us to evaluate the relevance of clustering, i.e. the degree to which subjects within the same Region have the same outcome [14]. The analyses were carried out by means of the R software, version 3.3.3.

Results

Prevalence of recurrent complaints

On considering all the Italian Regions included in this study, a mean of more than 40% of the adolescents surveyed reported suffering from recurrent headache; 27%

reported recurrent stomachache, and over 30% recurrent backache (Tab. II).
The prevalence of recurrent headache among girls ranged from 43.5% in the Autonomous Province of Bolzano to 67.5% in Campania, while among boys it ranged from 20% in the Autonomous Province of Bolzano to 36.9% in Piedmont, followed by Campania and Val d'Aosta, which displayed similar values (36.1%). In general, in all of the Regions considered, the prevalence of recurrent headache proved to be 1.5-2 times higher in females than in males, the difference being statistically significant.
The lowest prevalence values were recorded for stomachache, especially among males; in this group, the lowest value (14.1%) was seen in the Autonomous Province of Trento, and the highest (31.2%) in Val d'Aosta. Among girls, the highest prevalence of stomachache was recorded in Basilicata (43.2%) and the lowest in Bolzano (21.9%). The prevalence of stomachache also proved to be significantly higher among females than among males in practically all the Regions (except in Bolzano e Val d'Aosta, in which the difference was not significant).
Different types of recurrent pain were found to co-exist in individuals. All recurrent pains, nervousness and difficulty in getting to sleep were associated (the ORs were higher than 1 and statistically significant).

Prevalence of medicine use

The data on the prevalence of medicine use revealed that almost half of 15-year-old Italian adolescents use medicines to treat headache, followed by stomachache, the use of medicines to treat this complaint being about 1.5 times more frequent among girls than among boys in all the Regions (Tab. III). The use of medicines to alleviate insomnia and nervousness displayed markedly lower percentages, and in most of the Regions no significant difference emerged between males and females.
With regard to headache, the lowest prevalence of medicine use by boys was recorded in Lazio (26.7%), while the highest was seen in Calabria (37.9%). Among girls, by contrast, the prevalence proved to be much higher in almost all the Regions considered, with the highest percentage being recorded in Lombardy (57.6%) and the lowest in Marche (44.7%) and Molise (44.8%).
A marked difference was also seen between males and females with regard to the use of medicines to treat stomachache in most Regions, the greatest difference (almost 3-fold) being recorded in the Autonomous Province of Trento (males 9.6%, females 31.3%). Among males, the lowest percentage was seen in Trento (9.6%) and the highest in Campania (23.6%); among females, the lowest percentage was recorded in Friuli Venezia Giulia (28.4%) and the highest in Basilicata (41.6%).

Medicine use among adolescents with recurrent complaints

The odds of medicine use for headache and stomachache were substantially higher among adolescents with corresponding recurrent pain than among adolescents who reported pain less often (Tab. IV). Adolescents with recurrent headache or stomachache were more likely to use medications for other types of pain, too.
For both genders, the OR of medicine use for headache was 4. Furthermore, those who suffered from recurrent headache were twice as likely also to use medicines for nervousness and difficulties in getting to sleep. While for boys with recurrent stomachache there was four times increase in the odds of medicine use for stomachache, for girls the OR was 3. Furthermore, among adolescents of both genders, those who had stomachache and backache were 1.5 times more likely also to use medicines for headache. Regarding the use of medicines for nervousness and difficulties in getting to sleep, it can be seen in Table IV that, among boys, the ORs ranged from 2 to 2.5 for each complaint and among girls from 1.5 to 2.
Overall, little regional variation was seen in the association between medicine use and recurrent pain, as most of the calculated MORs were close to 1 and the ICCs were close to zero; thus, little of the total variance in the use of medicines is due to differences among the Italian Regions (Tab. IV).

Discussion

Medicines are commonly used to alleviate such complaints as headache, stomachache, backache, difficulty in getting to sleep and nervousness, and their use among adolescents is increasing [2-5, 15]. Given that habits acquired during adolescence tend to persist into adulthood, adolescents constitute a strategic study population from the point of view of public health [10, 16]. The purpose of the study was threefold. First, we aimed to investigate the prevalence of recurrent complaints, such as headache, stomachache and backache, among adolescents in 21 Italian Regions in a year time frame (2014). Second, we assessed the correlation between recurrent complaints and medicine use among males and females. Third, we conducted a comparison of data from the Italian Regions involved in our analysis. The fact that the HBSC utilises a common homogeneous method enabled us to compare the data gathered in the various Italian Regions. The data analysed in our study revealed that the prevalence of recurrent complaints among Italian adolescents was high in all the Regions considered, and that, in general, such complaints were more common among females than among males, as also reported in other studies [7, 8, 17-19]. The most frequent complaint proved to be headache, followed by backache and stomachache. Moreover, it emerged that the prevalence of backache was higher than that of stomachache, especially among males, in the majority of Regions. These data are in line with those of other studies, in which backache has been observed to be more common than stomachache among older adolescents [8].
Overall, about half of Italian adolescents make use of medicines, particularly in order to alleviate headache, as reported in other studies [18, 19], and, in second position, stomachache, with medicine use being more fre-

Tab. II. Prevalence of recurrent complaints, subdivided by sex and Region.

	Recurrent headache				Recurrent stomachache				Recurrent backache			
Regions	**Males**	**Females**	**p**	**Total**	**Males**	**Females**	**p**	**Total**	**Males**	**Females**	**p**	**Total**
Abruzzo	34.35	57.99	< 0.001	46.33	16.03	38.29	< 0.001	27.31	24.43	34.20	0.02	29.38
Basilicata	28.71	63.94	< 0.001	46.06	23.44	43.27	< 0.001	33.17	19.14	43.27	< 0.001	31.03
Bolzano	20.00	43.53	< 0.001	34.11	15.88	21.96	NS	19.53	21.76	27.84	NS	25.41
Calabria	34.92	64.87	< 0.001	49.76	21.27	41.27	< 0.001	31.12	25.88	34.29	0.03	29.86
Campania	36.10	67.48	< 0.001	51.00	21.15	43.36	< 0.001	31.67	29.39	31.82	NS	30.50
Emilia Romagna	26.76	59.94	< 0.001	42.77	20.59	35.02	< 0.001	27.55	25.88	40.69	< 0.001	33.03
Friuli Venezia Giulia	29.67	51.98	< 0.001	40.27	18.16	30.23	< 0.001	23.89	23.33	37.01	< 0.001	29.80
Lazio	24.48	62.91	< 0.001	42.51	18.26	38.50	< 0.001	27.75	21.58	44.60	< 0.001	32.38
Liguria	27.35	55.24	< 0.001	42.07	16.62	38.33	< 0.001	28.09	28.42	41.19	< 0.001	35.14
Lombardia	25.00	54.50	< 0.001	39.29	14.91	33.78	< 0.001	24.06	23.19	40.77	< 0.001	31.68
Marche	32.18	58.53	< 0.001	46.97	20.19	35.31	< 0.001	28.67	27.44	41.71	< 0.001	35.40
Molise	25.81	56.00	< 0.001	40.43	21.07	37.45	< 0.001	28.98	25.18	33.09	0.05	28.80
Piemonte	36.98	59.80	< 0.001	48.30	21.29	35.92	< 0.001	28.53	30.23	38.56	0.04	34.36
Puglia	33.22	56.16	< 0.001	44.64	20.49	33.56	< 0.001	26.99	20.14	32.88	< 0.001	26.47
Sardegna	30.20	59.22	< 0.001	44.63	21.78	40.29	< 0.001	30.98	33.17	40.29	NS	36.59
Sicilia	25.50	60.39	< 0.001	43.16	17.11	42.67	< 0.001	29.98	27.61	38.64	0.005	33.11
Toscana	27.48	61.93	< 0.001	45.23	14.73	38.83	< 0.001	27.19	23.23	39.09	< 0.001	31.30
Trento	24.92	52.48	< 0.001	39.07	14.14	29.50	< 0.001	22.03	24.58	37.27	< 0.001	31.03
Umbria	28.47	53.99	< 0.001	40.13	15.02	34.81	< 0.001	24.05	26.29	37.74	< 0.001	31.52
Val d'Aosta	36.17	59.46	< 0.001	47.77	31.21	40.54	NS	35.74	35.46	43.92	NS	39.52
Veneto	33.90	59.25	< 0.001	47.15	17.29	38.16	< 0.001	28.18	26.71	38.55	< 0.001	32.86
Total	29.80	57.92	< 0.001	43.94	18.44	36.54	< 0.001	27.54	25.75	38.00	< 0.001	31.86

NS = not significant

Tab. III. Prevalence of medicine use, subdivided by sex and region.

	Medicines for headache				Medicines for stomachache				Medicines for sleeping difficulties				Medicines for nervousness			
Regions	**Males**	**Females**	**p**	**Total**	**Males**	**Females**	**p**	**Total**	**Males**	**Females**	**p**	**Total**	**Males**	**Females**	**p**	**Total**
Abruzzo	32.31	48.13	< 0.001	40.11	15.89	32.46	< 0.001	24.11	3.88	4.85	NS	4.33	4.25	6.72	NS	5.46
Basilicata	30.43	49.76	< 0.001	39.38	19.42	41.67	< 0.001	29.83	5.83	3.40	NS	4.53	5.83	6.83	NS	6.21
Bolzano	31.76	46.27	0.004	40.47	14.12	32.55	< 0.001	25.18	7.06	7.45	NS	7.29	7.65	10.59	NS	9.41
Calabria	37.94	54.02	< 0.001	45.18	20.90	33.33	< 0.001	26.70	2.25	3.21	NS	2.69	4.50	6.75	NS	5.53
Campania	36.25	53.36	< 0.001	43.83	23.62	33.10	0.01	27.83	2.92	3.51	NS	3.17	5.18	7.75	NS	6.33
Emilia Romagna	29.97	55.70	< 0.001	42.16	16.12	28.98	< 0.001	22.07	2.68	4.49	NS	3.50	4.73	5.73	NS	5.18
Friuli Venezia Giulia	34.79	53.98	< 0.001	43.62	11.83	28.49	< 0.001	19.60	4.10	6.55	NS	5.23	2.05	6.82	0.003	4.30
Lazio	26.78	51.90	< 0.001	38.11	22.59	34.43	0.007	27.97	3.80	4.29	NS	3.96	6.72	10.90	NS	8.59
Liguria	31.27	50.72	< 0.001	41.18	15.55	33.73	< 0.001	24.94	4.07	7.91	0.04	6.05	3.23	7.25	0.02	5.29
Lombardia	31.21	57.69	< 0.001	43.82	15.42	33.63	< 0.001	24.17	4.41	5.42	NS	4.86	2.64	6.79	0.006	4.64
Marche	29.21	44.74	< 0.001	37.55	17.57	32.21	< 0.001	25.44	2.86	4.07	NS	3.50	4.13	6.99	NS	5.65
Molise	30.00	44.89	< 0.001	37.03	13.57	31.14	< 0.001	22.00	2.51	1.82	NS	2.15	5.36	4.74	NS	5.01
Piemonte	32.26	53.95	< 0.001	42.79	18.83	33.88	< 0.001	26.09	3.57	4.26	NS	3.89	4.85	5.61	NS	5.19
Puglia	32.62	54.64	< 0.001	43.43	19.08	30.24	0.003	24.57	2.14	4.11	NS	3.11	5.32	7.24	NS	6.23
Sardegna	27.86	55.61	< 0.001	41.46	16.42	36.45	< 0.001	26.10	1.99	7.84	0.01	4.88	2.51	7.80	0.03	5.12
Sicilia	36.86	46.25	0.02	41.19	20.34	40.98	< 0.001	30.48	3.74	3.30	NS	3.46	6.14	6.86	NS	6.43
Toscana	27.07	53.59	< 0.001	40.32	13.71	35.13	< 0.001	24.54	4.00	4.88	NS	4.38	2.85	7.69	0.006	5.31
Trento	31.96	48.90	< 0.001	40.03	9.69	31.35	< 0.001	20.58	2.76	6.25	NS	4.50	3.11	6.58	NS	4.82
Umbria	30.88	55.71	< 0.001	41.77	14.73	29.61	< 0.001	21.27	2.36	4.47	NS	3.29	3.77	7.80	0.02	5.57
Val d'Aosta	30.94	55.78	< 0.001	42.96	18.44	34.69	0.003	26.46	0	7.48	0.002	3.78	2.86	8.84	NS	5.84
Veneto	34.29	55.71	< 0.001	45.26	14.41	33.72	< 0.001	24.32	3.45	6.76	0.006	5.15	5.16	7.57	NS	6.37
Total	32.01	52.24	< 0.001	41.84	16.42	33.20	< 0.001	24.65	3.37	5.16	< 0.001	4.23	4.32	7.23	<0.001	5.73

t significant

Tab. IV. Association between propensity to recurrent complaints and medicine use.

	Medicines for headache		Medicines for stomachache		Medicines for sleeping difficulties		Medicines for nervousness	
Recurrent complaint	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Males								
Headache	4.14 MOR = 1.03	3.70-4.63 ICC = 0.00003	1.78 MOR = 1.19	1.56-2.04 ICC = 0.01	2.45 MOR = 1.05	1.87-3.21 ICC = 0.001	2.12 MOR = 1.19	1.67-2.70 ICC =0.01
Stomachache	1.80 MOR = 1.06	1.58-2.04 ICC = 0.001	3.85 MOR = 1.19	3.33-4.44 ICC = 0.01	2.67 MOR = 1.09	2.01-3.54 ICC = 0.003	2.52 MOR = 1.16	1.96-3.24 ICC = 0.007
Backache	1.33 MOR = 1.06	1.19-1.50 ICC = 0.001	1.48 MOR = 1.20	1.28-1.70 ICC = 0.01	2.08 MOR = 1.04	1.58-2.74 ICC = 0.0004	1.90 MOR = 1.18	1.49-2.43 ICC = 0.01
Females								
Headache	4.38 MOR = 1.14	4.07-4.72 ICC = 0.006	1.55 MOR = 1.03	1.39-1.72 ICC = 0.0004	2.06 MOR = 1.27	1.62-2.61 ICC = 0.02	2.13 MOR = 1	1.73-2.62 ICC = 0
Stomachache	1.67 MOR = 1.12	1.51-1.84 ICC = 0.004	2.89 MOR = 1	2.60-3.21 ICC = 0	2.25 MOR = 1.28	1.81-2.79 ICC = 0.02	1.93 MO = 1	1.61-2.32 ICC = 0
Backache	1.43 MOR = 1.11	1.29-1.58 ICC=0.004	1.42 MOR = 1.05	1.28-1.57 ICC = 0.0008	1.49 MOR = 1.24	1.20-1.85 ICC = 0.02	1.55 MOR = 1	1.29-1.87 ICC = 0

quent among females than males [3, 6, 7, 9]. These data support the conviction that adolescents now have easier access to medicines as a result of modifications in pharmaceutical regulations, more aggressive marketing and the changing attitudes of parents, who are the primary source of medicines [2, 6, 7]. In addition, the odds of medicine use for headache and stomachache were seen to be substantially higher among adolescents with corresponding recurrent pain than among those who reported less frequent pain in all the regions considered. Interestingly, boys with recurrent stomachache were seen to be much more likely to take medicines to treat this complaint than girls; although the medicine taken proved to be appropriate to the type of complaint, it should be stressed, as already pointed out by the results of national and international studies, that adolescent boys should not be underestimated as a subgroup at risk of excessive medicine use [20, 21].

In addition, it emerges from the present study that adolescents suffering from recurrent complaints are also more likely to use medicines that are not appropriate to the treatment of their specific complaint. This tendency, which was documented among adolescents of both sexes in our study, has been observed in international studies, in which adolescents with recurrent complaints have been seen to display a greater likelihood of using medicines in general and, in particular, medicines that are not specific to the treatment of their symptoms [20]. Those adolescents who most frequently take medicines to alleviate their pain not only report a higher frequency of pain, but also a higher frequency of anxiety, depression and functional disabilities than adolescents who make little or no use of medicines [22]. Moreover, the tendency to take medicines has been observed more frequently among young people with scant coping resources and poor self-rated health, and those of lower social class [8, 21].

A limitation of the present study is its lack of a clinical diagnosis of the intensity of each individual complaint and the unavailability of data on the social class of the respondents' parents; indeed, previous studies have found a correlation between the use of medicines and low parental social class, in addition to the conditions of having a scant sense of coherence and being a victim of bullying [21]. Nevertheless, the subjective perception of pain, regardless of its primary cause, is important in that it impacts on the adolescent's general well-being; indeed, recurrent pain has a detrimental effect on adolescents' daily functioning and quality of life [24-27]. Overall, the data reported in this study reveal that over 40% of 15-year-old adolescents in Italy report suffering from recurrent headache, followed by stomachache and backache. Adolescent girls are more likely to take medicines to treat headache and stomachache, while those of both sexes who suffer from recurrent complaints are more likely to take medicines that are not intended for the treatment of their specific complaint.

Adolescents' use of medicines to treat recurrent complaints is a complex phenomenon which needs to be properly studied. Indeed, this behaviour may be influenced by many factors, such as the attitudes, social class and educational level of parents, which should be taken into account. Given that behaviours acquired during adolescence tend to be carried over into adulthood, the recurrent complaints experienced by adolescents may increase the risk of chronic complaints and critical health disorders in adulthood [28]. Thus, the use of medicines in adolescence is a public health concern and constitutes an emerging issue that requires more attention and investigation on the part of scientific research and greater awareness among adults and health professionals.

Acknowledgements

HBSC is a World Health Organisation cross-national study. The international coordinator for the 2014/2015 study was Johanna Inchley, University of St Andrews; Data Bank Manager: Oddrun Samdal, University of Bergen. In Italy, the study has been carried out since 2001 under the coordination of the Universities of Torino, Padova, and Siena on the behalf of the Ministry of Education and the Ministry of Health.
Funding sources: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

GL wrote the first draft of the manuscript; AP conducted the statistical analyses; RS, FC, PD, PL, LC, PB, AB, AV, ML, LC and PB contributed to the paper revision and to the final manuscript editing. All authors have critically revised the manuscript and approved the final version. PD, AB, PL, FC, LC, PB, RS, AV, ML and GL participated in designing the study and data collection as members of the HBSC Italian team.

References

[1] World Health Organization. WHO – Recognizing adolescence. Available at: www.who.int/maternal_child_adolescent/topics/adolescence/development/en/

[2] Holstein BE, Andersen A, Fotiou A, Gobina I, Godeau E, Holme Hansen E, Iannotti R, Levin K, Gabhainn SN, Ravens-Sieberer U, Valimaa R and the Medicine Use Writing Group. Adolescents' medicine use for headache: secular trends in 20 countries from 1986 to 2010. Eur J Public Health 2015;(25 Suppl 2):76-9. https://doi.org/10.1093/eurpub/ckv035

[3] Andersen A, Holstein BE, Hansen EH. Is medicine use in adolescence risk behavior? Cross-sectional survey of school-aged children from 11 to 15. J Adolesc Health 2006;39:362-6. https://doi.org/10.1016/j.jadohealth.2005.12.023

[4] Hansen DL, Hansen EH, Holstein BE. Young women's use of medicines: autonomy and positioning in relation to family and peer norms. Health (London) 2009;13:467-85. https://doi.org/10.1177/1363459309103918

[5] Holstein BE, Holme Hansen E, Due P, Birna Almarsdottir A. Self-reported medicine use among 11- to 15-yearold girls and boys in Denmark 1988-1998. Scand J Public Health 2003;31(5):334-41. https://doi.org/10.1080/14034940210165082

[6] Levin KA, Whitehead R, Andersen A, Levin D, Gobina I, Holstein B. Changes in the association between health complaint frequency and medicine use among adolescents in Scotland between 1998 and 2010. J Psychosom Res 2015;78:371-6. https://doi.org/10.1016/j.jpsychores.2014.12.006

[7] Gobina I, Valimaa R, Tynjala J, Villberg J, Villerusa A, Iannotti RJ, Godeau E, Gabhainn SN, Andersen A, Holstein BE, H.M.U.W. Group, Griebler R, Borup I, Kokkevi A, Fotiou A, Boraccino A, Dallago L, Wagener Y, Levin K, Kuntsche E. The medicine use and corresponding subjective health complaints among adolescents, a cross-national survey. Pharmacoepidemiol Drug Saf 2011;20:424-31. https://doi.org/10.1002/pds.2102

[8] Gobina I, Villberg J, Villerusa A, Valimaa R, Tynjala J, Ottova-Jordan V, Ravens-Sieberer U, Levin K, Cavallo F, Borraccino A, Sigmund E, Andersen A, Holstein BE. Self-reported recurrent pain and medicine use behaviours among 15-year olds: results from the international study. Eur J Pain 2015;19:77-84. https://doi.org/10.1002/ejp.524

[9] Hansen EH, Holstein BE, Due P, Currie CE. International survey of self-reported medicine use among adolescents. Ann Pharmacother 2003;37:361-6.

[10] Andersen A, Holstein BE, Due P, Hansen EH. Medicine use for headache in adolescence predicts medicine use for headache in young adulthood. Pharmacoepidemiol Drug Saf 2009;18:619-23. https://doi.org/10.1002/pds.1748

[11] Lazzeri G, Giacchi MV, Dalmasso P, Vieno A, Nardone P, Lamberti A, Spinelli A, Cavallo F, H. Group, The methodology of the Italian HBSC 2010 study (Health Behaviour in School-aged Children). Ann Ig 2013;25:225-33. https://doi.org/10.7416/ai.2013.1925

[12] HBSC. Health Behavior in School-Aged Children. A World Health Organization Cross-National Study. Reasearch Protocol for the 2009/2010 survey. 2010. Available from: www.hbsc.org2010

[13] Roberts C, Freeman J, Sandal O, Schnohr CW, de Looze ME, Nic Gabhainn A, Iannotti R, Rasmussen M and the International HBSC Study Group. The Health Behaviour in School-aged Children (HBSC) study: methodological developments and current tensions. Int J Public Health 2009;54(Suppl 2):140-50. https://doi.org/10.1007/s00038-009-5405-9

[14] Austin PC, Merlo J. Intermediate and advanced topics in multilevel logistic regression analysis. Stat Med 2017;36:3257-3277. https://doi.org/10.1002/sim.7336

[15] Holstein BE. Hansen EH, Due P. Social class variation in medicine use among adolescents. Eur J Public Health 2004;14:49-52. https://doi.org/10.1093/eurpub/14.1.49

[16] Koushede V. Holstein BE. Sense of coherence and medicine use for headache among adolescents. J Adolesc Health 2009;45:149-55. https://doi.org/10.1016/j.jadohealth.2008.12.009

[17] Haugland S, Wold B, Stevenson J, Aaroe LE, Woynarowska B. Subjective health complaints in adolescence. A cross-national comparison of prevalence and dimensionality. Eur J Public Health. 2001;11:4-10. https://doi.org/10.1093/eurpub/11.1.4

[18] Roth-Isigkeit A, Thyen U, Raspe HH,Stoven H, Schmucker P. Reports of pain among German children and adolescents: an epidemiological study. Acta Paediatr 2004;93:258-63. https://doi.org/10.1111/j.1651-2227.2004.tb00717.x

[19] Torsheim T, Ravens-Sieberer U, Hetland J, Valimaa R, Danielson M, M. Overpeck M. Cross-national variation of gender differences in adolescent subjective health in Europe and North America. Soc Sci Med 2006;62:815-27. https://doi.org/10.1016/j.socscimed.2005.06.047

[20] Gobina I, Villberg J, Villerusa A, Välimaa R, Tynjälä J, Ottova-Jordan V, Ravens-Sieberer U, Levin K, Cavallo F, Borraccino A, Sigmund E, Andersen A, Holstein BE. Self-reported recurrent pain and medicine use behaviours among 15-year olds: results from the international study. Eur J Pain 2015;19:77-84. https://doi.org/10.1002/ejp.524

[21] Trombetta CM, Manini I, Pammolli A, Rossi S, Pozzi T, Montomoli E, Lazzeri G. Medicine use and recurrent complaints among 15-years-old adolescents in Tuscany. Ann Ist Super Sanità 2018;54:208-13. https://doi.org/10.4415/ANN_18_03_07

[22] Fichtel A, Larsson B. Psychosocial impact of headache and comorbidity with other pains among Swedish school adolescents. Headache 2002;42:766-75. https://doi.org/10.1046/j.1526-4610.2002.02178.x

[23] Gualano MR, Bert F, Passi S, Stillo M, Galis V, Manzoli L, Siliquini R. Use of self-medication among adolescents: a

systematic review and meta-analysis. Eur J Public Health 2015;25:444-50. https://doi.org/10.1093/eurpub/cku207

[24] Roth-Isigkeit A, Thyen U, Stőven H, Schwarzenberger J, Schmucker P. Pain among children and adolescents: Restriction in daily living and triggering factors. Pediatrics 2005;115:e152-e162.

[25] Powers SW, Gilman DK, Hershey AD. Headache and psychological functioning in children and adolescents. Headache 2006;46:1404-15. https://doi.org/10.1186/1129-2377-14-79

[26] Gauntlett-Gilbert J, Eccleston C. Disability in adolescents with recurrent pain: patterns and predictors across different domains of functioning. Pain 2007;13:132–141. https://doi.org/10.1016/j.pain.2006.12.021

[27] Larsson B, Sund AM. Emotional/behavioural, social correlates and one-year predictors of frequent pains among early adolescents: Influences of pain characteristics. Eur J Pain 2007;11:57-65. https://doi.org/10.1016/j.ejpain.2005.12.014

[28] Waldie KE. Childhood headache, stress in adolescence, and primary headache in young adulthood: a longitudinal cohort study. Headache 2011;41:1-10. https://doi.org/10.1046/j.1526-4610.2001.111006001.x

Received on August 27, 2019. Accepted on October 17, 2019.

Correspondence: Federica Centauri, Azienda Ospedaliera Universitaria Senese, strada delle Scotte 14, 53100 Siena - Tel. +39 0577 585168 - E-mail: federica.centauri@ao-siena.toscana.it

How to cite this article: Centauri F, Pammolli A, Simi R, Dalmasso P, Berchialla P, Borraccino A, Charrier L, Lenzi M, Vieno A, Lemma P, Cavallo F, Lazzeri G. Self-reported recurrent pain and medicine use among 15-year-olds: results from the HBSC Italian study. J Prev Med Hyg 2019;60:E368-E375. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1382

ORIGINAL ARTICLE

Young people, young adults and binge drinking

V. LA FAUCI[1], R. SQUERI[1], P. SPATARO[1], C. GENOVESE[2], N. LAUDANI[2], V. ALESSI[2]
[1] Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Italy; [2] Postgraduate Medical School in Hygiene and Preventive Medicine, University of Messina, Italy

Keywords

Young people • Binge drinking • Alcohol

Summary

Introduction and purpose. *The consumption of alcohol among young people and young adults has undergone, in recent decades, a sharp upsurge with the increasingly frequent intake of large quantities of alcohol. The aim of our study was to investigate socio-demographic, economic and behavioural factors that have a major impact on the voluntary alcohol habit in young people.*
Methods. *The survey was conducted via administration of an anonymous questionnaire based on "the WHO Alcohol Use Disorders Identification Test", disseminated on-line, to young people and young adults (aged 18 to 35).*
Results. *We examined a sample of 365 subjects. Consumption of wine and beer were predominant followed by super-alcohol mixes. We found correlations between alcohol use and the following variables: marital status ($p < 0.001$), parental education (mother $p < 0.05$; father $p < 0.001$), income level ($p < 0.05$), physical activity ($p < 0.05$) and voluptuous habits (smoke and coffee: $p < 0.001$.The 5% of men and 1% of women had car accidents during the previous year due to alcohol use and 15% said they did not remember what happened in an alcoholic evening once or twice a month.*
Conclusions. *The phenomenon of alcohol consummation is deeply ingrained in our reality, with dangerous episodes of binge drinking in young adults with a higher prevalence in the female sex.*

Introduction

The consumption of alcohol among adolescents has undergone a sharp upsurge in recent decades with behavioral changes and increasingly frequent intake of large quantities of alcohol. In community and adolescent social contexts in particular, this is called "binge drinking" or the so-called alcoholic binge which consists in taking five or six alcoholic beverages outside a meal and concentrated in a short time – two hours or less – for the purpose of getting drunk [1]. It is a recreational consumption away from the family context, but among friends, where drinking is associated with a state of well-being, joy and fun combined with emancipation, and where getting drunk becomes a fashion and a source of pride. There is no safe consumption for health, but if you drink alcohol, you should not drink on an empty stomach and you must not exceed the quantities considered at low risk, i.e. 2 units of alcohol per day for men, 1 unit of alcohol per day for women and for over 65s, zero units of alcohol for those under 18 [2]. The excess limits [3] correspond to the one-time consumption of more than 6 Alcoholic Units (UA = 12 grams of pure alcohol) of any alcoholic beverage. The alcoholic unit corresponds to about 10-12 grams of pure alcohol, corresponding to a standard glass of wine (12°, 125 ml), a can of beer (4.5°, 330 ml), an aperitif (18°, 80 ml), or a small glass of spirits (36°, 40 ml). The survey on the consumption of alcohol in adolescence and post-adolescence has aroused great concern in the scientific community as it is closely linked to social problems arising from it, as well as metabolic and behavioral disorders. The problems arising from alcohol consumption in young people, in fact, are different from those of adults [4]. In young people, the negative effects involve changes in the relationship with family members, classmates, colleagues and teachers, with poor academic performance and progressive lengthening of the time needed to complete primary education, aggression, anti-social behavior, crime, public disorder and other high-risk behaviors, such as driving while intoxicated, unprotected sexual activity involving a greater risk of unwanted pregnancy and transmission of sexual diseases [5]. Young people who consume a large amount of alcohol are on the whole more at risk than young people who do not drink at all [6]. Alcohol abuse therefore contributes directly and indirectly to the development of physical, mental and social harm in the drinker. Not only does all this combine to harm the young person physically, emotionally and socially, it is also a permanent negative modifier in the school and university environment, resulting in delayed training and entry to the world of labor.
The ISTAT data [7] report alcohol consumption in the 18-24 age group, compared to the older adult population, with a more episodic frequency, but with higher overall consumption, including heavy drinking prolonged for entire nights. Some authors report a greater likelihood of using psychoactive substances in adolescence and early adulthood, with a positive association between education level and socio-economic situation and consumption and substance abuse [8].

https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1309

Given these premises, it is essential to investigate the socio-demographic and economic factors that have an overall impact on the voluntary habit of alcohol consumption, especially since the data confirm that young Italians are in the top rank among drinkers worldwide, despite the measures predisposed to reduce this abuse [9].
The aim of the study was to investigate the consumption of alcoholic substances by young people and young adults resident in southern Italy in relation to the socio-economic and cultural situation.

Methods

An observational study was carried out between January 2018 and June 2018, by administration of an anonymous questionnaire, disseminated online to subjects aged between 18 and 35through social networks and based on multiple-choice questions.
The questionnaire was based on the WHO Alcohol Use Disorders Identification Test. Questions were asked concerning the subject's socio-economic condition, educational level and family nucleus, with particular attention to income. Habits and lifestyles were investigated regarding, apart from alcohol consumption, cigarette smoking, physical activity and coffee consumption. Also assessed were the frequency of alcohol intake, the amount taken daily, the excess consumption of spirits and the effects of alcohol on daily life, with particular attention to alcohol-induced driving accidents. Lastly, we investigated the type of alcohol consumed most frequently.
The sample was defined considering the prevalence of alcohol consummation of 73.3% for males with a n=75 and 44.7% and so a n=95 for women in Sicily, identifying a 95% Confidence Interval (CI) and an absolute accuracy of 10%.
We stratified the sample in relation to age (18- 4 years and 25-35 years) and sex, their education (graduate or undergraduate), parents' education (under or over eight years of education) and income level (0-10.000, 10.000-30.000, 30.000-50.000, 50.000-80.000, > 80.000 euros).

Statistical analysis

The mean and standard deviation (SD) were calculated with regard to the quantitative variable (age), while absolute and relative frequencies were obtained for categorical data. The Chi-Square test of independence with Yates correction (only for samples with n between 40 and 200) was used to determine any statistically significant associations between the alcohol consummation and all the categorical variables, adopting a relative partition model where the null hypothesis was rejected. Statistical significance thresh-old was set at $p = 0.050$; p-values of less than 0.050 on two-tailed tests were considered statistically significant. We chose to perform statistical analyses both on all samples (n = 365) and on samples stratified in two age groups (18-24 years and 25-35 years), to identify associations between young people and young adults. All statistical analyses were performed using R software.

Results

All respondents joined the questionnaire: we examined a sample of 365 subjects aged between 18 and 35 years with a mean of 24.70 years (4.5), of which 140/365 (38.6%) were males and 225/365 (62,4%) were females. Of these, 212/365 (58%) said they had used alcohol in the last year at least occasionally; of these, 40% of drinkers were males and 60% were women, with a mean age of 24.68 years (4.1) (Tab. I).
We stratified the sample in two age groups: 18-24 years and 25-35 years. The former represented 195/365 (53.4%) of the sample. Of these 131/195 (67.2%) were women and 64/195 (32.8%) were men. The age group of25-35 years made up 170/365 (46.6%) of the sample, of which 94/170 (55.3%) were women and 76/170 (44.7%) were men (Tab. II).

Demographic factors and education

We compared the responses of drinkers and non-drinkers, evaluating the presence of associations by gender and age. However, we found no statistical associations by sex (χ^2: 0.3431; $p = 0.55$) or age (χ^2: 0.4807; $p = 0.49$). It is important to underline the high percentage of alcohol use in women (71.15%) above the Sicilian average, while the percentage in males is lower (62.5%).
Other factors investigated were years of education and the presence of a paid job and, for students, which type of degree course they are attending (medical or not).
No associations emerged between the years of education, less than or equal to 8, between drinkers and non-drinkers ($\chi^2 = 1.488$; $p = 0.22$) and the presence of a paid job. However, more than half of those who joined the questionnaire were students. Within the subdivision for degree courses, it emerged that in this age group, 30/39 (77%) of medical students usually consume more alcohol than those who attend other degree programs, but we did not find any statistical difference (χ^2=0.5959; $p = 0.44$).
It also emerged that non-drinkers were both male and female, had a stable relationship or were married in greater numbers (of these 22% were males and 33% were women) than drinkers with high statistical associations ($\chi^2 = 7.1059$; $p < 0.001$).
We also investigated whether there were behavioral associations between young people and young adults, dividing the sample in two age groups, 18-24 yrs and 25-35 yrs.
Among the18-24-year-olds, the male drinkers represented 40/64 (62%) while female drinkers represented 70/131 (53%) of the sample. Among the 25-35-year-olds, males drinkers represented 58/94 (61%) of the sample while female drinkers were 44/76 (58%). We did not find any statistical difference by sex.

Tab. I. Distribution of the sample in "drinkers" and "non-drinkers" by sex, age, education, work (employed or not), type of work, sector of work, parents' education, income level, voluptuous habits.

Non-drinkers						
Male			**Female**			
15.34% (56)			26.58% (97)			
18-24 yrs			25-35 yrs			
23.29% (85)			18.63% (68)			
Single			**Married**			
36.44%(133)			5.48% (20)			
Less than 8 yrs			More than 8 yrs			
31.51%(115)			10.41%(38)			
Employed			**Unemployed**			
9.04%(33)			32.88%(120)			
Public employee	Private employee	Business owner	Self-employed	Housewife	Student	Worker
2.47%	2.19%	0.27%	2.74%	0.82%	30.96%	0.55%
Agriculture/fishing	Building	Industry	Trade/turism	Public health	Administration	Other
0.27%	0.55%	0.82%	0.55%	33.42%	0.27%	0.82%
Mother's education			**Father's education**			
Less than 8 yrs	**More than 8 yrs**		**Less than 8 yrs**	**More than 8 yrs**		
12.05%	29.86%		15.34%	26.57%		
< 10,000	10,000-30,000	30,000-50,000	50,000-80,000	> 80,000		
8.77%	18.90%	10.14%	3.56%	0.55%		
Physical activity						
No			**Yes**			
21.92% (80)			20% (73)			
Smoker						
No			**Yes**			
16.16% (59)			25.75%(94)			
Coffee drinker						
No			**Yes**			
28.22% (103)			13.70%(50)			
Drinkers						
Male			**Female**			
23.01% (84)			35.07% (128)			
18-24 yrs			25-35 yrs			
30.14% (110)			17.95% (102)			
Single			**Married**			
55.07% (201)			3.01% (11)			
Less than 8 yrs			More than 8 yrs			
0.27%(1)			57.81% (211)			
Employed			**Unemployed**			
15.89%(58)			42.19% (154)			
Public employee	Private employee	Business owner	Self-employed	Housewife	Student	Worker
2.74%	6.30%	1.37%	4.11%	0.55%	42.19%	0.27%
Agriculture/fishing	Building	Industry	Trade/turism	Public health	Administration	Other
1.10%	1.10%	0.27%	1.10%	44.38%	1.37%	1.91%
Mother's education			**Father's education**			
Less than 8 yrs	**More than 8 yrs**		**Less than 8 yrs**	**More than 8 yrs**		
10.96%	46.85%		11.23%	46.85%		
< 10,000	10,000-30,000	30,000-50,000	50,000-80,000	> 80,000		
7.40%	24.38%	15.07%	8.49%	2.74%		
Physical activity						
No			**Yes**			
38.08%(139)			20%(73)			
Smoker						
No			**Yes**			
38.90%(142)			19.18%(70)			
Coffee drinker						
No			**Yes**			
50.14%(183)			7.95%(29)			

*the percentage was calculated on the entire sample (n=365); the percentage does not represent "no answer".

Tab. II. Distribution of the sample in two ranges (18-25 yrs and 25-35 yrs) and in "drinkers" and "non-drinkers", by sex, education, work (employed or not), type of work, parents' education, income level and voluptuous habits.

Young people(18-24 years) n = 195							
Non-drinkers				Drinkers			
Male		Female		Male		Female	
24 (12.31%)		61 (31.28%)		40 (20.51%)		70 (35.90%)	
Single		Married		Single		Married	
84 (43.08)		1 (0.51%)		110 (56.41%)		0 (0%)	
Less than 8 yrs		More than 8 yrs		Less than 8 yrs		More than 8 yrs	
1 (0.51%)		84 (43.08%)		1 (0.51%)		109 (56.41%)	
Unemployed		Employed		Unemployed		Employed	
83 (42.56%)		2 (1.03%)		94 (48.21%)		16(8.21%)	
Mother's education		Father's education		Mother's education		Father's education	
< 8 yrs	> 8 yrs	< 8 yrs	< 8 yrs	< 8 yrs	> 8 yrs	< 8 yrs	> 8 yrs
21 (10.77%)	64 (32.82%)	31 (15.9%)	54 (27.69%)	23 (11.79%)	87 (44.62%)	25 (27.70%)	85 (43.59%)
< 30,000	30,000-50,000	> 50,000		< 30,000	30,000-50,000	> 50,000	
21 (10.7%)	57(29.23%)	7 (3.59%)		12 (6.15%)	80 (41%)	18 (9.23%)	
Physical activity							
No		Yes		No		Yes	
41 (21.03%)		44 (22.56%)		43 (22.05%)		67 (34.36%)	
Smoker							
No		Yes		No		Yes	
55 (28.21%)		30 (15.38%)		42 (21.54%)		68 (34.87%)	
Coffee drinker							
No		Yes		No		Yes	
31 (15.9%)		54 (27.69%)		15 (7.69%)		95 (48.72%)	
Young adults (25-35 years) n = 170							
Non-drinkers				Drinkers			
Male		Female		Male		Female	
32 (18.82%)		36 (21.18%)		44 (25.88%)		58 (34.12%)	
Single		Married		Single		Married	
49 (28.82%)		19 (11.18%)		91 (53.53%)		11 (6.47%)	
Less than 8 yrs		More than 8 yrs		Less than 8 yrs		More than 8 yrs	
3 (1.76%)		65 (38.24%)		0		81 (60%)	
Unemployed		Employed		Unemployed		Employed	
37 (21.76%)		31 (18.24%)		60 (35.29%)		42 (24.71%)	
Mother's education		Father's education		Mother's education		Father's education	
< 8 yrs	> 8 yrs	< 8 yrs	> 8 yrs	< 8 yrs	> 8 yrs	< 8 yrs	> 8 yrs
23 (13.53%)	45 (26.47%)	25 (14.71%)	43 (25.29%)	17 (10%)	84 (49.42%)	16 (9.41%)	86 (50.59%)
< 30,000	30,000-50,000	> 50,000		< 30,000	30,000-50,000	> 50,000	
11 (6.47%)	49 (19.%)	8 (4.7%)		15(8.8%)	64 (37.6%)	23 (13.5%)	
Physical activity							
No		Yes		No		Yes	
32 (18.82%)		36 (21.18%)		30 (17.65%)		72 (42.35%)	
Smoker							
No		Yes		No		Yes	
39 (22,94%)		29 (17,06%)		28 (16,47%)		74 (43,53%)	
Coffee drinker							
No		Yes		No		Yes	
19 (11.18%)		49 (21.82%)		14 (8.24%)		88 (51.76%)	

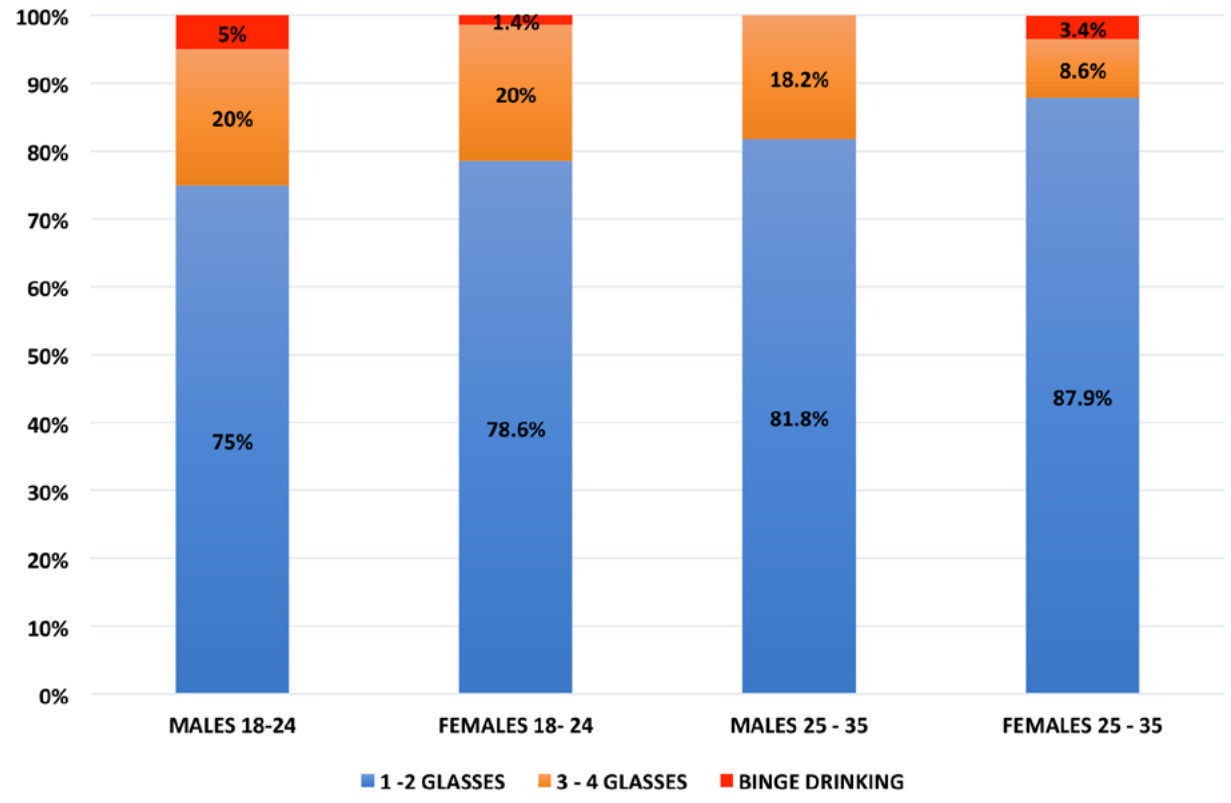
We investigated associations in the same age groups by education (graduate and undergraduate) and employment. For the former, we found no statistical difference, while a statistical difference emerged from the latter (χ^2 with Yates correction = 8.5072; $p < 0.01$), with medium statistical significance.

For marital status, we found medium statistical associations in young adults, with 60% of drinkers single and 36.67% married (χ^2 with Yates correction = 7.1255; $p < 0.01$).

Socio-economic factors

Regarding the parents' education, we observed that both fathers and mothers of non-drinkers have a lower educa-

Fig. 1. Number of glasses drunk during a single alcoholic evening.



tional qualification with high statistical associations (for mothers: $\chi^2 = 4.7991$; $p < 0.05$; for fathers: $\chi^2 = 13.0779$; $p < 0.001$).

As regards income level, we found statistical associations between income levels of drinkers and non-drinkers ($\chi^2 = 9.8957$; $p < 0.05$). Only 12.7% of drinkers, in fact, had an income of less than 10,000 euros per year, 42% had an income of between 10,000 and 30,000 euros, 25.9% between 30,000 and 50,000 euros, 19.1% between € 50,000 and € 80,000 and as many as 4.7% exceeding € 80,000 annually. Among non-drinkers, on the other hand, 20.9% had an income of less than 10,000 euros a year and only 9.8% have an income of between 50,000 and 80,000 euros or more.

For these factors, we also evaluated associations between young people and young adults and we found a correlation with income level (lower, medium or higher) only for the first group (χ^2 with Yates correction = 8.0836; $p < 0.05$). For parents' education we found statistical associations both in young people (for fathers χ^2 with Yates correction = 3.7782; $p < 0.05$, that in young adults (χ^2 with Yates correction = 8.7869; $p < 0.05$ for fathers; χ^2 with Yates correction = 5.5881; $p < 0.05$ for mothers).

Physical activity and voluptuous habits

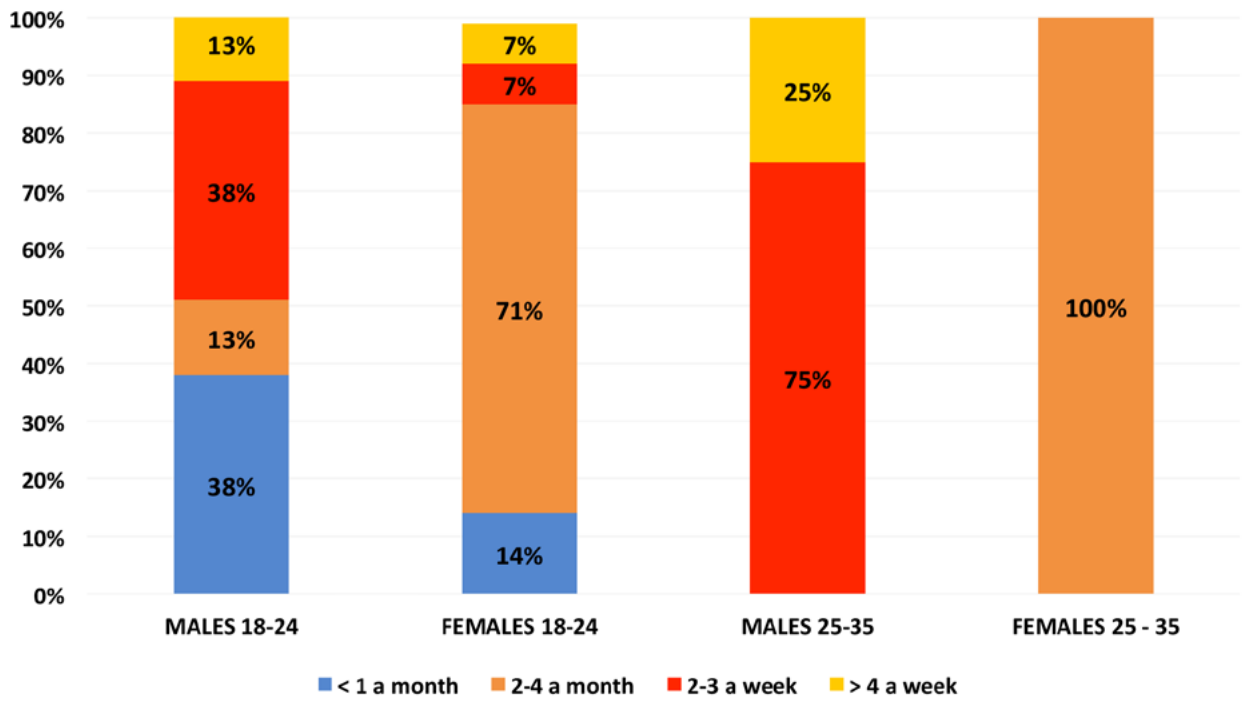
We investigated the presence of associations between drinkers and non-drinkers for voluptuous habits and we found high statistical associations between drinkers and non-drinkers for coffee consumption ($\chi^2 = 18.9172$; $p < 0.001$) and cigarettes smoking ($\chi^2 = 29.0068$; $p < 0.001$).Statistical associations were also found for physical activity ($\chi^2 = 6.5286$; $p < 0.05$), 63.8% in the drinkers' group not performing any compared with 50% that did. In the non-drinkers' group we found 47.2% that did not perform physical activity compared with 50% that did.

As regards lifestyle, drinkers aged 25-35 perform more physical activity than non-drinkers, with statistical associations (χ^2 with Yates correction = 4.7487; $p < 0.05$). Drinkers take coffee more frequently and in higher quantities non-drinkers (χ^2 with Yates correction= 12.6321; $p < 0.001$ for 18-25 yrs; χ^2 with Yates correction = 814.0507; $p < 0.001$). Lastly, drinkers are also smokers in significantly higher percentages than non-drinkers (χ^2 with Yates correction = 12.4535; $p < 0.05$ for 18-25 yrs; for 25-35 yrs χ^2 with Yates correction = 14.0507). It is interesting that among drinkers (but this can also be observed in non-drinkers), more women smoke (67%) than men (53%) (Tab. II).

Frequency and amount of alcohol intake

We stratified the sample by frequency of alcohol intake: never or less than once a month, twice/four times a month, twice/three times a week, four time or more per week. We investigated associations of consumption frequency by sex and age and we found statistical associations only in the first case (χ^2=10.2781; $p < 0.05$).

Fig. 2. Ratio between frequency and quantity for those who drink 3-4 glasses a night.



We evaluate alcohol intake according to each age group and sex (1-2 glasses, 3-4 glasses, over 4 glasses). The data are shown in Figure 1. We analyzed the presence of associations between sex and age group in the amount of alcohol intake, but we did not find any statistical associations, even when stratifying the samples between young people and young adults. Among those who drink from 3 to 4 glasses per night, it is interesting to evaluate the relationship between amount and frequency. There emerged a repetition of such high consumption 2-4 times a month, 2-3 times a week or even over 4 times a week, far exceeding the limits set by the WHO (Fig. 2).
In the 18-24 age group, women drank occasionally and monthly, respectively 24.3% and 60% against 20% and 52% for men. Instead, increasing the frequency of drinking, men drank more, respectively 22.5% drink weekly against 12.9% for women, while 5% of men in this age group drank daily against 2.9% for women. In the 25-35 age group, ratios remain similar, with women drinking less frequently than men. 13.8% drank occasionally against 9.1% for men, while 72.4% drank monthly compared with 27.3% for men. As the frequency increases, the results reverse, with 47.7% of men drinking weekly and 15.9% daily compared to 10.3% and 3.4% for women.

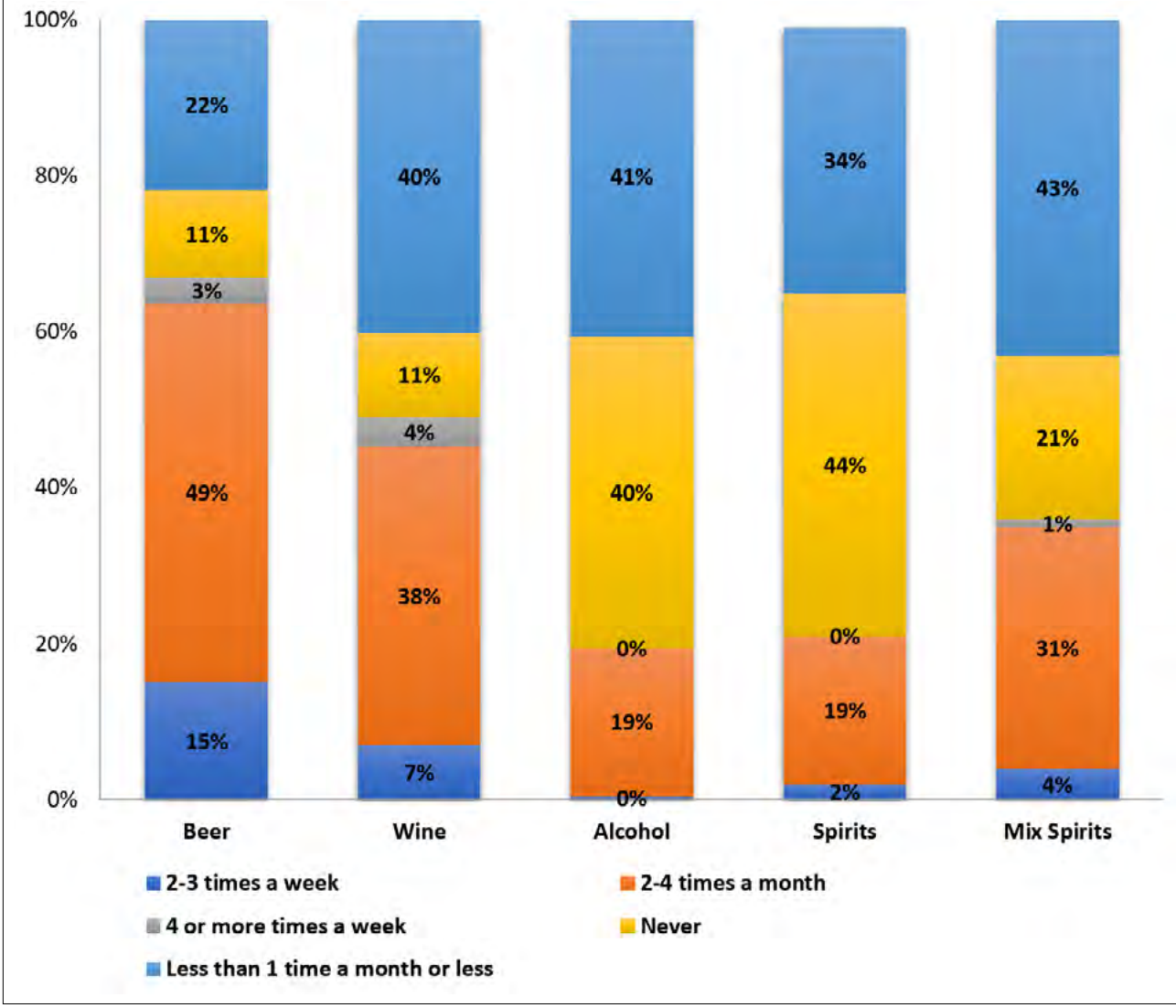
Type of alcohol consumed

The most frequently consumed types of alcohol, on the basis of the frequency range of 2-4 times a month, is beer, at 49%, followed by wine 38%, mix of spirits and, lastly, liqueurs and spirits (Fig. 3).

The effect of alcohol on everyday life

6% of men aged 18 to 24 yrs said they could not stop drinking once they started, both several times in the same month and in the same week. 15% felt remorse for the amount of alcohol taken while 3% said that several times a month they had no memory of what happened after an alcoholic evening. Among women between the ages of 18 and 24 years, 4% could not stop drinking once they started, 8% said they could not complete their daily activities due to drinking, 23% had remorse for the alcohol taken and 23% could not remember, several times in the same month, what happened the night before.
In the 25-35 age group, 4% of men failed to complete their daily activities, 7% needed a drink in the morning, 16% felt remorse for the alcohol taken and 12% could not remember what happened the night before. Among women of the same age group, 3% failed to perform normal daily activities, 12% felt guilty about the amount of alcohol taken and 10% had no memory, on several occasions, of what happened the night before.
Among those who responded to the questionnaire, 5% of men and 1% of women aged 18-24 reported having had an alcohol-related accident in the last year, 5% of men and 3% of women in previous years. While in the 25-35 age-band, only 2% of men had accidents in the last year due to drinking and 7% of women in previous years. Lastly, 5% and 3% of men and women aged 18-24, respectively, admitted having a relative concerned about their alcohol consumption and having received advice to stop drinking. In the 25- to 35-year age group, 2% of both men and women had received the same advice.

Fig. 3. Type of alcoholic beverages most frequently consumed.



Discussion and conclusion

Our results are in line with national data and in particular with Sicily, where the percentage of consumers of at least one alcoholic beverage in 2016 was 73.3%among men, 44.7% among women, compared to the national average for both genders [7, 8]. In our sample, women, in all age groups considered, had a higher educational qualification than men. Furthermore, when comparing drinkers and non-drinkers, drinkers showed to have a higher educational qualification and greater availability of financial resources, as they have more often a paid job. As regards socio-economic conditions, younger and older adult drinkers belong to economically more well-off and scholastically higher families, in line with international literature, though some studies reported more drinkers in families with low education [9, 10].

Non-drinkers tend to have stable relationships, but drinkers are predominantly single, according to international literature, with a decrease in drinking accompanying the transition from being single to a first marriage [11, 12].

The people that consume alcoholic beverages are also more often consumers of coffee and cigarettes, but paradoxically they perform more physical activity. Women of both categories smoke and drink more coffee and more often. These data confirm the literature and are worrying due to effects that these three factors can have on health [13].

When alcohol is mixed with caffeine, this can mask its depressant effects, making drinkers feel more alert than they would otherwise. As a result, they may drink more alcohol and become more impaired than they realize, increasing the risk of alcohol-attributable damage [14].

The effects of excessive consumption on respondents have more or less serious repercussions on daily life, influencing them in carrying out normal daily activities, reducing their prudence while driving. About this point, worrying is the fact that 8% of respondents in the last year alone had alcohol-related traffic accidents [15, 16],

a figure in line with the national one (ISTAT, 2018) where 7.8% of accidents are caused by drunkenness and the percentage is increasing [17]. The ISTISAN data then showed that among those who had a car accident in the 18-29 age group, the main cause turned out to be driving under the influence of alcohol and drugs (61.6% of cases) [18]. Alarming is the percentage of young people (3.4% compared to 0.7% of those over 30) who, although they know they have to drive, choose not to limit their consumption of alcohol [19]. The results of this study confirm that alcohol abuse is an important risk factor for public health.

It has been shown that reducing or stopping drinking produces health benefits at any age. The elimination of alcohol involves an inversion of the danger for all related chronic diseases, such as cirrhosis of the liver or depression, although there remains a level of risk due to protracted exposure [20]. Reduction or cessation of alcohol consumption is associated with a rapid improvement in physical conditions and a reduction in overall mortality [21]. In particular, young people who drastically decrease their alcohol consumption in the proximity of adulthood significantly reduce the risk of developing alcohol-related damage, particularly to the nervous system [22].

Limiting intake also allows subjects to keep their body weight under control: alcohol provides about 7 kilocalories per gram, requiring a certain activity in order to dispose of it [23-26].

Drinking alcohol, as well as smoking, also involves a considerable expense, not only for drinkers but for all society [27]. According to a recent review, in fact, the economic burden of alcohol on society is substantial, accounting for 0.45% to 5.44% of the Gross Domestic Product [28].

Moreover, we found in our study a statistical association between family income for young people but not for young adults, probably because the latter work more than the former and have a personal salary. Another factor is, as previously reported, that more people in this age group are more married and this could limit alcohol use.

Lastly, a recent study conducted by the University of Washington and published in September 2018 [29] highlighted that the amount of alcohol that should be taken, for safe consumption, is zero. This fact, taken with the due exceptions, highlights even more how policies aimed at reducing alcohol consumption should be an absolute priority. The WHO, too, after a study published recently, has established that there is no safe level to drink alcohol [30].

Obviously, there is low-risk consumption, but the WHO does not set particular limits, because the evidence shows that the ideal situation for health is not drinking at all. Research has shown a lower risk of ischemic events (heart disease, stroke and type 2 diabetes) among middle-aged and older light to moderate drinkers. However, the damaging effects of alcohol far outweigh any potential protective benefit. An older person will get much greater health benefits from being physically active and eating healthy foods that from alcohol [31].

The limit of our study is represented by the fact that self-reported data is often inaccurate, especially for adolescents and furthermore, we used an online survey, and this could have limited the effects of underestimation because the interviewer cannot view the respondents [32].

Diseases resulting from unhealthy behavior, facilitated by an unhealthy environment and solicited by commercial interests, are the dominant health problem of the 21st century. However, there is a clear need to motivate everyone, from young people to adults, so that we can start an important and radical cultural change [33], especially in behaviors adopted at a younger age. The model of drinking we have imported from northern Europe (binge drinking), must therefore be countered by the most effective means and in the shortest time possible.

Prevention remains the key element to combat this phenomenon. Highly necessary is the collaboration between families and the school. Indeed, the first signs of discomfort are among the school desks; it would therefore be very useful to train teachers to intercept this malaise in order to prevent the consequent possible use of tobacco, alcohol and drugs in young adolescents. Although this problem persists enormously on our territory with colossal consequences for the economic, psychic and general lives of the drinkers, prevention measures are lacking.

Promotion programs, such as vaccination, water potabilization, prevention of HAIs remain the most important weapons in the hands of public health: it is, therefore, imperative to establish structured forms of primary and secondary prevention at territorial level in order to prevent this phenomenon [34-43].

Other possible benefits could come from careful control policies and health programs, by increasing the taxation of alcoholic beverages, controlling their sales and sales hours and reducing the exposure of buyers to alcoholic beverage advertisements.

Acknowledgements

Funding sources: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

VLF conceived, designed and coordinated the research. VLF, RS, VA, PS, CG and NL contributed to the acquisition, analysis and interpretation of data . VLF, VA and CG evaluated the results. VLF, and VA wrote the manuscript. All Authors revised the manuscript and gave their

contribution to improve the paper. All authors read and approved the final manuscript.

References

[1] Stolle M, Sack PM, Thomasius R. Binge drinking in childhood and adolescence: epidemiology, consequences, and intervention. Dtsch Arztebl Int 2009;106:323-8, https://doi.org/10.3238/arztebl.2009.0323

[2] Ministry of Health. Available at: http://www.salute.gov.it/portale/temi/p2_6.jsp?lingua=italiano&id=2346&area=alcol&menu=problema (Last accessed: 2019, Jan 22).

[3] Istituto Superiore di Sanità. "L'impatto dei nuovi livelli stabiliti dalle rinnovate linee guide sul consumo alcolico sulla definizione del rischio e il monitoraggio dei consumi a rischio nella popolazione". Available at: http://www.epicentro.iss.it/alcol/apd2015/nuovo%20indicatore%20rischio(finale).pdf (Last accessed: 2019, Jan 22).

[4] Stueve A, O´Donnell LN. Early alcohol initation and subsequent sexual and alcohol risk behaviours among urban youths. Am J Public Health 2005;95:887-93. https://doi.org/10.2105/AJPH.2003.026567.

[5] Townshend JM, Kambouropoulos N, Griffin A, Hunt FJ, Milani RM. Binge drinking, reflection impulsivity, and unplanned sexual behavior: impaired decision-making in young social drinkers. Alcohol Clin Exp Res 2014;381143-50. https://doi.org/10.1111/acer.12333

[6] Hingson RW, Heeren T, Winter MR. Age at drinking onset and alcohol dependence: age at onset, duration, and severity. Arch Pediatr Adolesc Med 2006;160:739-46. https://doi.org/10.1001/archpedi.160.7.739

[7] Dati ISTAT, Available at: https://www.istat.it/it/files//2017/04/Consumo_alcol_in_Italia_2016.pdf (Last accessed: 2019, Jan 22).

[8] Velásquez J, Scoppetta O. Consumo de sustanciaspsicoactivas en estudiantes de carrerastécnicas y tecnológicas de Santa Fe de Bogotá. Bogotá: 1998, p. 80.

[9] Epidemiologia e monitoraggio alcol-correlato in Italia e nelle Regioni Valutazione dell'Osservatorio Nazionale Alcol sull'impatto del consumo di alcol ai fini dell'implementazione delle attività del Piano Nazionale Alcol e Salute Rapporto 2018 Emanuele Scafato, Silvia Ghirini, Claudia Gandin, Monica Vichi, Riccardo Scipione e il Gruppo di Lavoro CSDA (Centro Servizi Documentazione Alcol).

[10] Keyes KM, Hasin DS. Socio-economic status and problem alcohol use: the positive relationship between income and the DSM-IV alcohol abuse diagnosis. Addiction 2008;103:1120-30.https://doi.org/10.1111/j.1360-0443.2008.02218.x.

[11] Sargent JD, Wills TA, Stoolmiller M, Gibbons J, Gibbons FX. Alcohol use in motion pictures and its relation with early-onset teen drinking. J Stud Alcohol 2006;67:54-65. https://doi.org/10.15288/jsa.2006.67.54

[12] Prescott CA, Kendler KS. Associations between marital status and alcohol consumption in a longitudinal study of female twins. J Stud Alcohol 2001;62:589-604. https://doi.org/10.15288/jsa.2001.62.589

[13] Vinader-Caerols C, Monleón S, Carrasco C, Parra A. Effects of alcohol, coffee, and tobacco, alone or in combination, on phyrsiological parameters and anxiety in a young population, J Caffeine Res 2012;2:70-6. https://doi.org/10.1089/jcr.2012.0018

[14] McKetin R, Coen A, Kaye S. A comprehensive review of the effects of mixing caffeinated energy drinks with alcohol. Drug Alcohol Depend 2015;151:15-30. https://doi.org/10.1016/j.drugalcdep.2015.01.047

[15] Rehm J, Gmel G, Sempos CT, Trevisan M. Alcohol-related morbidity and mortality. Alcohol Res Health 2003;27:39-51.

[16] Taylor B, Rehm J. The relationship between alcohol consumption and fatal motor vehicle injury: high risk at low alcohol levels. Alcohol Clin Exp Res 2012;36:1827-34. https://doi.org/10.1111/j.1530-0277.2012.01785.x

[17] ISTAT, Incidenti stradali. Avaible at: https://www.istat.it/it/files/2018/07/Incidenti-stradali_2017.pdf (Last accessed: May 2019).

[18] Rapporti ISTISAN. Avaible at: http://old.iss.it/binary/publ/cont/11_4_web.pdf (Last accessed: May 2019).

[19] Istituto Superiore di Sanità: Epidemiologia e monitoraggio alcol-correlato in Italia e nelle Regioni. Avaible at: https://www.epicentro.iss.it/alcol/apd2018/Rapporto%20ISTISAN%20monitoraggio%20alcol%20correlato%20in%20Italia%202018.pdf (Last accessed: May 2019).

[20] Global status report on alcohol and health 2014. Avaible at: http://apps.who.int/iris/bitstream/handle/10665/112736/9789240692763_eng.pdf;jsessionid=EEFA1BEFABB665D290349718E3C76924?sequence=1 (Last accessed: Jan 2019).

[21] Charakida M, Georgiopoulos G, Dangardt F, Chiesa ST, Hughes AD, Rapala A, Davey Smith G, Lawlor D, Finer N, Deanfield JE. Early vascular damage from smoking and alcohol in teenage years: the ALSPAC study. Eur Heart J 2019;40:345-53. https://doi.org/10.1093/eurheartj/ehy524

[22] López-Caneda E, Cadaveira F, Correas A, Crego A, Maestú F, Rodríguez Holguín S. The brain of binge drinkers at rest: alterations in theta and beta oscillations in first-year college students with a binge drinking pattern. Front Behav Neurosci 2017;11:168. https://doi.org/10.3389/fnbeh.2017.00168

[23] Romano-Spica V, Macini P, Fara GM, Giammanco G; GSMS - Working Group on Movement Sciences for Health Italian Society of Hygiene Preventive Medicine and Public Health. Adapted physical activity for the promotion of health and the prevention of multifactorial chronic diseases: the Erice Charter. Ann Ig 2015;27:406-14. https://doi.org/10.7416/ai.2015.2028

[24] Squeri R, Genovese C, Palamara MAR, Trimarchi G, Ceccio C, Donia V, Pecoraro M, La Monica G, La Fauci V. Childhood obesity: risk factors involved An observational study on the effects of early and late risk factors on the development of childhood obesity in the South of Italy. EBPH 2018;15(4).

[25] Lazzeri G, Panatto D, Pammolli A, Azzolini E, Simi R, Meoni V, Giacchi MV, Amicizia D, Gasparini R. Trends in overweight and obesity prevalence in Tuscan schoolchildren (2002-2012). Public Health Nutr 2015;18:3078-85. https://doi.org/10.1017/S1368980015001676.

[26] Lazzeri G, Panatto D, Domnich A, Arata L, Pammolli A, Simi R, Giacchi MV, Amicizia D, Gasparini R. Clustering of health-related behaviors among early and mid-adolescents in Tuscany: results from a representative cross-sectional study. J Public Health (Oxf) 2018;40:e25-e33. https://doi.org/10.1093/pubmed/fdw134

[27] La Fauci V, Squeri R, Genovese C, Alessi V, Facciolà A. The 'Dangerous Cocktail': an epidemiological survey on the attitude of a population of pregnant woman towards some pregnancy risk factors. J Obstet Gynaecol. 2019 Aug 2:1-6 [Epub ahead of print]. https://doi.org/10.1080/0143615.2019.1621818

[28] Thavorncharoensap M, Teerawattananon Y, Yothasamut J, Lertpitakpong C, Chaikledkaew U. The economic impact of alcohol consumption: a systematic review. Subst Abuse Treat Prev Policy 2009;4:20. https://doi.org/10.1186/1747-597X-4-20

[29] GBD 2016 Alcohol Collaborators. Alcohol use and burden for 195 countries and territories, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 2018;392:1015-35. https://doi.org/10.1016/S0140-6736(18)31310-2

[30] Burton R, Sheron N. No level of alcohol consumption improves health. Lancet 2018;392:987-8. https://doi.org/10.1016/S0140-6736(18)31571-X

[31] Avaible on http://www.euro.who.int/en/health-topics/disease-prevention/alcohol-use/data-and-statistics/q-and-a-how-can-i-drink-alcohol-safely (Last accessed: Jan 2019).

[32] Bertol E, Vaiano F, Boscolo-Berto R, Fioravanti A, Palumbo D, Catalani V, Mari F, Patussi V, Serpelloni G. Alcohol, caffeine, and nicotine consumption in adolescents: hair analyrsis versus self-report. Am J Drug Alcohol Abuse 2017;43:341-9. https://doi.org/10.1080/00952990.2016.1216556

[33] Cannavò G, Delia,S, Grecò MC, Laganà P. Adolescents and alcohol: a survey in the city of Messina (Italy). Igiene e Sanità Pubblica 2009;65:53-8.

[34] Caselli E, Brusaferro S, Coccagna M, Arnoldo L, Berloco F, Antonioli P, Tarricone R, Pelissero G, Nola S, La Fauci V, Conte A, Tognon L, Villone G, Trua N, Mazzacane S; SAN-ICA Study Group. Reducing healthcare-associated infections incidence by a probiotic-based sanitation system: a multicentre, prospective, intervention study. PLoS One 2018;1:e0199616. https://doi.org/10.1371/journal.pone.0199616

[35] Squeri R, Genovese C, Trimarchi G, Palamara MAR, La Fauci V. An evaluation of attitude toward vaccines among healthcare workers of a University Hospital in Southern Italy. Ann Ig 2017;29:595-606. https://doi.org/10.7416/ai.2017.2188

[36] Genovese C, La Fauci V, Squeri A, Trimarchi G, Squeri R. HPV vaccine and autoimmune diseases: systematic review and meta-analysis of the literature. J Prev Med Hyg 2018;59:E194-E199. https://doi.org/10.15167/2421-4248/jpmh2018.59.3.998

[37] La Fauci V, Riso R, Facciolà A, Merlina V, Squeri R. Surveillance of microbiological contamination and correct use of protective lead garments. Ann Ig 2016;28:360-6. https://doi.org/10.7416/ai.2016.2116

[38] La Fauci V, Costa GB, Arena A, Ventura Spagnolo E, Genovese C, Palamara MA, Squeri R. Trend of MDR-microorganisms isolated from the biological samples of patients with HAI and from the surfaces around that patient. New Microbiol 2018;41:42-6.

[39] La Fauci V, Genovese C, Facciolà A, Palamara MAR, Squeri R. Five-year microbiological monitoring of wards and operating theatres in southern Italy. J Prev Med Hyg 2017;58:E166-E172.

[40] Genovese C, Picerno IAM, Trimarchi G, Cannavò G, Egitto G, Cosenza B, Merlina V, Icardi G, Panatto D, Amicizia D, Orsi A, Colosio C, Marsili C, Lari C, Palamara MAR, Vitale F, Casuccio A, Costantino C, Azara A, Castiglia P, Bianco A, Currà A, Gabutti G, Stefanati A, Sandri F, Florescu C, Marranzano M, Giorgianni G, Fiore V, Platania A, Torre I, Cappuccio A, Guillari A, Fabiani L, Giuliani AR, Appetiti A, La Fauci V, Squeri A, Ragusa R, Squeri R. Vaccination coverage in healthcare workers: a multicenter cross-sectional study in Italy. J Prev Med Hyg 2019;60:E12-E17. https://doi.org/10.15167/2421-4248/jpmh2019.60.1.1097

[41] La Fauci V, Costa GB, Facciolà A, Conti A, Riso R, Squeri R. Humidifiers for oxygen therapy: what risk for reusable and disposable devices? J Prev Med Hyg 2017;58:E161-E165.

[42] Squeri R, La Fauci V, Sindoni L, Cannavò G, Ventura Spagnolo E. Study on hepatitis B and C serologic status among municipal solid waste workers in Messina (Italy). J Prev Med Hyg 2006;47:110-3.

[43] La Fauci V, Sindoni D, Grillo OC, Calimeri S, Lo Giudice D, Squeri R. Hepatitis E virus (HEV) in sewage from treatment plants of Messina University Hospital and of Messina City Council. J Prev Med Hyg 2010;51:28-30.

Received on June 1, 2019. Accepted on October 24, 2019.

Correspondence: V. La Fauci, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, via Consolare Valeria, 98125 Messina, Italy - Fax +39 090 2213351 - E-mail: vlafauci@unime.it

How to cite this article: La Fauci V, Squeri R, Spataro P, Genovese C, Laudani N, Alessi V. Young people, young adults and binge drinking. J Prev Med Hyg 2019;60:E376-E385. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1309

ORIGINAL ARTICLE

Prevalence, trends and risk factors of thinness among Greek children and adolescents

K.D. TAMBALIS[1], D.B. PANAGIOTAKOS[1], G. PSARRA[1], L.S. SIDOSSIS[1,2]
[1] Department of Nutrition and Dietetics, Harokopio University, Athens, Greece; [2] Department of Kinesiology and Health, Rutgers University, New Brunswick, NJ 08901, USA

Keywords

Thinness • Trends • Risk factors • Children • Adolescents

Summary

Introduction. *Thinness affects more children and adolescents than obesity. Thus, the aim of the study is to examine the recent estimates of thinness and associated risk factors, and to identify trends in thinness, among Greek schoolchildren.*
Methods. *Epidemiological study. Population data from the recent estimates are derived from a school-based health survey polled in 2015 on 336,014 participants aged 4- to 17-years-old. To assess trends of thinness (1996-2015) we included a total of 300,104 children aged 8- to 9-years-old. Physical activity, dietary habits and sedentary activities were assessed through self-completed questionnaires. The gender and age-specific body mass index cut-off points proposed by International Obesity Task Force were used in order to define weight groups.*
Results. *Percent 8.4% of girls and 6.5% of boys were thin (all grades included). The prevalence of thinness decreased with age more in boys (from 13.8% at 4-years-old to 5.1% at 17-years-old, $p < 0.001$), than in girls (from 10.9% at 4-years-old to 8.7% at 17-years-old, $p < 0.001$). Sufficient dietary habits (OR: 0.87, 95% CI: 0.77-0.97) and adequate physical activity levels (OR: 0.92, 95% CI: 0.85-0.99) were associated with decreased risk of thinness. Thin schoolchildren performed better in aerobic fitness test than normalweight ones. Between 1996 and 2015, thinness rates decreased from 8.0% to 6.5% in boys ($p = 0.046$) and from 10.6% to 8.4% in girls ($p = 0.036$).*
Conclusions. *Our results suggest that thinness is a significant overlooked phenomenon. Although the prevalence of thinness has decreased the last two decades among Greek schoolchildren, actions need to be taken from public policy makers in order to establish and maintain a healthy body weight.*

Introduction

Thinness among children and adolescents can be described clinically as the low body mass index (BMI)-for-age [1]. In 2016, 192 million children worldwide were moderate and severe thin, while, in the same year, 124 million children worldwide were obese [2]. Thinness has a considerable impact on the health, development and well-being of children and adolescents which can also extend into adulthood [1]. Specifically, thinness is associated with stunting, menstrual irregularity, delayed maturation, nutritional deficiencies, and reduced cognitive capacity [3, 4].In addition, it is connected to frailty in muscular strength and work capacity, and lessens bone density into later life [1]. The problem of thinness concerns countries with different levels of socioeconomic status and is attributed to medical, social, and economic issues [5]. Especially in European countries, thinness is more prevalent among young females, potentially due to their desire to attain a dreamlike beauty of thinness displayed by fashion industry [6, 7]. Moderate or/and severe thinness could be a sign of malnutrition attributed to unhealthy eating behaviors. The factors that make susceptible a child/adolescent to undernutrition or malnutrition are complicated [8]. Thinness could be an indicator of malnutrition even though thin children and adolescents are not inevitably undernourished [9].

There is even less data accessible in the scientific literature on the prevalence of thinness among children and adolescents in Greece [10], while in developed countries trends in children's thinness have been scarcely documented [2, 7, 11, 12]. In addition, due to the lack of up to date data in the literature, research on the issues of factors associated with thinness in schoolchildren deserves further attention. Moreover, we hypothesized that thinness would have a negative impact on physical fitness measurements as compared to normalweight.
The aims of the present study were to: (i) describe the prevalence of graded thinness in Greek children and adolescents aged 4- to 17-years-old, using the three cut-off points proposed by Cole et al. [13]; (ii) investigate whether there is an association between lifestyle factors and thinness; (iii) identify trends in the prevalence of 3 grades of thinness in the last two decades in nationally representative samples of 8- to 9-years-old children; and (iv) explore association between thinness and physical fitness performances.

Methods

Main study (participants)

Data were derived from a nation-wide, school-based health survey under the auspices of the Ministry of Education. Anthropometric, physical activity, sedentary

 https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1374

habits, nutrition, and physical fitness data along with information on age and sex were collected from March 2015 to May 2015. In total, 336,014 (51% boys) children aged 4- to 17-years-old from pre-elementary (4- to 5-years-old), elementary (6- to 11-years-old) and middle (12- to 17-years-old) public and private schools agreed to participate in the study (participation rate was almost 40% of the total population). The working sample was representative of the entire Greek population(chi-square p-value as compared to the current sample with the age-sex distribution of all Greek areas = 0.93).

Assessment of demographic and anthropometric measurements

Demographic information of students (e.g., school, class, gender and date of birth) was obtained from each school headmaster. Children's height and weight were measured in the morning, using a standardized procedure. The exact ages of the participants were calculated from birth and examination dates. Weight was measured in the standing upright position with electronic scales with a precision of 100 g. Standing height was determined to the nearest 0.5 cm with the child's weight being equally distributed on the two feet, head back and buttocks on the vertical land of the height gauge. BMI was calculated as the ratio of body weight to the square of height (kg/m^2). Thinness (3 grades) and normal weight children were classified using the International Obesity Task Force (IOTF) age- and gender-specific BMI cut-off criteria as the most proper for epidemiologic studies [14]. We used the term thinness, instead of the term underweight in children, which the World Health Organization (WHO) uses to mean low BMI for age in children and adolescents. The WHO graded definition of thinness (adult cut-off points of 16, 17 and 18.5 kg/m^2 at age 18 years) corresponds to the three cut offs of thinness in IOTF criteria [1]. Specifically, using the IOTF age- and sex-specific criteria, the three categories of thinness were determined, coding grades I, II and III as cut-offs of 17 < BMI < 18.5 kg/m^2, 16 < BMI < 17 kg/m^2 and BMI < 16 kg/m^2, respectively. In each school, two teachers of physical education (PE) performed all anthropometric measurements. PE teachers followed a specific detailed protocol taught in corresponding seminars held by the Greek General Secretariat of Sports and followed a standardized procedure of measurements in order to minimize the inter-rate variability among schools.

Assessment of physical fitness levels

The Euro-fit physical fitness (PF) test battery was used to evaluate children's PF levels [15]. The battery consists of five tests: (a) a multi-stage 20 m shuttle run test (20 m SRT), to estimate aerobic performance; (b) a maximum 10 × 5 m shuttle run test (10 × 5 m SRT) to evaluate speed and agility; (c) a sit-ups test in 30 seconds (SUs), to measure the endurance of the abdominal and hip-flexor muscles; (d) a standing long jump (SLJ), to evaluate lower body explosive power; and (e) a sit and reach (SR) test to measure flexibility. All five PF tests were administered during the PE class by trained physical education professionals.

Assessment of dietary habits

Participating children's dietary, physical activity and sedentary habits were recorded via the use of an electronic questionnaire. It was completed at school with the presence and assistance of their teachers and/or information technology professors. Students' dietary habits were assessed through the KIDMED (Mediterranean Diet Quality Index for children and adolescents), developed by Serra-Majem et al. [16]. The total KIDMED score ranges from 0 to 12 and is classified into three levels: ≥8, suggesting an optimal adherence to the Mediterranean diet (MD); 4-7, suggesting an average adherence to the MD and an improvement needed to adjust dietary intake to guidelines; and ≤ 3, suggesting a low adherence to the MD and generally a low diet quality.

Assessment of self-reported physical activity and sedentary time

Physical activity (PA) patterns were also self-reported. The questionnaire has been previously used in children in other large-scale epidemiological studies [17] and included simple closed-type questions regarding children's frequency, time and intensity of participation in (i) school-related PA; (ii) organized sports activities; and (iii) PA during leisure time. The frequency of all reported PA was multiplied by the minutes of moderate to vigorous physical activities (MVPA) and then divided by seven to obtain the mean daily time children engaged in MVPA. Children who participated in MVPA at least for 60 minutes per day were considered as meeting the recommendation for PA [18].

Daily time (in hours) spent in sedentary activities (e.g. television viewing, use of Internet for non-study reasons, playing with computer or/and console games) was also calculated for each student. Students were classified as sedentary or not, i.e., exceeding (> 2 hours per day) or not (≤ 2 hours per day) the recommended daily time spent in sedentary activities [19, 20].

Based on the Consensus Statement of the American Academy of Sleep Medicine, we classified as meeting the recommendations of sufficient sleep those children who were sleeping at least nine hours daily and those adolescents who were sleeping at least eight hours per day. Children and adolescents that were sleeping daily fewer than the number of recommended hours were classified as having insufficient sleep [21].

Trends (1996-2015) of thinness

Population data derived from five waves of a nation-wide school-based health survey, carried out by the auspices of the Ministry of Education in 1996, 2001, 2006, 2010 and 2015.Specifically, anthropometric data and information on age, gender, city and area were collected yearly, between March 1 and June 15, in almost all schools of Primary Education (roughly 85%); schools that did not participate were from borderland areas, with small numbers of children. There were not differences in the survey methodology as well as measurement methods across time. Analytical presentation of anthropometric measurements is described above. The response rates of

participation were similar and the characteristics of the responders remained consistent, across time. Thus, from 1996 to 2015, a total of 300,104 children 8- to 9-years-old (51% boys and 49% girls, over 95% of the total student population) participated in the present study.

Ethical approval

Ethical approval for all health surveys was graded by the Ethical Review Board of the Ministry of Education and the Ethical Committee of Harokopio University. As the measurements were included in an obligatory school curriculum, verbal informed consent by the students was considered sufficient.

Data analysis

Normality was verified through the Shapiro-Wilk test. Descriptive statistics were expressed as means ± standard deviations. Prevalence of thinness and normal weight was calculated as the ratio of those children belonging in the corresponding class, based on the proposed cut-off points for BMI by IOTF and divided by the total number of children. Comparisons of the prevalence between genders were performed using the Pearson's chi-square test. Furthermore, simple regression analysis was used to evaluate the trends of each grade of thinness and in total (with lag 0). The independent variable was the year of birth. Serial dependency was evaluated using the partial autocorrelation function; no autocorrelation was observed for various lags tested. Results are presented as b-coefficient ± standard error (SE). In order to assess the potential effect of several lifestyle factors on thinness, binary logistic regression analysis was implemented and odds ratios (OR) with the corresponding 95% confidence intervals (CI) were calculated. The Hosmer and Lemeshow's goodness-of-fit test was calculated in order to evaluate the model's goodness-of-fit and residual analysis was implicated using the dbeta, the leverage, and Cook's distance D statistics in order to identify outliers and influential observations. Z-scores were calculated for each fitness test, by gender. All statistical analyses were performed using the SPSS version 23.0 software for Windows (SPSS Inc., Chicago, Il, USA). Statistical significance level from two-sided hypotheses was set at $p < 0.05$.

Results

Thinness in 2015 in children and adolescents

Prevalence of thinness (three grades and total) by age and gender is incorporated in Table I. More girls than boys aged 4- to 17-years-old were thin (8.4% vs. 6.5%, $p < 0.001$). In the whole population, proportions of thin-

Tab. I. Prevalence of thinness grades according to IOTF definitions, by gender and age, in 4- to 17- years-old Greek children.

	Boys				Girls			
Age†	Grade III (%)	Grade II (%)	Grade I (%)	Total (%)	Grade III (%)	Grade II (%)	Grade I (%)	Total (%)
Children								
4	2.3*	2.4*	9.1*	13.8*	1.1	2.3	9.0	10.9
5	1.1*	2.2*	9.3*	12.6*	1.3	2.4	7.2	10.9
6	0.7	1.8*	7.4*	9.9	0.8	2.0	7.0	9.8
7	0.7*	1.4*	6.3*	8.4*	0.9	1.6	6.8	9.3
8	0.4*	1.0*	5.5*	7.0*	0.6	1.4	6.4	8.4
9	0.4	0.8*	4.9*	6.1*	0.5	1.2	5.8	7.6
10	0.3*	0.7*	4.4*	5.4*	0.5	1.2	6.1	7.8
11	0.2*	0.6*	4.7*	5.5*	0.5	1.3	6.2	8.0
B ± SE per year change	-0.24 ± 0.06	-0.28 ± 0.03	-0.76 ± 0.10	-1.3 ± 0.15	-0.11 ± 0.02	-0.19 ± 0.03	-0.35 ± 0.09	-0.52 ± 0.08
P for trend = 0.008		< 0.001	< 0.001	< 0.001	= 0.003	= 0.001	= 0.008	= 0.014
Adolescents								
12	0.2*	0.7*	4.5*	5.3*	0.5	1.3	6.6	8.4
13	0.2*	0.8*	4.6*	5.6*	0.5	1.3	6.5	8.4
14	0.2*	0.7*	4.1*	5.0*	0.6	0.9	5.2	6.7
15	0.3*	0.6*	3.6*	4.4*	0.3	1.2	5.9	7.4
16	0.2*	0.7*	3.9*	4.8*	0.3	1.1	6.1	7.5
17	0.3*	0.5*	4.3*	5.1*	0.5	1.4	6.8	8.7
B ± SE per year change	0.02 ± 0.01	-0.04 ± 0.02	-0.10 ± 0.09	-0.11 ± 0.09	-0.02 ± 0.03	0.01 ± 0.05	0.01 ± 0.16	-0.01 ± 0.20
P for trend	= 0.188	= 0.104	= 0.302	= 0.293	= 0.414	= 0.910	= 0.931	= 0.979
All								
B ± SE per year change	-0.10 ± 0.03	-0.13 ± 0.2	-0.39 ± 0.06	-0.62 ± 0.11	-0.06 ± 0.01	-0.08 ± 0.02	-0.13 ± 0.05	-0.23 ± 0.06
P for trend	= 0.004	< 0.001	< 0.001	< 0.001	< 0.001	= 0.001	= 0.02	< 0.001

B: beta; SE: standard error; IOTF: International Obesity Task Force; Thinness Grade III (BMI < 16.0); Thinness Grade II (BMI = 16.0-16.9); Thinness Grade I (BMI = 17.0-18.49); †Age: completed age, e.g., 4 years = 4.00-4.99 years; * p-value < 0.05 for differences between boys and girls from the same thinness grade.

ness decreased with age, in both genders (all p-values for trend < 0.05).The findings regarding the trend of thinness by gender during childhood and adolescence are presenting in Table I. In childhood, thinness (three grades and total) rates were decreasing with age, in both genders (all p-values for trend < 0.05). In adolescence, no significant differences were observed in thinness rates.

Health behaviors of children/adolescents on thinness

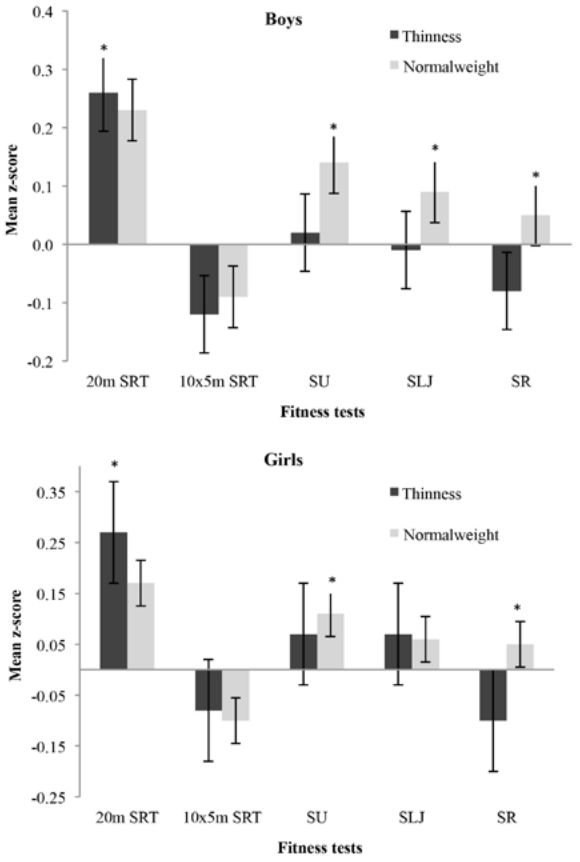
Data were analyzed only for those children who filled their questionnaire (177,091 children) from a whole sample of 232,401 schoolchildren 8- to 17-years-old.In unadjusted binary logistic regression (normalweight vs. thinness), adherence to the Mediterranean diet was associated with lower odds of thinness in both genders (Tab. II, Model 1). After adjusting for potential covariates (e.g. sleeping hours and screen time), the food habits index, previously reported remained significantly associated with thinness, in both genders (Tab. II, Model 2). Additional further adjustment for PA levels holds onto the same results regarding nutrition habits, while it appears that adequate PA level was associated with lower odds of being thin, in both genders. (Tab. II, Model 3). Finally, when PF measurements were added in the analysis (Tab. II, Model 4), the influence of previous factors did not changed significantly, while improved performances in aerobic fitness (20 m SRT) measurements were related to lower probabilities of being thin, in both genders (all p-values < 0.05). In opposite, better performances in SLJ, SR and SUs among boys and SR, SUs among girls were connected to higher odds of being thin (all p-values < 0.05). Specifically, performances of thin boys was significantly poorer than normalweight ones on SR

Tab. II. Associations between health behaviors and odds of normalweight vs. thinness, by gender.

Predictors	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)	Model 4 OR (95% CI)
Boys				
Adherence to the Mediterranean diet (low vs. moderate/high)	0.875 (0.871-0.880)	0.816 (0.741-0.898)	0.823 (0.747-0.906)	0.857 (0.760-0.967)
Sleeping hours (insufficient vs. sufficient)		0.964 (0.963-1.028)	0.963 (0.902-1.067)	0.931 (0.862-1.006)
Screen time (increased vs. acceptable time)		1.018 (0.949-1.092)	1.025 (0.955-1.100)	1.004 (0.922-1.094)
Physical activity levels (inadequate vs. adequate)			0.902 (0.846-0.961)	0.919 (0.850-0.993)
Sit and reach test (per 1cm)				0.924 (0.979-0.989)
20 meters shuttle run test (per 1 stage)				1.006 (1.004-1.008)
10x5 meters shuttle run test (per 1 sec)				0.991 (0.979-1.004)
Sit-ups in 30 seconds (per 1 sit-up)				0.973 (0.966-0.981)
Standing long jump (per 1 cm)				0.999 (0.989-0.999)
Girls				
Adherence to the Mediterranean diet (low vs. moderate/high)	0.911 (0.844-0.978)	0.908 (0.831-0.993)	0.909 (0.831-0.994)	0.890 (0.792-0.999)
Sleeping hours (insufficient vs. sufficient)		0.949 (0.897-1.004)	0.949 (0.896-1.003)	0.945 (0.880-1.014)
Screen time (increased vs. acceptable time)		0.979 (0.916-1.046)	0.979 (0.916-1.047)	0.963 (0.885-1.048)
Physical activity levels (inadequate vs. adequate)			0.912 (0.875-0.960)	0.924 (0.862-0.990)
Sit and reach test (per 1 cm)				0.981 (0.977-0.985)
20 meters shuttle run test (per 1 stage)				1.009 (1.007-1.012)
10x5 meters shuttle run test (per 1 sec)				1.003 (0.992-1.014)
Sit-ups in 30 seconds (per 1 sit up)				0.982 (0.986-0.999)
Standing long jump (per 1 cm)				1.000 (0.999-1.001)

OR: odds ratio; CI: confidence interval;; Model 1: KIDMED index; Model 2: Model 1 + Sleeping status and screen time; Model 3: Model 2 + Physical activity levels; Model 4: Model 3 + Physical fitness measurements

Fig. 1. The differences between the physical fitness tests measurements of normalweight boys and girls aged 6- to 17-years-old as compared to thin ones (IOTF criteria); SR: sit-and-reach; SLJ: standing long jump; SU: sit up; 10 x 5 m SRT: 10 X 5 m shuttle run test; 20 m SRT: 20 m shuttle run test. *p < 0.05 for differences between normalweight and thin.



(p < 0.001), SLJ (p < 0.001) and SUs (p < 0.001), while thin boys outperformed on 20mSRT in comparison to normalweight (p < 0.05) (Fig. 1). Thin girls presented better performances in 20 m SRT(p < 0.001), but poorer in SR and SUs(p < 0.001) than normalweight ones (Fig. 1).There were no significant differences between thin and normalweight ones in 10 × 5 m SRT test, for both genders.

Trends of thinness (1996-2015)

The trends of thinness (1996-2015) by grades and in total are presented in Table III. Rates of thinness as total in boys decreased, from 8.0% in 1996 to 6.5% in 2015 (p = 0.036), while a significant decreasing trend per 0.03 ± 0.01% (p < 0.001) per year was observed only in thinness grade II, the same time period. For girls, a decrease in thinness rates of grade II and in total from 2.2% to 1.4% (p = 0.01) and from 10.6% to 8.4% (p = 0.046), was evident between 1996 and 2015.

Discussion

The main findings of our study, indicate that: (a) thinness rates seems to decrease in the transitioning from childhood to adolescence, (b) compliance with recommendations in Mediterranean diet and adequate physical activity levels decreased the odds of thinness, in both genders, (c) thin schoolchildren performed better in aerobic fitness test than normalweight ones, and (d) the trends of thinness the last two decades presented a significant decrease in both genders.

The current results indicated that the overall prevalence of thinness was higher in girls than in boys. In general, the prevalence of thinness among Greek schoolchildren is similar to countries such as Brazil and Russia, higher to that in the USA, and lesser than in developing areas such as south Asia, southeast Asia, China and Africa, on the basis of the same definitions of thinness (IOTF criteria) [10, 22]. In similar, the mean prevalence of thinness in girls and boys aged 2-18 years from The Netherlands in 2009 were 10.1% and 9.5%, respectively [11]. Also, the presented findings are in line with those of study among ten European countries (thinness rates equal to 7.2% and 5.4%, in boys and girls, respectively) [23] and with those of Spanish and United Kingdom children aged 9- to 10-year-old that refereed percentages 9.0% among boys and 9.5% among girls (IOTF criteria) [12, 24].Potentially, dieting with advanced age, particularly in adolescent girls, might be an explanation for the higher prevalence of thinness among girls.

Thinness rates in the transitioning from childhood to adolescence seem to significantly decreased, in both genders. The finding that thinness in schoolchildren

Tab. III. Trends (1996-2015) of thinness (3 Grades) among boys and girls 8- to 9-years-old.

	1996 (n = 61995)	2001 (n = 65332)	2006 (n = 71227)	2011 (n = 60251)	2015 (n = 41299)	B±SE per year change	P for trend
Boys							
Grade III (%)	0.7	0.8	0.7	0.5	0.4	-0.01 ± 0.01	0.379
Grade II (%)	1.5	1.4	1.3	1.1	0.9	-0.03 ± 0.01	0.010
Grade I (%)	5.7	5.7	5.9	5.5	5.2	-0.02 ± 0.02	0.249
Total (%)	8.0	7.9	7.9	7.1	6.5	-0.08 ± 0.02	0.036
Girls							
Grade III (%)	1.0	1.5	1.1	0.9	0.6	-0.03 ± 0.02	0.181
Grade II (%)	2.2	2.3	1.9	1.7	1.4	-0.05 ± 0.01	0.010
Grade I (%)	7.4	6.8	6.7	6.5	6.4	-0.05 ± 0.01	0.032
Total (%)	10.6	10.6	10.7	9.1	8.4	-0.13 ± 0.04	0.046

is declining across age has been reported and elsewhere [12, 25, 26]. Furthermore, results in schoolchildren from 10 European countries and the USA refereed increased prevalence of thinness in younger schoolchildren compared to older ones [7].

To our knowledge, this study is the first to examine the association of thinness with several modifiable lifestyle factors simultaneously. Adequate dietary habits and PA levels related to decreased risk of being thin among Greek children and adolescents. Thin children are more prone to poor dietary habits (e.g. dislike of fish, vegetables, legumes etc.) compared to normalweight counterparts [27]. An unbalanced diet could be a reason of malnutrition, which is related to holdup in somatic growth and development among children [28]. Thin children may consume fewer dietary nutrients such as minerals and vitamins that are required for optimal health [29]. Moreover, increase in PA levels with the adequate energy and protein intake can promote children's healthy body composition [30].

The relationships between thinness and physical fitness are less well investigated. Our results indicated that thin children performed poorer than normalweight ones on SU, SLJ and SR tests, but better on 20 m SRT. Previous study of our laboratory in 8- to 9-years-old children revealed that underweight children (IOTF criteria) had better performances on 20 m SRT as compared to normalweight ones [31]. In accordance, study among 10,285 children shown that underweight children performed better on 20 m SRT than normalweight ones ($p = 0.017$) but poorer on SU and SLJ tests [32]. Also, thin children aged 6- to 18-years-old from Mozambique performed worse in absolute strength tests, better in endurance tests, and equally in agility test, as compared to normalweight group [33]. In contrast, Malina et al., did not found significant differences between stunted (z-score below -2.00) and normalweight children aged 6- to 13-years-old in the distance covered in 8 to 12 min runs [34]. Potentially, the lower weight of thin children could be an advantageous parameter for aerobic fitness performance, especially for weight-bearing physical activities like running.

In our study thinness rates were decreasing steadily between 1996 and 2015. Globally, the prevalence of moderate and severe thinness decreased from 9.2% to 8.4% between 1975 and 2016 in girls and from 14.8% to 12.4% in boys, the same time period [2]. In line, data regarding the trends of thinness during childhood has been shown a decline in the USA, Brazil, China and The Netherlands [11, 22]. Furthermore, study included adolescents from ten European countries and the USA, indicated that the prevalence of thinness has declined in almost all countries from 1998 to 2006 [7].

Our study has several strengths. It was conducted in a wide age-range group and examined several anthropometric and lifestyle factors. Furthermore, primary and secondary education is compulsory in Greece and, therefore, we were able to study a great proportion of 4- to 17-years-old children and adolescents.

Limitations of the present study include methodological issues and the fact that potential confounding factors (e.g. genetic factors, socioeconomic status, sexual maturation, etc.) have not been evaluated. In addition, information on the health status of the schoolchildren (e.g. infectious disease) has not been assessed. Dietary habits, physical activity and sedentary time status are based on self-reported data that could be subject to socially desirable reporting bias.

Conclusions

Despite the previously mentioned limitations, our results revealed that almost 7.5% of schoolchildren population was thin, while thinness rates in both genders were transitioning at more favorable levels from childhood to adolescence. Poor dietary habits and inadequate PA levels were associated with increased risk of thinness. Thin schoolchildren presented better performances in aerobic fitness test than normalweight ones. The trends of thinness the last two decades showed a significant decrease. This study suggests that health actions should be adopted for the treatment of thinness, with emphasis in primary caregivers of children who can support and establish behaviors to achieve and maintain a healthy weight for the child.

Supplementary Table (Tab. SI).

Acknowledgements

Funding sources: this study was supported by the Hellenic Ministry of Education and Religious Affairs, Secretariat General of Sports, OPAP S.A., Nestlé Hellas S.A., and the Department of Nutrition and Dietetics Graduate Program, Harokopio University of Athens.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

KDT designed the study, performed the data collection and analysis and wrote the paper. DBP and GP participated in the design of the study and critically reviewed the paper. LSS was involved in the study design, manuscript writing and in overall supervision of the study.

References

[1] World Health Organization. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser1995;854:1-452.

[2] NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents,

and adults. Lancet 2017;390:2627-42. https://doi.org/10.1016/S0140-6736(17)32129-3

[3] Misra M, Aggarwal A, Miller KK, Almazan C, Worley M, Soyka LA,Herzog DB, Klibanski A. Effects of anorexia nervosa on clinical, hematologic, biochemical, and bone density parameters in community-dwelling adolescent girls. Pediatrics 2004;114:1574-83. https://doi.org/10.1542/peds.2004-0540

[4] Crimshaw NS, San Giovanni JP. Synergism of nutrition, infection, and immunity: an overview. Am J Clin Nutr 1997;66:464S–77S. https://doi.org/10.1093/ajcn/66.2.464S

[5] Akseer N, Al-Gashm S, Mehta S, Mokdad A, Bhutta ZA. Global and regional trends in the nutritional status of young people: a critical and neglected age group. Ann N Y Acad Sci 2017;1393:3-20. https://doi.org/10.1111/nyas.13336

[6] Lawrie Z, Sullivan EA, Davies PS, Hill RJ. Media influence on the body image of children and adolescents. Eat Disord 2006;14:355-64. https://doi.org/10.1080/10640260600952506

[7] Lazzeri G, Rossi S, Kelly C, Vereecken C, Ahluwalia N, Giacchi MV. Trends in thinness prevalence among adolescents in ten European countries and the USA (1998-2006): a cross-sectional survey. Public Health Nutr 2014;17:2207-15. https://doi.org/10.1017/S1368980013002541

[8] Chisuwa N, O'Dea JA. Body image and eating disorders amongst Japanese adolescents. A review of the literature. Appetite 2010;54:5-15. https://doi.org/10.1016/j.appet.2009.11.008

[9] Uzogara S. Underweight, the less discussed type of unhealthy weight and its implications: a review. American Journal of Food Science and Nutrition Research 2016;3:126-42.

[10] Tambalis KD, Panagiotakos DB, Kavouras SA, Kallistratos AA, Moraiti IP, Douvis SJ, Toutouzas PK, Sidossis LS. Eleven-year prevalence trends of obesity in Greek children: first evidence that prevalence of obesity is leveling off. Obesity (Silver Spring) 2010;18:161-66. https://doi.org/10.1038/oby.2009.188

[11] Schönbeck Y, van Dommelen P, HiraSing RA, van Buuren S. Thinness in the era of obesity: trends in children and adolescents in The Netherlands since 1980. Eur J Public Health 2015;25:268-73. https://doi.org/10.1093/eurpub/cku130

[12] Martínez-Vizcaíno V, Sánchez López M, Moya Martínez P, Solera Martinez M, Notario Pacheco B, Salcedo Aguilar F, Rodríguez-Artalejo F. Trends in excess weight and thinness among Spanish schoolchildren in the period 1992-2004: the Cuenca study. Public Health Nutr 2009;12:1015-18. https://doi.org/10.1017/S1368980008003571

[13] Cole T, Flegal K, Nicholls D, Jackson A. Body mass index cut offs to define thinness in children and adolescents: international survey. BMJ 2007;335:194-98. https://doi.org/10.1136/bmj.39238.399444.55

[14] World Health Organization (WHO). Physical Status: the use and interpretation of anthropometry: Tech. Rep. Series 854, 1995. Geneva: WHO. Available at:http://apps.who.int/iris/bitstream/handle/10665/37003/WHO_TRS_854.pdf;jsessionid=8C99B71EB29E26FD65B5DA357C8798C0?sequence=1. Accessed on 12/03/2019.

[15] Council of Europe Committee of Experts on Sports Research. Eurofit: handbook for the Eurofit tests of Physical fitness. Athens: Salto1993, pp. 1-75.

[16] Serra-Majem L, Ribas L, Ngo J, Ortega RM, García A, Pérez-Rodrigo C, Aranceta J. Food, youth and the Mediterranean diet in Spain. Development of KIDMED, Mediterranean Diet Quality Index in children and adolescents. Public Health Nutrition 2004;7:931-5.

[17] Grigorakis DA, Georgoulis M, Psarra G, Tambalis KD, Panagiotakos DB, Sidossis LS. Prevalence and lifestyle determinants of central obesity in children. Eur J Nutr 2016;55:1923-31. https://doi.org/10.1007/s00394-015-1008-9

[18] World Health Organization (WHO): Global Recommendations on Physical Activity for Health. Geneva, World Health Organization, 2010. Available at: http://whqlibdoc.who.int/publications/2010/9789241599979_eng.pdf. Accessed on12/03/2019.

[19] Colley RC, Janssen I, Tremblay MS: Daily step target to measure adherence to physical activity guidelines in children. Med Sci Sports Exerc 2012;44:977-82. https://doi.org/10.1249/MSS.0b013e31823f23b1

[20] Tremblay MS, Leblanc AG, Janssen I, Kho ME, Hicks A, Murumets K, Colley RC, Duggan M. Canadian sedentary behaviour guidelines for children and youth. Appl Physiol Nutr Metab 2011;36:59-64. https://doi.org/10.1139/H11-012

[21] Paruthi S, Brooks LJ, D'Ambrosio C, Hall WA, Kotagal S, Lloyd RM,Malow BA, Maski K, Nichols C, Quan SF, Rosen CL, Troester MM, Wise MS. Consensus Statement of the American Academy of Sleep Medicine on the recommended amount of sleep for healthy children: methodology and discussion. J Clin Sleep Med 2016;12:1549-61. https://doi.org/10.5664/jcsm.6288

[22] Wang Y, Monteiro C, Popkin BM. Trends of obesity and underweight in older children and adolescents in theUnited States, Brazil, China, and Russia. Am J Clin Nutr 2002;75:971-7. https://doi.org/10.1093/ajcn/75.6.971

[23] Yngve A, De Bourdeaudhuij I, Wolf A, Grjibovski A, Brug J, Due P, Ehrenblad B, Elmadfa I, Franchini B, Klepp KI, Poortvliet E, Rasmussen M, Thorsdottir I, Perez Rodrigo C. Differences in prevalence of overweight and stunting in 11-year olds across Europe: the Pro Children Study. Eur J Public Health 2008;18:126-30. https://doi.org/10.1093/eurpub/ckm099

[24] Boddy LM, Hackett AF, Stratton G. The prevalence of underweight in 9- and 10-year-old schoolchildren in Liverpool: 1998-2006. Public Health Nutr 2009;12:953-56. https://doi.org/10.1017/S136898000800311X

[25] Bovet P, Kizirian N, Madeleine G, Blössner M, Chiolero A. Prevalence of thinness in children and adolescents in the Seychelles: comparison of two international growth references. Nutr J 2011;10:65. https://doi.org/10.1017/S136898000800311X

[26] Lazzeri G, Rossi S, Pammolli A, Pilato V, Pozzi T, Giacchi MV. Underweight and overweight among children and adolescents in Tuscany (Italy). Prevalence and short-term trends. J Prev Med Hyg 2008;49:13-21.

[27] Lee G, Ham OK. Factors Affecting underweight and obesity among elementary school children in South Korea. Asian Nurs Res (Korean SocNursSci) 2015;9:298-304. https://doi.org/10.1016/j.anr.2015.07.004

[28] Borowitz KC, Borowitz SM. Feeding problems in infants and children: assessment and etiology. Pediatr Clin North Am 2018;65:59-72. https://doi.org/10.1016/j.pcl.2017.08.021

[29] Caballero B. A nutrition paradox-underweight and obesity in developing countries. N Engl J Med 2005;352:1514-16. https://doi.org/10.1056/NEJMp048310

[30] Doak CM, Adair LS, Bentley M, Monteiro C, Popkin BM. The dual burden household and the nutrition transition paradox. Int J Obes (Lond) 2005;29:129-36. https://doi.org/10.1038/sj.ijo.0802824

[31] Tambalis KD, Panagiotakos DB, Psarra G, Sidossis LS. Inverse, but independent trends in obesity and fitness levels among Greek children: a time-series analysis from 1997 to 2007. Obes Facts 2011;4:165-74. https://doi.org/10.1159/000327994

[32] Armstrong MEG, Lambert MI, Lambert EV. Relationships between different nutritional anthropometric statuses and health-related fitness of South African primary school children. Ann Hum Biol 2017;44:208-13. https://doi.org/10.1080/03014460.2016.1224386

[33] Prista A, Maia JA, Damasceno A, Beunen G. Anthropometric indicators of nutritional status: implications for fitness, activity, and health in school-age children and adolescents from Maputo, Mozambique. Am J Clin Nutr 2003;77:952-59. https://doi.org/10.1093/ajcn/77.4.952

[34] Malina RM, Reyes MP, Tan SK, Little BB. Physical fitness of normal, stunted and overweight children 6-13 years in Oaxaca, Mexico. Eur J Clin Nutr 2011;65:826-34. https://doi.org/10.1038/ejcn.2011.44

Tab. SI. Anthropometric indices (means ± SE) of population by gender and age.

	Boys				Girls			
Age†	N	Height (cm)	Weight (kg)	BMI (kg/m²)	N	Height (cm)	Weight (kg)	BMI (kg/m²)
4	3157	107.9 (5.0)*	18.7 (2.8)*	16.0 (1.9)	3091	106.6 (5.1)	18.3 (2.8)	16.0 (1.9)
5	4550	113.1 (5.4)*	20.8 (3.8)*	16.3 (2.1)*	4477	111.8 (5.3)	20.2 (3.7)	16.1 (2.2)
6	11361	120.0 (5.6)*	24.0 (3.7)*	16.4 (2.4)*	11139	118.8 (5.5)	23.3 (4.6)	16.4 (2.4)
7	21034	125.1 (5.8)*	26.6 (5.5)*	16.9 (2.6)*	21165	124.1 (5.8)	26.0 (5.3)	16.8 (2.6)
8	21159	131.4 (6.0)*	30.4 (6.7)*	17.6 (3.0)*	20140	130.0 (6.1)	29.7 (6.5)	17.4 (3.0)
9	21387	136.9 (6.4)*	34.5 (8.0)*	18.3 (3.3)*	20524	135.6 (6.5)	33.7 (7.7)	18.1 (3.2)
10	21162	142.1 (6.8)*	38.5 (9.0)*	18.9 (3.5)*	20424	141.8 (7.7)	38.0 (8.9)	18.7 (3.4)
11	19875	147.3 (7.1)*	43.2 (10.2)*	19.6 (3.6)*	18910	148.4 (7.6)	43.0 (9.9)	19.3 (3.6)
12	16349	153.7 (7.9)*	47.5 (11.3)*	20.1 (3.8)*	15465	154.8 (7.4)	47.9 (10.6)	19.9 (3.6)
13	8515	160.5 (8.7)*	54.0 (12.9)*	20.7 (3.9)*	7819	159.2 (6.7)	52.1 (10.8)	20.5 (3.7)
14	7635	167.4 (8.5)*	60.5 (13.6)*	21.4 (3.9)*	6734	162.0 (6.2)	55.6 (10.5)	21.2 (3.6)
15	6273	172.5 (7.5)*	65.2 (13.1)*	21.8 (3.8)*	5425	163.2 (6.1)	57.5 (10.2)	21.6 (3.5)
16	3695	175.5 (7.0)*	69.3 (13.0)*	22.5 (3.8)*	3413	164.2 (6.3)	58.9 (10.4)	21.8 (3.5)
17	2358	177.0 (7.1)*	72.0 (13.2)*	22.9 (3.7)*	2228	164.7 (6.3)	60.1 (10.8)	22.1 (3.6)
P for trend		< 0.001	< 0.001	< 0.001		< 0.001	< 0.001	< 0.001

† Age: completed age, e.g., 4 years = 4.00-4.99 years; * p-value < 0.01 between boys and girls; BMI: Body Mass Index.

Received on August 17, 2019. Accepted on August 19, 2019.

Correspondence: Labros S. Sidossis, Department of Kinesiology and Health, 70 Lipman Drive, New Brunswick, NJ 08901-8525, USA - E-mail: lsidossis@kines.rutgers.edu

How to cite this article: Tambalis KD, Panagiotakos DB, Psarra G, Sidossis LS. Prevalence, trends and risk factors of thinness among Greek children and adolescents. J Prev Med Hyg 2019;60:E386-E393. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1374

ORIGINAL ARTICLE

Regione Lombardia: a tool for improving quality in hospitals

M. NOBILE[1], E. LUCONI[2], R. SFOGLIARINI[3], M. BERSANI[4], E. BRIVIO[4], S. CASTALDI[1, 2]
[1] Dept. Biomedical Science for Health, University of Milan, Italy; [2] Fondazione IRCCS Ca' Granda Ospedale Maggiore di Milano Policlinico, Italy; [3]ASST Crema, Italy; [4] Direzione Generale Wellfare Regione Lombardia, Italy

Keywords

Quality improvement • Health structures • Quality tool

Summary

Introduction. *The regional healthcare system of the Lombardy Region pay great attention to monitoring the effectiveness and quality level with which its services.The aim of this paper is to describe the method adopted by the Lombardy Region to create a governance tool for the healthcare system that would be applied within hospitals to create value at financial-economic level, to achieve continuous quality improvement and to increase patient/customer satisfaction levels. It was called: Piano Integrato del Miglioramento dell'Organizzazione (PIMO), i.e. Integrated Plan for Hospital Improvement.*
Metods. *The approach for the definition of the PIMO was based on: the Plan Do Check Act methodology; the management requirements introduced by the UNI EN ISO 9001:2008 and UNI EN ISO 9004:2005 standards; the regulations and indications made for the Public Administration; the Guidelines for planning and monitoring improvement proposed by the CAF (Common Assessment Framework).*
Results. *The evaluation of the scores for all the health structures shows a good level of quality and qualifies PIMO as a strategic tool for hospitals.*
Conclusions. *It will be necessary to allow this tool to operate for some time in order to make an overall assessment of the results achieved.*

Introduction

A solution to provide quality health care in an efficient way that has proved effective, especially at higher decision-making levels, is performance management [1].
The cardinal element of performance management is the quality improvement in order to promote the best possible quality level [2].
One of the most widespread strategies for the pursuit of a quality improvement is the creation of accreditation and performance monitoring systems. Examples of performance monitoring systems in hospitals are the Joint Commission on Accreditation of Healthcare Organization (JCAHO) [3] and the Australian Council of Healthcare Standards (ACHS) [4].
Since 1997, with the recommendation n. R 97.17 "On the development and activation of systems for the improvement of the quality of healthcare", adopted on 30 September 1997, Europe recommends a higher quality of the health system for all Member States and the presence of an improvement system that is known and understood by all [5].
Despite this, in Europe hospital performance monitoring seems a relatively new sector in the field of healthcare sciences and hospital management [6].
In 2005, a project named PATH (Performance Assessment Tool for quality improvement in Hospitals) was promoted by the WHO Regional Office for Europe. Its goal was to develop a framework for performance monitoring and quality improvement in hospitals by creating and developing a set of monitoring indicators [7, 8].
This project involved several States and it aimed to improve the quality of their services, in their public healthcare departments. A network system was developed to facilitate exchange of information, to identify best practices and to pinpoint key elements in the monitoring/accreditation programmes [9].
With the entry into force of Directive 2011/24/EU concerning cross-border healthcare, it has become essential to monitor the quality of individual structures so that the patient has guarantees concerning the quality of the facilities present in the different Member States [10].
Since 1978, Italy has organized a public national healthcare service based on availability to all (universal). Various subsequent laws have introduced reforms that have gradually established the concept of company-style management [11, 12]. Starting from the end of the last century a strong trend towards decentralization has led to an increasingly regional basis for the provision of healthcare services; the result has been that healthcare services have been organized and managed in very different ways from one region to another, but the principle of a national health service was not denied.
The regional healthcare system of the Lombardy Region, in Northern Italy, was approved in 1997. From its inception, it pays great attention to monitoring the effectiveness and quality level with which the services were provided [13].
The Lombardy Region aimed to adopt a governance tool for the healthcare system that would be applied within

https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1298

hospitals to create value at the financial-economic level, to achieve quality improvement in the internal processes. The aim of this paper is to describe the results of the self-assessment checklists filled twice a year by all the healthcare structures of the Lombardy region in the years 2016 and 2017 to monitor their quality improvement.

Methods

This tool was created by the clinical and research healthcare facilities themselves through the organization of work groups and applying a bottom up logic within a system that had acquired, over the years, solid experience in relation to Quality Improvement.
The structures themselves named the project *Piano Integrato del Miglioramento Ospedaliero* (PIMO), *i.e.* Integrated Plan for Hospital Improvement.
PIMO project is directed to all healthcare facilities, public and private, accredited and contracted within the Regional Health Service, in particular the main objective of PIMO is to highlight the priorities for improving the quality of each organizations fixing medium and long-term strategic objectives [14].
The self-assessment check list of the PIMO of the single health structure is collected in a regional database (after the approval by the health structure's Strategic Direction), so this is an important monitoring tool also for the Region (as well as for the single structure and for territorial level).
The self-assessment checklist has 17 areas with 85 standards and 370 items (Tab. I).
For each item there are 6 possible scores:
- 1 (systematic application);
- 0.75 (applied everywhere);
- 0.5 (applied in part);
- 0.25 (applied in experimental or initial phase);
- 0 (not applied);
- NA (Not Applicable).

For documental items there are four scores:
- 1 (document prepared according to the content of the item);
- 0.5 (document partially respects the expected contents);
- 0 (no document);
- NA (Not Applied)..

The Regional Health Authority of Lombardy provided data of 4 self-assessment checklists (first semester of 2016, second semester of 2016, first semester of 2017 and second semester of 2017) for all the healthcare structures who took part in the project in the considered period (ASST (*Aziende Socio Sanitarie Territoriali*, i.e. hospital and community trusts), IRCCS (*Istituto di Ricovero e Cura a Carattere Scientifico*, i.e. Scientific Institute for Research and Healthcare), and private structures).
We had not the possibility to access to the dataset of each structure collected by Lombardy Region. We could only use information provided by the report of Lombardy region. The reports available contained only graphical representation of data and, in particular, we decided to use histograms with the following information:
- the average value of self-assessment for each type of structures (IRCCS, ASST and private structures) in the two semesters of 2016 and 2017;
- the percentages of zero responses for each area for the first and the second semesters of 2016 and 2017. In this case it was not possible to distinguish between structures because in the histogram there was not a distinction between different types of structures, but the data was aggregated for information of interest;
- the average value of self-assessment checklists for each type of structures (IRCCS, ASST e private structures) for each area in the two semesters of 2016 and 2017.

Data were synthesized by the average value for self-assessment checklists of each type of healthcare structures, the zero percentage responses for each area and, for each semester and the number of areas in which the type of structures obtained a higher value respect to the other structures for the same area. Moreover, we made differences about the second and the first semester of the same year and the first and the second semester of different years about the zero percentage responses for each area. We also made difference from the second and the first semester of the same year for the average value of self-assessment for each type of structures.
To obtain numerical values needed for the descriptive statistical above cited, for each histogram, the average values of self-assessment for each type of structures, the percentages of responses with a value of zero per area of interests and the average values of self-assessment for each institution was obtained through the program "PDF Xchange Viewer" (Tracker Software) [15].
In particular the program measurement tool allowed to obtain the height of the bars of the histogram, from which the value of the average or of the percentage was obtained thanks to a proportion with the measurement of a tick marks.
In this study, even considering the available data, only descriptive statistic was made, as the population was considered as the set of health structures that were interested by the PIMO in the considered period.
The analyses with numerical data (obtained from histograms as described above) were carried out using R (ver. 3.5.1, R: A Language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria) and KNIME (KNIME Analytics Platform, ver. 3.5.3, KNIME AG, Zurich, Switzerland).

Results

In Lombardy Region there are 27 ASST and 4 IRCCS and 86 private hospitals. For all type of healthcare structures, it was possible to evaluate the results of the 4 self-assessment checklists for the years 2016 e 2017.
Table II presents the average value of ASST, IRCCS and private structures for each semester of 2016 and 2017; the percentage of zero responses for each area for each

Tab. I. The 17 areas of the self-assessment checklist

Acronym	Area	Standards
AAC	Anaestheesiological and surgical assistance	Sedation; anesthesia; surgical planning; surgery; post-operative care
AAS	Acquisition of equipment and supervision of contracts	Appropriate use of equipment, devices and medications recommended by professional associations or, alternatively, by other authoritative sources; contracts for services entrusted to external persons
ACA	Access to care and assistance services	Screening and reception; Patient acceptance and hospitalization process and management of ambulatory patients; evaluation of patients with urgent needs; linguistic, cultural and structural barriers; Access criteria and transfer to intensive care units
CCC	Coordination and continuity of care	Coordination of care for clinical-assistance continuity; sharing of clinical and assistance information
DCR	Clinical and rehabilitative documentation	Patient's medical record; contents of the patient's medical record; health documentation checks; symbols codes and definitions
DIM	Discharge area	Appropriate discharge of the patient; territorial network; discharge letter; follow-up instructions
EPF	Education of patients and family	Assessment of each patient's educational needs and registration; essential areas of the educational process
IDP	Information and rights of the patient	Protected categories; patient information and informed consent; privacy and confidentiality
OBI	International goals for patient safety	Identification of the patient; telephone and verbal communications; management of high-risk drugs; safe surgery; prevention of infections related to care practices; prevention and management of damage resulting from falls
PDC	Care process	Planning of care and assistance; planning rehabilitation treatment; care for high risk patients; high risk processes; pain management
PGF	Drug management process	Prescription and transcription of drugs: policies and procedures; requirements and criteria for acceptability of drug therapy prescriptions; the organisation identifies qualified professionals who are authorized to prescribe or order drugs; Registration of prescription and administration of drugs; drug preparation management; authorization to administer drugs; management of drug administration; regulation of self-administration of drugs and samples of medicinal specialities; monitoring and measurement of the effects of drugs on the patient; LASA drugs (look-alike drugs)
PGM	Management process for improving the organization	Development and dissemination of documentation; plan for the improvement of the organization and its realization; communication and feed back to the staff about information on improvement; monitoring and control activities and data analysis; guidelines for clinical practice and clinical pathways to guide clinical care; key indicators to monitor the structures, the processes,and the clinical and managerial, processes and outcomes; management of sentinel event; reporting and management of near misses and adverse events; analysis of trends and unwanted variations; planning of information requirements
PVP	Patient Assessment Process	Initial evaluation of the patient; timeliness of the initial evaluation process; personalized evaluations; presurgical evaluations; resignation planning; patient revaluation
QDP	Qualification of the staff	Plan of the organic amenities; the responsibilities of each member of staff are defined in an updated document (job description); insertion of the newly-hired or newly-assigned person and his evaluation; evaluation of managerial staff; evaluation of the operators belonging to the health professions and the technical administrative area; personal file; credentials: degree of study and qualifications;training in the techniques of emergency cardiopulmonary resuscitation; training, updating and development of skills
SDI	Diagnostic services through images	Pre-diagnostic phase; diagnostic phase and refertation
SML	Laboratory medicine services	Pre-analytical phase; quality controls; analytical phase; post-analytical phase
TDP	Patient transfer	Patient transfer; suitability of the receiving structure; transfer letter; monitoring during the transfer; documentation of the transfer process; transport service of patients

semester of the considered periods is presented in Table III.

The biggest difference between the second and the first semesters of the 2016 was for the area PVP (patient assessment process), with -1.039% of answers with value zero, so in the second semester of 2016 the quality of this area increased respect to the first semester of 2016 (Tab. III). The other areas that in the second half of 2016 increased their quality were: DIM (discharge area), CCC (coordination and continuity of care), OBI (international goals for patient safety), AAS (acquisition of equipment and supervision of contracts), IDP (information and rights of the patient) and AAC (anaestheesiological and surgical assistance) (Tab. III). For PDC (care process) the percentage of responses with zero value was the same between the first and the second semester of 2016. In the other areas the percentage of zero-responses score increased between the first and the second semester of 2016 (Tab. III).

Tab. II. The average value for self-assessment checklists of each type of healthcare facility.

	I sem. 2016	II sem. 2016	I sem. 2017	II sem. 2017
ASST	0.891	0.878	0.858	0.855
IRCCS	0.91	0.881	0.883	0.884
Private structures	0.887	0.880	0.879	0.882

The average value for self-assessment checklists for each type of healthcare facility (ASST (hospital and community trusts), IRCCS (Scientific Institute for Research and Healthcare) and private structures) for each semester (I sem.2016 (first semester of 2016), II sem.2016 (second semester of 2016), I sem.2017 (first semester of 2017), II sem.2017 (second semester of 2017)).

Tab. III. The zero percentage responses for each area.

Area	I sem. 2016 (%)	II sem. 2016 (%)	I sem. 2017 (%)	II sem. 2017 (%)
AAC	0.615	0.5	0.769	0.731
AAS	1.231	0.962	1.308	1.308
ACA	1.154	1.5	2.154	2.538
CCC	1.615	1.192	2.846	3.5
DCR	0.423	0.538	1.077	0.962
DIM	4.423	3.808	4.538	4.654
EPF	1.423	1.577	2.615	2.577
IDP	1.769	1.615	2.423	2.423
OBI	1.385	1.115	1.615	1.577
PDC	0.692	0.692	1.154	1.077
PGF	1.038	1.115	1.346	1.038
PGM	2.115	2.423	2.962	3.346
PVP	2.962	1.923	2.769	2.577
QDP	3.5	4.154	4.654	5.423
SDI	2.615	2.961	5.192	5.538
SML	0.462	0.538	1	1.192
TDP	3.423	3.577	5.577	6.192

The zero percentage responses for each area for each semester. For the explanation of the acronyms of the areas see Table I. I sem. 2016 (first semester of 2016), II sem. 2016 (second semester of 2016), I sem. 2017 (first semester of 2017), II sem. 2017 (second semester of 2017).

Between the second and the first semester of 2017 the biggest difference was for the area PGF (drug management process) (-0.308%), so in the second semester of 2017 the quality increased for this area, respect to the first semester of 2017. The other areas that in the second half of 2017 increased their quality were: PVP (patient assessment process), DCR (clinical and rehabilitative documentation), PDC, EPF (education of patients and family), AAC and OBI.

For AAS and IDP the percentage of responses with zero value was the same between the first and the second semester of 2017. In the other areas the quality decreased between the first and the second semester of 2017.

The biggest difference between the first semester of 2017 and the first semester of 2016 was for PVP (patient assessment process) area (-0.193%), so in the first semester of 2017 the quality was better. For all other areas there was a decrease in quality in the first semester of 2017 compared to the first semester of 2016.

The only area where there was an improvement in the quality from the second semester of 2017 compared to the second semester of 2016 was the PGF. For the other areas there were a decrease in the level of hospital quality.

In the first semester of 2016, for 9 areas out of 17 the highest score (compared to the scores of the other type of structures in the same period for the same area) was for IRCCS structures, for 6 areas it had been registered for private structures and for 2 areas was detected for ASST.

In the second semester of 2016, for 8 areas out of 17 the highest recorded score (compared to the scores of the other type of structures in the same period for the same area) was for private structures, for 4 areas was detected for IRCCS, for 3 areas it has been registered for ASST; for 1 area the highest average score was found to be the same between IRCCS and ASST and for 1 area out of 17 the maximum recorded score was found to be the same between private structures and IRCCS.

In the first semester of 2017, for 8 areas out of 2017 the highest recorded score (compared to the scores of the other type of structures in the same period for the same area) was for IRCCS, for 7 areas it has been registered for private structures, for 1 area was detected for ASST; for 1 area the highest average score was found to be the same between private structures and IRCCS.

In the second semester of 2017, for 9 areas out of 2017 highest recorded score (compared to the scores of the other type of structures in the same period for the same area) was for private structures, for 7 areas was detected for IRCCS, for 1 area it has been registered for ASST.

In the second half of 2016 there was the decrease of all the scores for all the structures compared to the first half of the same year, and the biggest difference was for IRCCS structures. In 2017 it was the same for the ASST, but the average of the scores of IRCCS structures and the average of the private structures

increased from the first to the second semester of the considered year; the increase was greater for the private structures (Tab. II).

Discussion

The checklist as a self-assessment tool makes possible to identify most of the areas which need a plan of quality improvement that enable the standards to be achieved. It is worth emphasizing that the checklist is not merely a set of standards to be monitored: it is a planning tool. It should be conceived and implemented to bridge the gap between medium/long-term strategic decisions and the implementation tools which are, as of now, principally aimed at the short-term. Its planning function, unlike other tools, it could overcome certain limits/risks linked to the introduction of management performance systems, such as tunnel vision and compliance mood, since it steers the unit's attention towards finding answers to its needs [16]. It encourages healthcare facilities to identify long-term goals as well as short-term ones, so it could overcome the risk of short-sightedness. The scores differences looking at the same year or at the trend in the two years might be explained with the meetings of the working group and with their attempts to make comparable their scores. Also some peculiarities of the national and regional health services might explain some differences and trends, for example IRCCS must be certified to obtain Ministry of Health fundings and private structures try to be appeal for patients and increase the level of their services.

In any case, the goal of this approach of the Lombardy Region has been achieved as all the health structures involved have shown that they take into great consideration the level of quality of their services. It is worth to underlines some limitations of this study:

- the tool we describes has only two years of life and for this reason we propone our results as preliminary ones;
- the tool we presented need for its application of a health service with a quality background it is not feasible to be used as the starting method to implement quality improvement.

It si quite strange in the international scenario that the quality improvement starts from a single health regional service but it should be read looking at the organization of the Italian National Health Service which in 1992 and 1993 allowed the single Regional Authorities to organize their health service complying with the National one [17, 18].

The are many types of quality improvement tools because they must manage the specific health service organization as happened in the Lombardia Regional Health Authority also in many other Countries the quality improvement used different strategies the most succeful are the ones able to involve all the staff and the leadership [3-5].

Conclusions

In response to the increased attention towards quality, many Countries have developed improvement programmes based on both an external auditing approach, such as accreditation systems, and an internal assessment approach, such as self-assessments and plans merging both strategies, such as indicator-based systems.

Starting from the standards proposed by the accreditation systems, performance monitoring programmes based on specific indicators have often been created.

The process presented is the result of experience gained by the Lombardy region from 2010 onwards in the performance monitoring all the healthcare facilities both public and private.

This tool was created by the clinical and research healthcare facilities themselves through the organization of work groups and applying a bottom up logic within a system that had acquired, over the years, solid experience in relation to Quality Improvement.

The checklist as a self-assessment tool makes possible to identify most of the areas which need a plan of quality improvement that enable the standards to be achieved. The internal monitoring system enables control of all areas and processes and identification of areas for improvement.

It is worth emphasizing that the checklist is not merely a set of standards to be monitored, it is a planning tool. It should be conceived and implemented to bridge the gap between medium/long-term strategic decisions and the implementation tools which are, as of now, principally aimed at the short-term. Its planning function, unlike other tools, it could overcome certain limits/risks linked to the introduction of management performance systems, such as tunnel vision and compliance mood, since it steers the unit's attention towards finding answers to its needs.

It encourages healthcare facilities to identify long-term goals as well as short-term ones, so it could overcome the risk of short-sightedness.

The methodology used to support these activities responds to the need to involve first and foremost the Regional Government in drawing up programmes for quality improvement, in order to ensure consistency between the activities undertaken and the needs of the system, involving the healthcare providers directly in proposing ways to improve the systems for which they are responsible. Sharing knowledge is a cardinal aspect at both healthcare unit and regional levels. This latter has created a dedicated network that will continue to support the improvement process. Quality improvement, especially in the healthcare field, requires multiple approaches, often in apparent contradiction with one another, strong leadership combined with a sense of participation, orientation and control, but also flexibility in implementing actions on the basis of local needs, and a willingness to learn from feedback that is constructively critical of the services provided.

Declarations

Ethical approval and consent to partecipate - not applicable.
Consent form - not applicable.
Availability of data and materials - Data used for this paper were given to the authors by the regional authorities and Dr Maurizio Bersani and Elisabeta Brivio controlled they were used correctly. Data are about the results of the check lists of all the health structures of the Lombardia region for the year 2016 and 2017.

Acknowledgements

All the authors would like to thank very much Maria Aloia.
Funding sources: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

MN, SC and RS designed the study EL anlysed and evaluated data, EB and MB revised the manuscript. All the author approved the final version

References

[1] Dilley JA, Bekemeier B, Harris JR. Quality improvement interventions in public health systems: a systematic review. Am J Prev Med 2012;42(Suppl 1):58-71. https://doi.org/10.1016/j.amepre.2012.01.022

[2] Beitsch LM, Yeager VA, Moran J. Deciphering the imperative: translating public health quality improvement into organizational performance management gains. Annu Rev Public Health 2015;36:273-87. https://doi.org/10.1146/annurev-publhealth-031914-122810

[3] The Joint Commission. What is accreditation? https://www.jointcommission.org/accreditation/accreditation_main.aspx (2018). Accessed 3 September 2018.

[4] The Australian Council on Healthcare Standards (ACHS). Programs & Services. https://www.achs.org.au/programs-services/ (2018). Accessed 3 September 2018.

[5] Consiglio d'Europa, Raccomandazione N. R (97) 17 del Comitato dei Ministri agli Stati Membri. Sullo Sviluppo e l'attivazione di Sistemi di Miglioramento della Qualità (SMQ) dell'Assistenza Sanitaria" (i.e. "On the Development and activation of healthcare quality improvement systems (SMQ)"). Adottato dal Consiglio dei Ministri il 30 settembre 1997 alla 602° riunione dei Ministri. http://www.salute.gov.it/imgs/C_17_pubblicazioni_28_allegato.pdf (p.8). Accessed 31 October 2018.

[6] Groene O, Skau JK, Frølich A. An international review of projects on hospital performance assessment. Int J Qual Health Care 2008;20:162-71. https://doi.org/10.1093/intqhc/mzn008

[7] National Center for Quality Assessment in Health Care, WHO Collaborating Centre for Development of Quality and Safety in Health Systems. PATH Performance Assessment Tool for Quality Assessment in Hospital. http://www.pathqualityproject.eu/what_is_path.html (2018). Accessed 20 August 2018.

[8] Shaw CD, Groene O, Botje D, Sunol R, Kutryba B, Klazinga N, Bruneau C, Hammer A, Wang A, Arah OA, Wagner C; DUQuE Project Consortium.. The effect of certification and accreditation on quality management in 4 clinical services in 73 European hospitals. Int J Qual Health Care 2014;26(Suppl 1):100-7. https://doi.org/10.1093/intqhc/mzu023

[9] Brewer RA, Joly B, Mason M, Tews D, Thielen L. Lessons learned from the multistate learning collaborative. J Public Health Manag Pract 2007;13:388-94. https://doi.org/10.1097/01.PHH.0000278033.64443.2a

[10] Directive 2011/24/EU of the European Parliament and of the Council of 9 march 2011 on the application of patients' rights in cross-border healthcare. Official Journal of the European Union, 4/4/2011, L 88/45.

[11] Legge 23 dicembre 1978, n. 833: Istituzione del servizio sanitario nazionale, GU n.360 del 28-12-1978, Suppl. Ordinario.

[12] D. Lgs 19 giugno 1999, n. 229. Norme per la razionalizzazione del Servizio Sanitario Nazionale, a norma dell'articolo 1 della legge 30 novembre 1998, n. 419. Gazzetta Ufficiale n. 165 del 16 luglio 1999 - Supplemento Ordinario n. 132.

[13] Legge Regionale 11 luglio 1997, n. 31. Norme per il riordino del servizio sanitario regionale e sua integrazione con le attività dei servizi sociali. BURL n. 28, 2° suppl. ord. del 11 Luglio 1997, urn:nir:regione.lombardia:legge:1997-07-11;31.

[14] Regione Lombardia, BOLLETTINO UFFICIALE, Serie Ordinaria n. 25, Lunedì 15 giugno 2015, Allegato 1,Programma Integrato di Miglioramento dell'Organizzazione.

[15] Xchange PDF, version 2.5.322.6, Tracker Software Products, web site: www.trackersoftware.com/product/pdf-xchange-viewer.

[16] Dixon-Woods M, McNicol S, Martin G. Ten challenges in improving quality in healthcare: lessons from the Health Foundation's programme evaluations and relevant literature. BMJ QualSaf 2012; 21:876-84. https://doi.org/10.1136/bmjqs-2011-000760

[17] Decreto Legislativo 30 dicembre 1992, n. 502.Riordino della disciplina in material sanitaria, a norma dell'articolo 1 della legge 23 ottobre 1992, n. 421.

[18] Decreto Legislativo 7 dicembre 1993, n. 517.Modificazioni al decreto legislativo 30 dicembre 1992, n. 502, recante riordino della disciplina in materia sanitaria, a norma dell'articolo 1 della legge 23 ottobre 1992, n. 421.

Received on May 25, 2019. Accepted on September 26, 2019.

Correspondence: Silvana Castaldi, Dept Biomedical Science for Health, University of Milan, Section of Public Health, Fondazione IRCCS Ca' Granda OMP, Quality Unit, via Pascal 36, 20133 Milan, Italy - Tel +39 02 55038342 - Fax +39 02 55033144 - E-mail: Silvana.castaldi@unimi.it – silvana.castaldi@policlinico.mi.it

How to cite this article: Nobile M, Luconi E, Sfogliarini R, Bersani M, Brivio E, Castaldi S. Regione Lombardia: a tool for improving quality in hospitals. J Prev Med Hyg 2019;60:E394-E399. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1298

ORIGINAL ARTICLE

The prevalence and predictive factors of somatization and its relationship with anxiety and depression in Iranian population

B. GARRUSI[1], M. DANAEI[2], R. ABOOSAEIDI[3]

[1] Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran; [2] Department of Community Medicine, Social Determinants of Health Research Center, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran; [3] Department of Community Medicine, HIV/STI Surveillance Research Center, WHO Collaborating Center for HIV Surveillance, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran

Keywords

Somatoform disorders • Anxiety • Depression • Prevalence • Risk factors

Summary

Introduction. *Today mental disorders are important concerns of health care system in all countries. Among different mental disorders; depression, anxiety, and somatization are more frequent. This manuscript was conducted to evaluate the frequency of somatization symptoms, its related factors and the correlation between somatization symptoms and anxiety and depression disorders in Iranian population.*

Methods. *The cross-sectional study was conducted in Kerman, Iran, 2017. Participants were selected from patients who referred to the Clinics of Educational Hospitals using convenience sampling method. The PHQ-15 and HADS questionnaire were used to assess the somatization and depression and anxiety, respectively. The univariate and multivariate logistic regression was used to determine the predictive factors of somatization symptoms. The correlations between each PHQ-15 item score and anxiety and depression score were expressed.*

Results. *The frequency of mild, moderate and severe levels of somatization was 66.3%, 20.5% and 13.1%, respectively. Considering multivariate logistic regression analysis; age was associated with somatic symptoms, significantly. The risk of somatic symptoms was 3.4 times more in Divorced/Widowed participants than single ones (p-value: 0.035). There were significant positive correlations between anxiety and depression scores. Each additional score of anxiety and depression were associated with 1.14 times more likely (p-value: < 0.001) and 1.11 times less likely (p-value: 0.003) of having somatic symptoms, respectively.*

Conclusion. *The burden of somatization, depression and anxiety is high in Iranian population. Psychologists and policy-makers should consider these predictive factors for primary prevention of somatization at the personal and community level, respectively.*

Introduction

Today mental disorders are important concerns of health care system in all countries, with a major burden of disease and catastrophic socio-economic effects [1]. The prevalence of psychological morbidity in north Amira and Western Europe were estimated by different studies from 14% to 50% among primary health care patients [2, 3]. Approximately 80% of mental disorders occur in low or middle-income countries [1]. Among different mental disorders; depression, anxiety, and somatization are more frequent [4].

A population-based study on 36,000 Iranian adult populations in 2015 showed that 23.4% of them have mental disorders. Among cases, the prevalence of somatization (29.8% of cases) and anxiety (29.5% of cases) was higher. The prevalence of mental disorder was higher in females, individuals living in urban areas and older age peoples. Single individuals, students, employed and more educated people suffered less frequent from mental disorders comparing other groups [5].

Somatization is identified by multiple and recurrent complaints regarding somatic symptoms. Somatization has a chronic course and high psychiatric co-morbidity especially anxiety and depression. This situation causes the suffering of the patients and their family [6].Different studies concluded that 20.4% [7], or 30% [8] of patients with somatoform disorders suffer from anxiety and depressive disorders, concurrently. Women, older people, and widowed or divorced individuals reported somatic symptoms more than others, significantly [7]. Also, the results of studies show somatization occurs commonly in people with low socio-economic status and low educational level [9].

Somatization has direct and indirect consequences on society. Direct consequences are resources that use to treat and manage it such as costs of drugs, laboratory tests, and health care personnel. Indirect consequences are absenteeism from work, reduction or loss of productivity and quality of life. To the best of our knowledge, there are limited studies about somatization and its risk factors among Iranian population [5, 10]. Due to the importance of somatization and the concurrence of somatization with other mental disorders, this study was conducted to evaluate the frequency of somatization symptoms, its related factors. Also, the correlation between somatization symptoms and anxiety and depression disorders was assessed in Iranian population.

https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1006

Material and methods

This cross-sectional study was conducted in Kerman, Iran, from July to December 2017. Kerman Province covers about 3% of Iran population and is located in the Southeast of Iran. Participants were selected from patients who referred to the Clinics of Educational Hospitals. Considering Municipality district (4 districts), one Clinic of Educational Hospitals in each Municipality district was selected randomly. Individuals who have at least 18 years old, and did not have any dementia or mental retardation or medical condition requiring hospitalization were included in the study using convenience sampling method. Verbal informed consent was obtained. Considering the prevalence of somatization symptoms in general population as 22% [9] type I error as 0.05, confidence level as 0.95, and precision as 0.04 the total sample size was calculated as 412. Considering the design effect as 1.25, the total sample size was calculated as 515.

After obtaining the permission from the Ethics Committee of Kerman Medical University, trained interviewers described the importance and the aim of this study and asked participants to complete the questionnaires carefully. The process of interview was continued until the completeness of sample size. Two questionnaires were used. The valid and reliable Patient Health Questionnaire-15 (PHQ-15) questionnaire (Cronbach's alpha: 0.76) was used to assess the somatization symptoms [9].

Thirteen symptoms (including stomach pain, back pain, pain in arms, legs, and joints, headache, chest pain, dizziness, fainting spells, palpitation, shortness of breath, pain during sexual intercourse, Constipation/diarrhea, nausea and menstrual pain or problem) were asked for the last four weeks and participants answered them as "not bothered at all", "bothered a little", and "bothered a lot". These answers were coded as a Likert scale from 0 to 2, respectively. Feeling tired and Sleep problems were asked, additionally. Participants answered the questions as the Likert scale as 0 ("not at all"), 1 ("several days"), and 2 ("more than half the days" or "nearly every day"). Therefore the minimum and maximum score of the PHQ_15 questionnaire were 0 and 30, respectively. The total score < 5 was considered as no somatization disorder. Also, the total score classified to the three level of somatization as mild (Score ≥ 5), moderate (Score ≥ 10) and severe (Score ≥ 15) [11-13]. Finally, for regression analysis, the total score of PHQ-15 was coded as 0 "< 10 scores" and 1 "≥ 10 scores". The menstruation item was excluded, because its function is only for women and other studies excluded this item from their study, therefore we can compare the results of this study to other studies [14].

The Hospital Anxiety and Depression Scale questionnaire (HADS) was used to assess the anxiety and depression. The validity and reliability of this questionnaire were assessed later with Cronbach's alpha 0.83 for anxiety subscale and 0.82 for depression subscale. This questionnaire measures the severity of anxiety and depression through last week. This questionnaire includes 14 items, 7 items for measuring the severity of depression and 7 items for measuring the severity of anxiety. The responses were coded as a Likert scale from 0 to 3. Therefore a total score of HADS questionnaire ranged from 0 to 21 for each anxiety and depression subscale. It is classified as normal (score: 0-7), mild (score: 8-10), moderate (score: 11-14), severe (score: 15-21) [15]. Completed questionnaires with more than 10% non-response questions were excluded from the analysis.

HADS and PHQ are frequently used questionnaires. These questionnaires are self-administered, but studies demonstrated that these questionnaires could diagnose disorders with an accurate estimation [16, 17].

The socio-demographic questions including age, gender, marital status, occupational status, level of education, living condition and socio-economic status (participants' estimation of their socio-economic status) were asked at the end of questionnaires. The severity of somatization, anxiety and depression disorders were described. The data were analyzed using IBM SPSS 20.0 software (IBM SPSS Inc. Chicago, IL, USA). The univariate and multiple logistic regressions were used to determine the effects of demographic factors, and anxiety and depression score on somatization symptoms. After conducting univariate logistic regression analysis, all variables with P-value ≤ 0.2 were included in the multiple logistic regression analysis (Enter Method).The correlation between each PHQ-15 item score and anxiety and depression score were expressed using Pearson correlations. The significance level was set at 0.05(Two-tailed analysis).

Results

Sample characteristics

A total of 502 questionnaires were completed (Response rate: 97.5%). The socio-demographic characteristics of samples are described in Table I. The mean ± SD of the age was 44.78 ± 9.6.About 60% of participants were women. Majority of participants were married. Approximately 28.2% of male participants and 25.8% of female participants had a university education. Majority of them were housewife and lives in the urban area. From the point of view of participants, only 14% of participants reported their socio-economic status as bad (Tab. I).

The mean ± SD of the total PHQ-15 score was 8.53 ± 5.6. The median and interquartile range was 7.9 and 7. According to the classification of PHQ-15 score, the frequency of no, mild, moderate and severe levels of somatization were 23.7% (n = 79), 42.6% (n = 254), 20.5% (n = 103) and 13.2% (n = 66), respectively. Comparing different symptoms, feeling pain in arms, legs, and joints, stomach pain and headache have a highest mean score, also shortness of breath, chest pain, pain during sexual intercourse and palpitation have a lower mean score, respectively (Tab. II).

Anxiety and depression

The mean ± SD of anxiety and depression scores were 7.20 ± 4.9 and 5.72 ± 4.8, respectively. Nearly 56.2% of participants did not have any anxiety disorder and 66.2% of participants did not experienced depression disorders.

Tab. I. Socio-demographic characteristics of participants.

Socio-demographic characteristics	Males (n = 204)		Females (n = 298)		Total sample (n = 502)	
	n	%	n	%	n	%
Age group						
10-30 years	60	30.2	100	34.2	161	32.7
31-50 years	99	49.7	141	48.3	241	48.9
51-70 years	35	17.6	44	15.1	79	16.0
71-90 years	5	2.5	7	2.4	12	2.4
Marital status						
Single	49	24.3	47	15.8	96	19.2
Married	145	71.8	219	73.7	366	73.1
Divorced/Widowed	8	4.0	31	10.4	39	7.8
Education						
Illiterate or primary school	19	9.4	42	14.1	61	12.2
Secondary school	27	13.4	44	14.8	71	14.1
High school	99	49.0	135	45.3	235	46.8
University	57	28.2	77	25.8	135	26.9
Occupational status						
Government job	64	33.2	46	16.1	111	23.1
Non-government job	100	51.8	36	12.6	137	28.5
Retired	27	14.0	12	4.2	39	8.1
Housewife/Unemployed	2	1.0	192	67.1	194	40.3
Living Condition						
Urban	173	89.2	259	91.2	434	90.4
Rural	21	10.8	25	8.8	46	9.6
Socio-economic status						
Bad	30	14.9	40	13.6	70	14.1
Moderate	51	25.4	91	31.0	142	28.6
Good	62	30.8	80	27.2	142	28.6
Very good	58	28.9	83	28.2	143	28.8

PHQ-15 mean scores

Tab. II. The mean score, Standard Deviation and frequency of answers to PHQ-15 items.

Complaint	Mean	SD	Not at all N (%)	A little N (%)	A lot N (%)
Stomach pain	0.85	0.7	181 (36.1)	213 (42.4)	108 (21.5)
Back pain	0.60	0.6	262 (52.2)	177 (35.3)	63 (12.5)
Pain in arms, legs, joints	0.93	0.7	155 (30.9)	232 (46.2)	115 (22.9)
Headache	0.82	0.6	161 (32.1)	276 (55.0)	65 (12.9)
Chest pain	0.37	0.6	351 (69.9)	114 (22.7)	37 (7.4)
Dizziness	0.58	0.6	255 (50.8)	203 (40.4)	44 (8.8)
Fainting spells	0.68	0.6	216 (43.0)	230 (45.8)	56 (11.2)
Palpitation	0.37	0.6	362 (72.1)	94(18.7)	46 (9.2)
Shortness of breath	0.30	0.5	377 (75.1)	97 (19.3)	28 (5.6)
Pain during sexual intercourse	0.35	0.5	400 (79.7)	71 (14.1)	31 (6.2)
Constipation, diarrhea	0.55	0.6	276 (55.0)	183 (36.5)	43 (8.6)
Nausea	0.79	0.6	195 (38.8)	226 (45.0)	81(16.1)
Feeling tired	0.72	0.6	198 (39.4)	249 (49.6)	55 (11.0)
Sleep problems	0.54	0.6	290 (57.8)	154 (30.7)	58 (11.6)

The prevalence of severe anxiety and depression were 9.8% and 4.3%, respectively (Tab. III).

Associated factors of somatic symptoms

The results of univariate logistic regression analysis showed the significant association between age, marital status, education, occupational status, living condition, socio-economic status, anxiety and depression with somatic symptoms Results of multiple analyses showed that age was associated with somatic symptoms, significantly. The 71-90 years old people were 36.10 times more likely to have somatic symptoms than 10-30 years old one (p-value: 0.004), also 51-70 years old participants were 2.81 times more likely to have these symp-

Tab. III. The measures of central tendency and dispersion of anxiety and depression score and the Frequency of severity of anxiety and depression disorders in participants.

HADS questionnaire subscales	Mean	Standard Deviation	Median	Inter quartile range	Minimum	Maximum
Anxiety	7.20	4.9	7	7	0	21
Depression	5.72	4.8	4	8	0	21
	Classification Frequency (percent)					
	Normal		Mild		Moderate	Severe
Anxiety	278 (56.2)		97 (19.6)		71 (14.1)	49 (9.8)
Depression	321 (66.2)		65 (13.4)		78 (16.1)	21 (4.3)

Tab. IV. The associated factors of somatic symptoms using univariate and multivariate logistic regression.

Variable	OR	95% CI	OR	95% CI
Age group				
10-30 years	1.00	-	1.00	-
31-50 years	1.72	1.08-2.74	1.250	0.704-2.219
51-70 years	4.94	2.76-.87	2.817	1.240-6.401
71-90 years	18.68	3.91-89.29	36.101	3.192-408.238
Gender				
Male	1.00	-	1.00	-
Female	1.03	0.70-1.49	0.88	0.45-1.76
Marital status				
Single	1.00	-	1.00	-
Married	2.41	1.37-4.25	2.44	1.08-5.53
Divorced/Widowed	9.29	3.98-21.69	3.58	1.13-11.37
Education				
Illiterate or primary school	6.96	3.57-13.59	1.11	0.40-3.17
Secondary school	2.83	1.51-5.28	2.08	0.88-4.92
High school	1.52	0.92-2.49	1.16	0.59-2.30
University	1.00	-	1.00	-
Occupational status				
Housewife/Jobless	1.00	-	1.00	-
Government job	0.80	0.48-1.33	2.12	0.95-4.73
Non-government job	0.75	0.47-1.21	1.02	0.48-2.27
Retired	4.27	2.03-8.95	2.55	0.85-7.68
Living condition				
Urban	1.00	-	1.00	-
Rural	2.55	1.38-4.71	1.30	0.58-2.93
Socio-economic status				
Bad	3.53	1.92-6.49	1.178	0.487-2.850
Moderate	2.37	1.41-3.96	1.065	0.532-2.133
Good	1.31	0.76- 2.22	0.793	0.395-1.592
Very good	1.00	-	1.00	-
Anxiety	1.19	1.19-1.26	1.14	1.06-1.22
Depression	1.18	1.13-1.23	1.11	1.04-1.19

toms (p-value: 0.013). The risk of somatic symptoms was 3.58 times more likely in Divorced/Widowed participants than single ones (p-value: 0.031). There were significant positive correlations between anxiety and depression scores. Each additional score of anxiety and depression were associated with 1.14 times more likely (p-value: <0.001) and 1.11 times less likely (p-value: 0.003) of having somatic symptoms, respectively (Tab. IV).

The correlations between the PHQ-15 items and anxiety and depression scores are described in Table V. All items of PHQ-15 have significant positive correlations with the anxiety and depression scores. Among different items "fainting spells" was most moderately correlated with anxiety score (r = 0.36) and "sleep problems" was most moderately associated with depression score (r = 0.41).

Tab. V. Correlations between PHQ-15 items and anxiety and depression scores.

Complaint	Anxiety score		Depression score	
	r	P-value	r	P-value
Stomach pain	0.32	< 0.001	0.30	< 0.001
Back pain	0.31	< 0.001	0.36	< 0.001
Pain in arms, legs, joints	0.17	< 0.001	0.13	0.006
Headache	0.25	< 0.001	0.17	< 0.001
Chest pain	0.24	< 0.001	0.34	< 0.001
Dizziness	0.31	< 0.001	0.24	< 0.001
Fainting spells	0.36	< 0.001	0.30	< 0.001
Palpitation	0.20	< 0.001	0.33	< 0.001
Shortness of breath	0.19	< 0.001	0.28	< 0.001
Pain during sexual intercourse	0.19	< 0.001	0.18	< 0.001
Constipation, diarrhea	0.20	< 0.001	0.16	< 0.001
Nausea	0.25	< 0.001	0.27	< 0.001
Feeling tired	0.25	< 0.001	0.28	< 0.001
Sleep problems	0.266	< 0.001	0.41	< 0.001

Discussion

Physicians in primary health care settings visit many patients with perplexing complaints. Often these people had multiple visits and various treatment but they are unsatisfied. Somatic complaints play as the junction between physical and psychosocial aspects of illness and health; accordingly, physicians must be learned regarding variant aspects of diseases.

In this study, the frequency of no, mild, moderate and severe levels of somatization were 23.7%, 42.6%, 20.5%, and 13.2%, respectively. The results of a similar study in Germany showed that the frequency of no, mild, moderate and severe levels of somatization in the general population in Germany was 46.8%, 38.3%, 11.8%, and 3.1%, respectively [14]. Our study showed prevalence of somatic symptoms in primary care is high and about 13.2% of patients had severe symptoms. Katon and colleagues introduced "The systems model of somatization" in the patient's socio-cultural background. These social and cultural factors were included in cultural attitudes regarding health and illness, illness behavior, and availability of health care facilities. Interaction between these factors could be affected demonstration of somatization in different groups [18]. On the other hand, Pain experience has inter-individual variability and many factors including genetic factors, ethnicity, age, and gender could affect it [19].

Previous studies in Asian societies have shown that somatic complaints may be an appropriate way for psychological distress presentation and help-seeking rather than direct expression [20]. Therefore it is not surprising, the prevalence of somatic complaints varies in different societies as 1.5 to 21.9% or even greater in the adult population [21].

In this study, most complaints were Pain in limbs, Stomach pain, headache, and nausea. In some of the researches, the most common symptoms were a pain in limbs and joints, GI symptoms and headache [22, 23]. According to our results older age, being divorced or widowhood, and having anxiety or depression were associated with somatization symptoms. There was no difference regarding kinds of somatic complaints based on genders. Glise demonstrated the nearly equal prevalence of somatic complaints in men and women [21]. The results of a review article demonstrated that women reported more frequently somatic symptoms then men. In addition mental disorders in women were greater than men [24]. Biological factors, more limitations in society, gender social roles and traditional social stressors may play a role in its differences [25].

Our results showed about 43.8% and 33.8% of respondents had some of degree of anxiety and depression respectively. The World Health Organization (WHO) estimated the lifetime prevalence of mental disorders ranged from 18.1 to 36.1% [26]. Based on the last Mental Health Survey of the Iranian Adult Population in 2015, somatic complaints and anxiety (29% for both) were greater than depression (10.39) [6]. This difference may be due to different setting, different questionnaire for detection of mental disorders and or study design [4].The prevalence of mental disorders was notable in this study. The statistical society in this study was the patients who referred to the Clinics of Educational Hospitals; therefore the prevalence of mental disorders was higher than the total population. Also, changing economic situations in recent years could affect Iranian mental disorders.

WHO data demonstrated in the primary care setting about 70% of patients had diagnostic criteria for depression that present with somatic symptoms [27]. Since the psychosocial stressors could affect health, some of the researches were showed that anxiety and depression increase unexplained somatic complaints more than twice [28]. Depression and anxiety could because of somatic complaints. Mental disorders such as depression and anxiety are associated with a higher misperception of somatic complaints. Also, it may be the existence of depression or anxiety was causing more vulnerability to disease [22]. In other words, the somatic complaint may be a presentation of invisible mental disorders. Mental disorders were being affected by some of the variables such as age and marital status [5].

In present study, increasing of age was the one of important contributing factor, in somatic complaint presentation. Impaired of function, social network changes such as social withdrawn are associated with poor health in old age persons [29]. The results of this study showed that the age of more than 50-year-old is a significant predictor factor for somatization. The effect of age was maximized at the age of ≥ 70-year-old. The results of another similar study also showed the maximum score of somatization was observed in ≥70-year-old participants [14]. In late life, some of mental health disturbances (depression, dementia) could worsen and may be affect somatic complaint. Patient-doctor relation, also may be affected

by patient's age. This matter may be act as contributing factors in attention to patients' complaints [30].
In this study, the risk of somatic symptoms was 3.4 times more in Divorced/Widowed participants than single ones. There are different findings regarding the relationship between marriage and mental disorders, but one Epidemiologic Catchment Area (ECA) study revealed married men and women had the lowest depression than divorced men and women [31].
Anxiety also was similar. Loss of spouse and widowed has the high rate of stress and individual could be prone to mental disorders such as depression, Generalized Anxiety Disorders and panic disorders [32]. Our finding may be due to indirect effect of marital status on depression and anxiety.
Multiple somatic complaints that associated with psychiatric co morbidities are the large portion of burden of disease. It is expected, physician who had trained bio psychosocial -oriented have more appropriate approach to somatic complaints. Future researches regarding physicians' diagnostic ability and their therapeutic plan are advised.
There were significant positive associations between anxiety (OR: 1.14) and depression scores (OR: 1.11) with somatization in this study. The results of a large population study in Norway showed the strong association between anxiety, depression and somatic symptoms in both men and women [33]. Also, the results of a systematic review in women in low/middle income countries suggested a strong correlation between anxiety/depression and somatization (OR ranging 2.5-3.5). This association has multidimensional etiology including existence the risk factors for all mental diseases or being anxiety/depression as a risk factor for somatization [34].
This study has a number of limitations. We used self-report questionnaires, cross-sectional design and convenience sampling method. Limited sociodemographic data especially family and social support were other limitations of this study. These limitations could be a negative effect on the generalization of study.

Conclusion

With considering of results, having old age, being divorced or widow and suffering from anxiety or depression are moderately associated with somatization. Family physicians, psychiatrists, mental health workers and policy-makers should consider these predictive factors for primary prevention of somatization at the personal and community level, respectively**.** Physicians should consider the overlap syndrome between depression/anxiety and somatization in their primary care visits.

Acknowledgements

The present article is based on the thesis by Rezvan Aboosaeidi.

Funding sources: the present article was financially supported by Kerman University of Medical Sciences (grant code: 96000110).

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

BG and MD designed the study, RAboosaeidi performed the literature Searches and data gathering, Mina Danaei performed the statistics, and BehshidGarrusi and Mina Danaei wrote the manuscript. All authors commented on a first draft, contributed to and have approved the final manuscript.

References

[1] Jacob K, Patel V. Classification of mental disorders: a global mental health perspective. Lancet 2014;383:1433-5. https://doi.org/10.1016/S0140-6736(13)62382-X

[2] Richards J, Ryan P, McCabe M, Groom G, Hickie I. Barriers to the effective management of depression in general practice. Aust N Z J Psychiatry 2004;38:795-803. https://doi.org/10.1080/j.1440-1614.2004.01464.x

[3] Pini S, Perkonnig A, Tansella M, Wittchen H, Psich D. Prevalence and 12-month outcome of threshold and subthreshold mental disorders in primary care. J Affect Disord 1999;56:37-8. https://doi.org/10.1016/s0165-0327(99)00141-x

[4] Hanel G, Henningsen P, Herzog W, Sauer N, Schaefert R, Szecsenyi J, Lowe B. Depression, anxiety, and somatoform disorders: vague or distinct categories in primary care? Results from a large cross-sectional study. J Psychosom Res 2009;67:189-97. https://doi.org/10.1016/j.jpsychores.2009.04.013

[5] Noorbala AA, Faghihzadeh S, Kamali K, Bagheri Yazdi SA, Hajebi A, Mousavi MT, Akhondzadeh S, Faghihzadeh E, Nouri B. Mental health survey of the Iranian adult population in 2015. Arch Iran Med (AIM) 2017;20(3). https://doi.org/0172003/AIM.003

[6] De Waal MW, Arnold IA, Spinhoven P, Eekhof JA, van Hemert AM. The reporting of specific physical symptoms for mental distress in general practice. J Psychosom Res 2005;59:89-95. https://doi.org/10.1016/j.jpsychores.2005.02.011

[7] Ritsner M, Ponizovsky A, Kurs R, Modai I. Somatization in an immigrant population in Israel: a community survey of prevalence, risk factors, and help-seeking behavior. Am J Psychiatry 2000;157:385-92. https://doi.org/10.1176/appi.ajp.157.3.385

[8] Löwe B, Spitzer RL, Williams JB, Mussell M, Schellberg D, Kroenke K. Depression, anxiety and somatization in primary care: syndrome overlap and functional impairment. Gen Hosp Psychiatry 2008;30:191-9. https://doi.org/10.1016/j.genhosppsych.2008.01.001

[9] Abdolmohammadi K, Ghadiri Sourman Abadi F, Sadat Seyed Pourmand N, Falsafinejad M R. The validation of somatization inventory in the students of Tabriz Universities (Iran). Qom Univ Med Sci J 2018;11:61-7.(Persian)

[10] Heidari Z, Feizi A, Roohafza H, Keshteli AH, Adibi P. Somatoform symptoms profiles in relation to psychological disorders. A population classification analysis in a large sample of general adults. Psychiatry Res 2017;254:173-8. https://doi.org/10.1016/j.psychres.2017.04.064

[11] Van der Leeuw G, Gerrits M, Terluin B, Numans M, van der Feltz-Cornelis C, van der Horst H, Penninx BW, van Marwijk

HW. The association between somatization and disability in primary care patients. J Psychosom Res 2015;79:117-22. https://doi.org/10.1016/j.jpsychores

[12] Shabbeh Z, Feizi A, Afshar H, Hassanzade Kashtali A, Adibi P. Identifying the profiles of psychosomatic disorders in an iranian adult population and their relation to psychological problems. Journal of Mazandaran University of Medical Sciences. 2016;26:82-94. (Persian)

[13] Kocalevent R-D, Hinz A, Brähler E. Standardization of a screening instrument (PHQ-15) for somatization syndromes in the general population. BMC Psychiatry 2013;13:91. https://doi.org/10.1186/1471-244X-13-91

[14] Hinz A, Ernst J, Glaesmer H, Brähler E, Rauscher FG, Petrowski K, Kocalevent RD. Frequency of somatic symptoms in the general population: normative values for the Patient Health Questionnaire-15 (PHQ-15). J Psychosom Res 2017;96:27-31. https://doi.org/10.1016/j.jpsychores

[15] Lukaviciute L, Navickas P, Navickas A, Grigaitiene J, Ganceviciene R, Zouboulis C. Quality of life, anxiety prevalence, depression symptomatology and suicidal ideation among acne patients in Lithuania. J Eur Acad Dermatol Venereol 2017; 31:1900-6. https://doi.org/10.1111/jdv.14477

[16] Djukanovic I, Carlsson J, Årestedt K. Is the Hospital Anxiety and Depression Scale (HADS) a valid measure in a general population 65-80 years old? A psychometric evaluation study. Health Qual Life Outcomes 2017;15:193.

[17] van Ravesteijn H, Wittkampf K, Lucassen P, van de Lisdonk E, van den Hoogen H, van Weert H, Huijser J, Schene A, Van Weel C, Speckens A. Detecting somatoform disorders in primary care with the PHQ-15. Ann Fam Med 2009;7:232-8. https://doi.org/10.1370/afm.985

[18] Katon W, Ries RK, Kleinman A. The prevalence of somatization in primary care. Compr Psychiatry 1984;25:208-15.

[19] Fillingim RB. Individual differences in pain: understanding the mosaic that makes pain personal. Pain 2017;158(Suppl 1):S11. https://doi.org/10.1097/j.pain.0000000000000775.

[20] Xiaolu Zhou A N, SeonghoMin B, JiahongSun C, SeJooKim D, Joung-sookAhn B, YunshiPeng E, Samuel Noh F, Ryder AG. Extending a structural model of somatization to South Koreans: Cultural values, somatization tendency, and the presentation of depressive symptoms. J Affect Disord 2015;176:151-4. https://doi.org/10.1016/j.jad.2015.01.040

[21] Glise K, Ahlborg G Jr, Jonsdottir IH. Prevalence and course of somatic symptoms in patients with stress-related exhaustion: does sex or age matter. BMC Psychiatry 2014;14:118. https://doi.org/10.1186/1471-244X-14-118

[22] Haftgoli N, Favrat B, Verdon F, Vaucher P, Bischoff T, Burnand B, Herzig L. Patients presenting with somatic complaints in general practice: depression, anxiety and somatoform disorders are frequent and associated with psychosocial stressors. BMC Fam Pract 2010;11:67. https://doi.org/10.1186/1471-2296-11-67

[23] Hekmatravan R, Samsum Shariat M, Khani F, Khademi M. The relationship between anxiety and depression with Somatization in Blind people of Isfahan city. 4th International Congress on Psychosomatic. Azad University, Isfahan, Iran. 2012; Oct, 17-19 (Persian).

[24] Barsky AJ, Peekna HM, Borus JF. Somatic symptom reporting in women and men. J Gen Intern Med 2001;16:266-75.

[25] Eagly AH. Sex differences in social behavior: a social-role interpretation. Psychology Press 2013 May 13.

[26] Kessler RC, Aguilar-Gaxiola S, Alonso J, Chatterji S, Lee S, Ormel J, Üstün TB, Wang PS. The global burden of mental disorders: an update from the WHO World Mental Health (WMH) surveys. Epidemiol Psychiatr Sci 2009;18:23-33.

[27] Simon GE, VonKorff M, Piccinelli M, Fullerton C, Ormel J. An international study of the relation between somatic symptoms and depression. N Engl J Med 1999;341:1329-35. https://doi.org/10.1056/NEJM199910283411801

[28] Katon W, Sullivan M, Walker E. Medical symptoms without identified pathology: relationship to psychiatric disorders, childhood and adult trauma, and personality traits. Ann Intern Med 2001;134(9 Pt 2):917-25. https://doi.org/10.7326/0003-4819-134-9_part_2-200105011-00017

[29] Hilderink PH, Collard R, Rosmalen JG, Oude Voshaar RC. Prevalence of somatoform disorders and medically unexplained symptoms in old age populations in comparison with younger age groups: a systematic review. Ageing Res Rev 2013; 12:151-6. https://doi.org/10.1016/j.arr.2012.04.004

[30] Andreas S, Schulz H, Volkert J, Dehoust M, Sehner S, Suling A, Ausín B, Canuto A, Crawford M, Da Ronch C, Grassi L. Prevalence of mental disorders in elderly people: the European MentDis_ICF65+ study. Br J Psychiatry 2017;210:125-31. https://doi.org/ 10.1192/bjp.bp.115.180463

[31] O'Leary CA. Infidelity and separations precipitate major depressive episodes and symptoms of non-specific depression and anxiety. J Clin Psychol 2000;68:774-81.

[32] Keyes KM, Pratt C, Galea S, McLaughlin KA, Koenen KC, Shear MK. The burden of loss: unexpected death of a loved one and psychiatric disorders across the life course in a national study. Am J Psychiatry 2014;171(8):864-71. https://doi.org/10.1176/appi.ajp.2014.13081132

[33] Haug TT, Mykletun A, Dahl AA. The association between anxiety, depression, and somatic symptoms in a large population: the HUNT-II study. Psychosom Med 2004;66:845-51. https://doi.org/10.1097/01.psy.0000145823.85658.0c

[34] Shidhaye R, Mendenhall E, Sumathipala K, Sumathipala A, Patel V. Association of somatoform disorders with anxiety and depression in women in low and middle income countries: a systematic review. Int Rev Psychiatry 2013;25:65-76. https://doi.org/10.3109/09540261.2012.748651

Received on July 15, 2018. Accepted on November 5, 2019.

Correspondence: Mina Danaei, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Imam Highway, Shahid Bahonar University, Afzalipour Faculty of Medicine, Community and Family Medicine Department, 7616914115 Kerman, Iran - Tel. +98 913 3409727 - Fax +98 34 33257671 - E-mail: danaei.mina@gmail.com

How to cite this article: Garrusi B, Danaei M, Aboosaeidi R. The prevalence and predictive factors of somatization and its relationship with anxiety and depression in Iranian population. J Prev Med Hyg 2019;60:E400-E406. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1006

ORIGINAL ARTICLE

Health impact of exposure to asbestos in polluted area of Southern Italy

L. VIMERCATI[1], D. CAVONE[1], F. MANSI[1], E.S.S. CANNONE[1], L. DE MARIA[1], A. CAPUTI[1], M.C. DELFINO[1], G. SERIO[2]
[1] Interdisciplinary Department of Medicine, Section of Occupational Medicine "B. Ramazzini", University of Bari Medical School, Bari, Italy; [2] Department of Biomedical Science and Human Oncology, University of Bari Medical School, Bari, Italy

Keywords

Bari • Asbestos • Contaminated town-site of national interest • Environmental exposure • Mesothelioma

Summary

The three main sources of asbestos pollution in the city of Bari, Puglia, the former Fibronit asbestos factory, the Torre Quetta beach, the former Rossani barracks and the history of their reclamation are described. The results of cohort studies on factory workers and case-control studies on asbestos exposure to the resident population and the onset of mesothelioma are also reported. Finally, the data of the regional register of mesothelioma related to residents in the city of Bari and four new cases with environmental exposure due to the former Rossani barracks are presented.

Introduction

Environmental pollution is one of the most serious global challenges. It can induce effects on human health. In particular, some polluting substances (asbestos, polycyclic aromatic hydrocarbons, carbon monoxide, heavy metals, gases) can cause respiratory and oncological diseases in the general population [1-6]. Among these, asbestos is a naturally occurring fibrous mineral found in the ground and mines all over the world of which there are different mineralogical varieties (actinolite, amosite, antophyllite, chrysotile, crocidolite and tremolite) [7]. Because of its mechanical, electrical, chemical and thermal resistance characteristics, asbestos fibers are exploited in numerous commercial and industrial settings [8].
Since the 1960s, several studies have shown the relationship between asbestos and cancer [9, 10]. In 1964, the conference on the biological effects of asbestos organized by the New York Academy of Sciences unanimously recognized the carcinogenic effects of asbestos [11]. In 1973, the International Agency for Research on Cancer (IARC) classified all types of asbestos as carcinogenic to humans (Group 1) [12].
In contrast to other professional air pollutants (e.g. formaldehyde, wood and leather dusts) [13-15] that cause tumors mainly in the upper airways, asbestos fibers, when mechanically disturbing, tend to divide longitudinally, generating thinner fibers (fibrils) that can penetrate deeply into the lung and reach the pulmonary air spaces. Exposure to them can cause serious diseases such as fibrotic (asbestosis) and neoplastic processes [i.e. malignant mesothelioma (MM), lung cancer] [16-18]. These disorders are characterized by a long latency interval between the beginning of exposure and the onset of the disease (usually decades) [19].
In addition to occupational exposure, the risk of asbestos-related diseases is also linked to environmental exposure, both of human origin (i.e. pollution by industrial sites, presence of asbestos in buildings) or of natural origin (i.e. areas where there are natural outcrops of asbestos-like minerals) [20, 21].
Several studies have reported a significant risk of mesothelioma for environmental asbestos exposure. Other studies have shown an increased risk in the general population associated with relatively low exposure to asbestos [22, 23].
Environmental exposure is defined as a neighbourhood exposure based on residence close to industrial /mining sources of asbestos or residence in municipal or polluted areas. It is also described as any exposure that occurs during the period of residence in a city in which asbestos processing plants were located [24], nevertheless it can come from the presence of asbestos in buildings and from natural contamination of the soil [25].
In Italy, raw asbestos has been used in a wide range of industrial activities such as industrial production of asbestos-cement products, textile articles containing asbestos, shipbuilding, repair and\or demolition of railway rolling stock, construction and many other sectors. Therefore, the number of workers occupationally exposed is very significant [26].
Despite the Directive 2003/18/CE of the European Parliament and of the Council of 27 March 2003 that bans the use of asbestos, it is still used in developing countries and even in some of the twenty-five countries of the European Union [27].
In Italy, Law n. 257/1992 decreed the "cessation of asbestos use"; in particular, it prohibits the extraction, importation, exportation and use of asbestos and products containing asbestos. The law also provides measures to decontaminate and reclaim areas affected by asbestos pollution [28].

Although Italian legislation concerning asbestos is one of the most advanced in Europe, on national territory there are still present several million tonnes of compact materials containing asbestos and many tonnes of brittle asbestos in a large number of public and private and industrial sites [29].
According to World Health Organization, *contaminates sites* are defined as: "Localized areas hosting or having hosted large and / or hazardous industrial facilities, producing or with strong potential to produce environmental contamination resulting in health impacts" [30].
Despite in 2001, the municipality of Bari was included among Italian Polluted Sites, environmental remediation interventions only started in 2016, after fifteen years, and it is still in progress [31].
The area of Bari municipality, Apulia region of southern Italy, is one of the most contaminated sites nationwide and in 2001 was classified as site of national interest for remediation. The three main sources of asbestos pollution in the city of Bari are represented by the former Fibronit asbestos factory, the Torre Quetta beach and the former Rossani barracks.
Since the 1990s, several epidemiological studies have been conducted in exposed workers as well as in resident population. The aim of this review is to assess the impact on public health of exposure to asbestos in both occupationally and non-occupationally exposed individuals in the area of Bari municipality.

Methods

The published literature was searched using MEDLINE, accessed via PubMed (the U.S. National Library of Medicine). Additional studies were also hand-searched from bibliographies of the selected studies. Key words included environmental pollution, asbestos (and specific fiber types, including crocidolite, amosite, and chrysotile), occupational exposure, municipality of Bari, health effects, mesothelioma, or asbestosis.
Analytical and descriptive epidemiologic studies were considered in this study, including cohort, case-control and case reports. The medical conditions of interest were pleural and peritoneal mesothelioma.
Information extracted from each study included first author, publication year, geographic area, study type, total number of cases, and controls, fiber type, industry type, measurement of asbestos, definition of asbestos exposure and/or period of employment/exposure and classification of outcome. The exposure studies reviewed included a variety of study types conducted in the area of Bari municipality, divided into 3 major groups:

1. SENTIERI Project - Epidemiological study of residents in national priority contaminated sites: incidence of mesothelioma.
2. Cohort studies - Occupational exposure assessment-Fibronit (Bari).
3. Environmental exposure.

The sites of interest are listed below.

Fibronit asbestos cement factory

The former Fibronit placed close to a densely populated area, occupies a surface area of 39,000 square meters and it is composed of several industrial buildings and offices. The plant, with about 400 employees produced various products containing asbestos, from 1933 until 1985 [32, 33]. In particular, it manufactured pipes of various lengths, corrugated sheets and special pieces, such as square rods, ridges, sleeves or tanks, water tanks. The production cycle was divided into several phases which, until the beginning of the 1970s, were mostly dry and manually. The most common type of asbestos used in the factory was crysotile (80%), followed by crocidolite (15%) and amosite (5%).
The dusts initially sucked were carried to the outside generating a high environmental risk. Exhaust systems used in 1974 only partially had blast chillers.
The wards were cleaned with a shovel and a broom. The processing residues were accumulated on one side and once a week a company passed that took everything and took it away. Some of the waste material was in some cases thrown into natural troughs of soil existing in the Fibronit area and were then covered first with soil and then with asphalt. In Fibronit, there were no waste collection and disposal systems and no processing waste, other areas of the city outside the factory were repeatedly used as an illegal dump. There is no news of any use of personal protective equipment (PPE) by Fibronit workers who have testified that they returned home with clothes and hair soaked in dust.
From 1970 to 1974 some industrial hygiene surveys were carried out with measurement of the concentration of airborne fibers, in a range between 5 ff/l and 20 ff/l, with the highest values measured in the milling department and the monolithic tube and glass department. The first survey carried out by the National Institution for the Prevention of Accidents (ENPI) in 1970 revealed concentrations up to 20 ff/cc near the "molazza". In the second survey, carried out in 1972 by the Labor Inspectorate, the maximum concentrations reached were 10 ff/cc near the mullers and in the monolithic tube and glass department. Finally, in 1974, an expert opinion prepared by the judiciary carried out withdrawals on three consecutive days, finding concentrations that, where the most risky operations were performed, ranged from 4 to 19 ff/cc [32].
The operations that resulted with the greatest exposure to asbestos were emptying and rattling of the bags, milling, pipe turning and slab cutting. In the seventies, the ACGIH working exposure limit (TLV) was 5 ff / cc (fibers / cubic centimeter).
In 1975, the same year of the first complaints of the total non-existence of safety measures in the workplace, by the unions, the presence of dust was detected in the air not only of the factory, but also in the neighboring areas. Average concentration values equal to 16.06 x 10-4 "particles (< 5 microns in size) per cc of air" [34] were reported.
In 1994 the hygiene and public health service set up for Fibronit a work plan for the removal of some materials containing asbestos that had been piled up outside

the factory buildings since 1985 (cessation of the company's activities), there were high quantities of asbestos not only in outdoor sheds, but also in the basement of the plant and for several meters of depth. In the most serious cases, the contaminated areas also reached a thickness of 6 meters, for a total volume of about 90,000 cubic meters, even affecting the land on which the sheds are located. Even the underground utilities and the sewage system are contaminated by dust and asbestos processing residues.
From 1997 to 2001, provisional safety has started with the removal of shed roofs, the ground breach and with fixing paint to prevent asbestos fibers from continuing to disperse in the air.
In 2001, the Fibronit industrial area was included among Italian Polluted Sites (IPS) and in 2002 the Bari Public Prosecutor seizes the polluted area, still recognizing the presence of serious risks for public health.
After the closure of the factory in 1985, the work of partially securing the overpass was only completed in 2007. In 2011, the decision-making conference between the Ministries of the Environment, Health and Economic Development and the Apulia Region approves the project of putting Fibronit into permanent safety. To date, remediation activities are ahead. At the same time, the procedure for awarding the works for the construction of the park that will be built instead of the factory was started.
Currently, the area of the former Fibronit plant covers over ten hectares in a central area of the city of Bari. Nearby, there are both construction-free soils and densely built-up soils, as well as other areas with different destinations.
This area is defined as *brownfields* that is "sites that have been affected by the former uses of the site and the surrounding land; are derelict or underused; have real or perceived contamination problems; to bring them back to beneficial use" [35, 36].

Torre Quetta Beach

Former workers have testified that the residues of processing were unloaded not only in the subsoil of the industrial area, but also in the surrounding areas and along the coastal strip, in particular on the coast south of the city "Torre Quetta", used as a landfill for waste and mixtures of asbestos processing.
At the beginning of 2001, a technical report by the Environmental Protection Agency of Apulia (ARPA) highlighted, in the stretch of the Bari coast of "Torre Quetta", the widespread presence of asbestos, both in the form of asbestos cement scrap embedded in the ground scattered everywhere, and in material friable with the presence of free asbestos fibers, present in the filling layers. The total amount of asbestos collected amounted to around 40,000 kilograms.
The materials had an absolutely peculiar typology, different from that of asbestos cement waste, which is often found in areas subject to uncontrolled waste dumping and deriving from building demolition, consisting of roof slabs, flues or fragmented downspouts. In 2004, the entire stretch of coast of "Torre Quetta" was precautionary seizure by the Judicial Authority.
For many years, Torre Quetta area has been used in public bathing and caused an unconscious exposure to asbestos in general population. As regards the permanent safety and reclamation of the area there are problems due to the continuous action of the sea, which over time has produced the erosion of the filling layers and the spread of asbestos materials on a very extensive stretch of coast [37].

The Military Barrack "Rossani"

The military barracks "Rossani" were built in the early decade of the 20th century. The barracks, located near the central station – in the heart of city –, stands on a total area of 80,000 square meters of which 14,000 square meters of covered area. All the roofs and chimneys of the barracks buildings were constructed of asbestos. In 2001 the reclamation of asbestos roofing started, estimating the removal of about 5000 square meters of Eternit.
In 2004, an Inspection of the Environmental Protection Agency of Puglia (ARPA) suspended the first works for the removal of hazardous materials because the remains of the removal remained on the ground. In particular, the defaults would concern fragments of the dispersed artefacts, bags containing poorly kept asbestos, failure to use decontamination units and a pollution higher than that hypothesized in the operational program.
In 2005, despite the strong protests from citizens, the work resumes and were completed at the end of 2006 [38].

Results

SENTIERI Project

As part of the SENTIERI project, a national epidemiological study of the territories and settlements exposed to pollution risk, coordinated by the National Institute of Health, the mortality rate of IPS Bari-Fibronit relative to the years 1995-2002 was analyzed.
Among the main causes of death, there is an excess of mortality for all causes, for all tumors and for respiratory system diseases in both women and men. In women, there is an excess of mortality for diseases of the digestive system. Once adjusted for deprivation rate index, mortality is also higher than expected for cardiovascular diseases in both the genders, for digestive system in men and for genito-urinary system in women.
For causes of death for which there is an evidence sufficient or limited in association with the sources of environmental exposures of IPS, there is an excess for lung cancer in women. There is an excess for malignant pleural cancer among men and women.
Respectively among men and women, 49 and 17 deaths from pleural cancer with standardized mortality ratio (SMR) equal to 199 (confidence interval (CI) 90% 155-253) and 192 (CI 90% 122-287) [39].

SENTIERI-ReNaM

The SENTIERI-ReNaM project described the incidence of mesothelioma in IPS in the period 2000/2011. In this period, 123 cases (88 men, 35 female) of MM were recorded in subjects residing in IPS. In men the average age at diagnosis was 66.4 (DS ± 11.4) and the median at 66.5; in women they were respectively 67.3 [standard deviation (SD) ± 12.4] and 70. The standardized incidence ratios (SIR) of MM (certain, probable, possible), for all the sites, were equal to 271 (CI 90% 228- 323) in men and 322 (CI 90% 244-426) in women [40].
The settlement, in the urban context, of the Fibronit factory for the production of asbestos cement has configured both direct asbestos exposure panels, airborne fibers during processing, and indirect exposure to environmental exposure. The cases of MM with environmental exposure, in the period considered, are 9 in men and 18 in women, with M/W ratio of 0.5. For these environmental cases, residence near the aforementioned Fibronit plant was established. In the only case with family exposure (female gender) it is an exhibition of cohabitants with employees in the manufacture of fiber cement products [40].

Cohort studies - Occupational exposure assessment- Fibronit (Bari)

A first cohort study involved 233 Fibronit workers with disability pensions for asbestos on 12/31/1979. The data was retrieved from the National Institute of Occupational Accident Insurance (INAIL) archive. The mortality observed in the cohort was then compared with that expected based on mortality data from Apulia and was higher than expected, with significant excesses for asbestosis and for lung, pleural, mediastinal and peritoneal mesothelioma [41] (Tab. I).
Coviello et al. [32] analysed mortality in Fibronit workers present in the factory from 1972 to the closure of the same: the cohort included all male subjects, for a total of 417 people.
In terms of latency it is observed that, while the first cases of pulmonary neoplasia begin to appear already around 1975, the first case of pleural mesothelioma does not arise before 1990.

Tab. I. Fibronit cohort - National Institute of Occupational Accident Insurance – INAIL (Bari).

Follow-up	1980-1997
Follow-up completed	98,3%
Cause of death known	96,6% of deaths
All deaths	SMR: 117 (87 deaths)
Pneumoconiosis	SMR: 11238 (14 deaths, p < 0.05)
Malignant tumors	SMR: 163 (38 deaths, p < 0.05)
Circulatory diseases	SMR: 64 (18 deaths, p < 0.05)
Lung tumors	SMR: 206 (17 deaths, p < 0.05)
Pleura and peritoneum tumors	SMR: 2551 (8 d., P < 0.05)

In the investigated cohort, the average latency for pleural mesothelioma is about 42 years, with a minimum latency of 26 and a maximum of 52 years. The results of the study concerning the entire cohort of 417 workers show excess mortality for all causes, for pneumoconiosis, for all tumors, for malignant lung, pleura and peritoneum tumors [42] (Tab. II).
In 2016, the follow-up of 414 former Fibronit workers at 31 December 2012 was published. The subjects in the study, all male, number 414 (377 workers, 29 employees and 8 transacted from worker to employee duties), of which 325 (78%) were already present at February 1, 1972 in the Fibronit plant and 89 (22%) were hired later. No information is available about workers who left the job before the aforementioned date, which we define as baseline. The workers were hired in the factory between 1934 and 1982, with a median age at the first recruitment of 27.1 years [43] (Tab. III).
The analysis disaggregated by ten-year latency classes shows a significant excess for the lung tumor starting from the latency class 20-29 years, while the excess for the malignant tumor of the pleura occurs from the 30-39 year class and for peritoneum cancer from the 40-49 year class.
Among the non-neoplastic causes, pneumoconiosis shows extremely high values that are already evident starting from the 10-19 year latency class.
In the analysis along the latency axis, for lung cancer, we note an increase in SMRs up to the latency class 30-39 years, with subsequent decrease; in the case of pleural cancer the first cases appear 30 years after the beginning of the exposure with a growing SMR up to the latency class 50-59 years [43].

Tab. II. Fibronit cohort (1975-2000).

Follow-up	1972-1995 (105 deaths) 2000 (145 deaths)
All causes	1995 SMR (CI 95%) 118 (97-143), 2000 SMR (CI 90%) 121 (102-142)
Asbestosis	1995 SMR (CI 95%) 14.705 (9.519-21.708) 2000 SMR (CI 95%) 15.650 (11.010-22.250)
All tumors	1995 SMR (CI 95%) 139 (100-189) 2000 SMR (CI 95%) 148 (114-191)
Lung tumors	1995 SMR (CI 95%) 191 (116-294) 2000 SMR (CI 95%) 175 (116-259)
Pleural tumors	1995 SMR (CI 95%) 1.578 (325-4.613) 2000 SMR (CI 95%) 2.963 (1.594-5.507)
Peritoneal tumors	1995 SMR (CI 95%) 95 1667 (222-6018) 2000 SMR (CI 95%) 1165 (264-4.007)

Tab. III. Fibronit cohort (1972-2012).

Follow-up	1972-2012 (232 deaths)
All causes	SMR (CI 95%) 120 (105-136)
Asbestosis	SMR (CI 95%) 13.268 (9.481-18.570)
All tumors	SMR (CI 95%) 194 (159-237)
Lung tumors	SMR (CI 95%) 201 (146-276)
Pleural tumors	SMR (CI 95%) 4.033 (2.541-6.401)
Peritoneal tumors	SMR (CI 95%) 2.945 (1.404-6.177)

Environmental exposure

In 2003 Bilancia et al. [44] analyze, with explorative methods based on geographical analysis, the relationship between the presence of the asbestos cement factory in the urban area of Bari and the mesothelioma cases that occurred between 1980 and 2001 among the residents. Subjects who have had a permanent residence in the city of Bari for a period of at least 20 years prior to the onset of the disease. The estimate of the SIR shows that within an area having a radius of approximately 1 km, centered on the industrial plant, the risk level was higher than expected. The data source of the 64 cases studied is the National mesothelioma Registry (ReNaM), the Regional Operations Center of Apulia (COR-Apulia). The data was analyzed with the S + SpatialStats software. Both the single data analysis and the exploratory geographical analysis showed an increase in the risk of disease among the people who lived near the asbestos cement factory: within an area centered on the location of the company and with a radius of about 1 km, the estimated risk was 2.38 times the normal level.

The impact of environmental exposure to asbestos, in neighborhoods bordering the production site, was also estimated with a case-control study that assessed the spatial distribution of 48 cases of mesothelioma of non-professional origin residing for more than 15 years time of diagnosis, from the data of the mesothelioma register of Puglia referring to the years 1993-2003, and of 273 controls also residing for over 15 years in the city of Bari. The complete residential histories of both cases and controls were analysed. The study compared the distribution of addresses between cases of MM with the corresponding distribution of controls, residents who died on the same calendar date as cases for causes other than mesothelioma. Residential history and distance from Fibronit has been considered as a proxy for environmental exposure to asbestos.

The disease risk was estimated using a logistic regression model, in which the probability of disease occurrence is expressed as a function of the distance classes from the Fibronit site. A non-parametric method was applied to estimate the total area of the risk relative. The study observed a significant increase in risk within the resident population within 500 meters of the plant [Relative Risk (RR) = 5.29 (95 CI: 1.18-23.74)] as the distance between the patients' home and the factory decreased. A cluster of six cases of MM has been identified east of Fibronit, near the urban beach "Torre Quetta", where unauthorized waste disposal occurred during 1950-70 years.

The results also show that the odds ratio (OR) for the lowest exposure group (in terms of distance from the plant, 1,500-2,000 m) is remarkably high but not significant (OR = 2.31, 95% CI: 0.88-6.06): a reasonable explanation is that this distance band includes the secondary cluster '' Torre Quetta" [33].

In the 2012 study [45] a high pulmonary fiber load is described in five mesothelioma cases, two women and three men, subjects who after an accurate reconstruction of the exposure circumstances were professionally unexposed but resident near Fibronit. The subjects of age at diagnosis between 36 and 65 years, diagnosed between 2005 and 2009, had lived in periods between 2 and 24 years, between 1960 and 1997, at distances between 200 and 2000 meters from the factory.

Lung tissue samples used to measure the pulmonary load of asbestos fibers were taken 10-38 years after the last residence (after the cessation of exposure). To provide information on the intensity of environmental exposure of asbestos, semi-quantitative and quantitative indices of cumulative environmental exposure to asbestos were calculated, based on the distance of the residence from the factory and its duration. The pulmonary load of fibers ranges from 110 000 to 2 300 000 fibers per gram of dry lung (f/g). In two cases, a 51-year-old woman and a 53-year-old man found concentrations greater than 1 000 000 f/g of amphibole fibers, a value proposed as a cut off to identify subjects with occupational exposure to asbestos, even when no evidence of such exposure is present in their work histories [46]. The semi-quantitative indices of asbestos exposure intensity assume that the intensity of exposure is proportional to the contamination of the environment surrounding the factory and that the contamination decreases with distance.

Amphiboles were found in the patients' lungs, presumably due to the slower clearance of amphiboles in the lungs. Considering the clearance of asbestos fibers and the average of 22.5 years of delay between the cessation of exposure and the collection of tissue samples, during exposure or immediately after the pulmonary load was probably much higher.

A linear relationship was observed between the pulmonary fiber load and the cumulative dose indices in the five subjects with environmental exposures. In the absence of systematic measurements of asbestos fiber concentrations in the Bari ambient air when the factory was active, these results provide information on past exposure to asbestos associated with contamination of the urban environment. Environmental exposure to a mixture of asbestos fibers can lead to a high pulmonary load of amphiboles even years after the cessation of exposure. The epidemiological data of an increased risk of mesothelioma for the general population of Bari, associated with asbestos contamination of the living environment, is confirmed [45].

In addition, from the data of COR Apulia on the years 1993/2015, there are 367 cases of mesothelioma among the residents of the city of Bari. 69% in males and 31% in females. For 70% of cases registered among residents (255) it was possible to reconstruct the exposure. 58% of reconstructed cases have occupational exposure, 3% family / domestic or extra-work exposure occurred as a result of leisure activities. 26% of the cases among the residents have environmental exposure, of these 45% are men and 55% are women. Among the cases resident in Bari, 107 occasions of environmental exposure were recorded due to Fibronit or Rossani. Among these cases, 62% was classified as environmental exposure in the absence of other exposures. On the contrary, for 38% of subjects who also had occupational exposure, despite having residences close to the two sources of exposure,

they were not classified as environmental exposures as they are multiple exposures.
Six family clans in blood relatives are present in the cases of COR Apulia related to occupational, family or environmental exposures due to Fibronit. Three sisters who lived inside the plant in the apartments available to employees [47]. Two brothers who worked in Fibronit and a couple, a father who worked in Fibronit and son with environmental exposure for residence near the plant for 36 years [48]. A mother and daughter couple both lived for 11 years near Fibronit [45]. A mother and son couple living near the plant for over 24 years, and a brother couple who worked in Fibronit and sister with family exposure, had washed his brother's suits for 11 years.
Recently from the regional registry data four new cases of MM emerged, one peritoneal and three pleural caused by environmental exposure to asbestos due to the presence of a military barracks located in a semi-central urban area [49, 50].

Discussion

This study, consistent with literature, confirms the adverse health effects of asbestos environmental pollution in the Municipality of Bari resulting from the presence of the three contaminated sites in this area.
Since 1960, some authors showed the risk of pleural mesothelioma associated with asbestos exposure in South Africa, some of the cases reported have been attributed to environmental exposure [9].
Malignant mesothelioma cases found in the city of Bari are emblematic of unconscious environmental exposure in a large polluted city. In a recent review, Liu et al. [24] summarized the latest studies on the association between MM and environmental exposure to asbestos.
The role of non-occupational asbestos exposure (paraoccupational, domestic or environmental) in the occurrence of MM has already been demonstrated in numerous studies. In a recent meta-analysis Marsh et al. [23] confirmed an increased risk of pleural MM from non-occupational exposure to asbestos RR = 6.9 (95% CI 4.2 to 11.4).
In this regard, IARC (2012, No. 100) [51] states that "In studies of asbestos concentrations in outdoor air, chrysotile is the predominantly detected fiber. Low levels of asbestos have been measured in rural areas (typical concentration, 10 fibers/m3 [f/m3]). Typical concentrations are about 10 fold higher in urban locations and about 1000 times higher in proximity of industrial sources of exposure (e.g. asbestos mine or factory, demolition site, or improperly protected asbestos-containing waste site)".
Moreover, when mesothelioma is due to environmental exposure, the M: F ratio is 1:1 and the median age at diagnosis is 60 years. The duration and intensity of exposure to asbestos are positively associated with the risk of MM [52, 53].
Because of the long latency period and the limited number of cases, the study of the environmental risk of MM is challenging [54]. Furthermore, considering that it is difficult to obtain an early diagnosis with radiological techniques alone, it is important to use biological indicators especially in the early stages [55]. Among these, mesothelin is one of the several well-know biomarkers used in the diagnosis of pleural malignant [56].
Other studies have found a significant risk of MM caused by residence near asbestos cement production plants without an occupational exposure [57, 58]. These studies highlight the importance of assessing the impact of exposure to asbestos not only among workers but also among their cohabiting family members and in the general population.
Orenstein and Schenker [59] studied the association between the distance of residence from the source of environmental exposure, the decrease in the duration of exposure and the risk of MM. Environmental exposure studies have shown that the increase in the distance from pollution sources is associated with a decreased MM risk [33, 60]. Furthermore, in 1989 Spurny established that fibers released from asbestos cement products have the same carcinogenic charge as standard chrysotile [61].
Regarding the four cases of MM selected from Apulia Regional Mesothelioma Register, all patients lived or worked at distances from 12 to 100 meters from the military barracks and from 200 to 1200 meters from Fribronit plant. In particular, patient n. 4 had an important exposure because he lived and worked closely with the two sources of pollution.
Instead, the patient n. 1 with peritoneal mesothelioma lived close to the source of pollution continuously from birth for 36 years and his long exposure, even if not professional, is in agreement with what was claimed by Hodgson and Darnton [62]. The patient was 36 at diagnosis. He was treated at a specialized center in France (Gustave Roussy Institute) where he underwent cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy with oxaliplatin (HIPEC). An exposure to residential/environmental asbestos was ascertained: he had lived in a building less than 12 meters from the barracks for 36 years from birth: the apartment had overlooked the military barracks and since he was a child he had played to soccer in the camp of the barracks. Following a follow-up in April 2017, more than 18 years (221 months) after diagnosis and 216 months after treatment, the patient is alive and without recurrence. Currently, he has chronic diarrhea and chronic abdominal pain.
When the tumor occurs in younger people, generally, a genetic predisposition and environmental exposure to asbestos or other mineral fibers are implicated. Cytogenetic studies have shown chromosomal and genetic alterations in patients with MM could play an important role in the initial development and subsequent progression of tumor [63 64]. Molecular analysis with comparative genomic hybridization (CGH-array), performed on tumor samples embedded in paraffin, revealed multiple chromosomal anomalies (copy number alterations CNAs), with prevalent amplifications. Deletions have

been found in 1q21, 2q11.1 → 2q13, 8p23.1, 9p12 → 9p11, 9q21.33 → 9q33.1, 9q12 → 9q21.33 and 17p12 → 17p11.2. Chromosomes 3p21 (BAP1), 9p21 (CDKN2A) and 22q12 (NF2) were not affected. Such findings are rare in malignant peritoneal mesothelioma. Some chromosomal aberrations that in this case seem to be random, could justify the response to therapy and long survival, thus revealing useful prognostic factors in the peritoneum MM [50].

The authors report that peritoneal mesotheliomas increase with the square of cumulative exposure to asbestos and conclude that any further unit of exposure would have added lower risk for pleural MM and greater risk for peritoneal MM. Furthermore, Welch et al. [65], found a strong association (OR, 5.0; 95% CI: 1.2-21.5) between exposure in a population control case-study. of asbestos and peritoneal mesotheliomas in cases with relatively low exposures and conclude that intermittent or low asbestos exposure is associated with peritoneal mesothelioma. More recently, Dragon et al. [66] found that the difference in the incidence of pleural and peritoneal MM, in the same conditions of exposure to asbestos, is due to the different physiology of mesothelial cells in charge of a different inflammatory response.

This study, in agreement with the literature, confirms the negative health effects of environmental asbestos pollution in the city of Bari [33, 44]. Data on the environmental concentration of asbestos in the barracks and on the Fibronit site are not available. Fibronit fiber concentration measurements were only available with data limited to the 1970s. At that time up to 20 ff/cc of asbestos fibers in the air were measured (length > 5 μm and diameter> 0.3 μm). In 1972, up to 10 ff / cc and in 1974, after reclamation, concentrations ranging from 4 to 19 ff/cc were reported [32]. In the mid-1970s, environmental measurements near the plant in areas away from roads, without urban traffic pollution, reported average concentration values of 16.06 x 10-4 "particles (< 5 microns in size) per cc d 'air' [34], but were certainly not representative of the high level of pollution in previous decades.

The level of environmental pollution present at the time in the city of Bari, corresponding to the years of exposure in the cases discussed here, can also be estimated from the comparison with the currently recognized background levels. The exposure level at the background level corresponds to an average cumulative exposure of less than 0.1 fibers/mL-y, an average concentration of about 0.1 fibers/l, as reported by the monograph of the International Research Agency on Cancer (IARC) n. 100 (2012) [51]. Furthermore, the World Health Organization (WHO) has estimated that with continuous exposure at 0.4-1 fibers / l, the risk of MM would be from (4 to 10) × 100,000. The linear extrapolation to 0.1 fibers/l (the current background level) would correspond to an excess in the order of one case (from 0.4 to 2.5) of MM per 100,000 people [67].

The process of spreading asbestos fibers, both from the factory and from the military barracks, to the surrounding areas was favored by physical mechanisms and was confirmed by the history of urban expansion in the city of Bari around the two sites. Urban development and natural weather conditions have potentially contributed to release asbestos fibers into the environment. The wind direction influences the concentration of asbestos fibers in specific areas and the concentrations of asbestos in the air surrounding the point of emission depend on the direction and speed of the wind [68]. Furthermore, Abakay et al. [69] studied the risk of MM in relation to meteorological and geological conditions and the distance from the natural source of asbestos (naturally occurring asbestos – NOA) and found a greater risk near NOA and in the direction of the wind (downwind of the source). The study showed that the distance of a residence from a natural source of asbestos contamination and the prevailing wind conditions in the area can influence the risk of developing environmental mesothelioma. Other authors, Kurumatani, Kumagai [70] and Tarres et al. [71], also studied the effect of meteorological conditions on MM. In their studies, the dominant wind direction influenced the MM risk and therefore meteorological factors could be related to pleural MM deaths through environmental exposure. A high standardized mortality ratio (SMR) has been reported among people living in areas of relatively high concentration levels. Therefore, the authors conclude that a parameter that includes the meteorological conditions is a better *proxy* of the exposure dose than the distance of residence from the source of pollution and could be useful for a more accurate investigation of the effects of asbestos exposure between the residents [70]. Fazzo et al. [72], in a study on the incidence of tumors, reported that the highest values in the polluting sectors were consistent with the directions of the prevailing winds and confirmed that the air quality in the areas defined as "contaminated sites" (CS) is influenced by industrial atmospheric emissions.

In this study the meteorological data of the period of interest, from 1912 for the Rossani barracks and from 1933 for the Fibronit, were not available, so the meteorological data and the direction of the winds of the last years (1961-1990) were considered as in our previous study [33]. The diffusion of the wind does not seem to play an important role due to the absence of clearly dominant winds.

Furthermore, in the study conducted by Barbieri on the loading of asbestos fibers in the lungs of five patients who had lived in Bari (age at diagnosis from 36 to 65 years) and residence at distances ranging from 200 to 2000 m from Fibronit (from1960 to 1997), a linear relationship between the pulmonary load and the environmental exposure indices based on the distance between the residence and the factory has been demonstrated [2]. These results can provide information on the past exposure to asbestos associated with the contamination of the urban environment of Bari, in particular considering that the cases discussed lived at a distance between 200 and 1200 meters from Fibronit.

A systematic review of the quantitative relationship between MM and asbestos exposure was carried out for the second Italian Consensus Conference on Malignant Mesothelioma of the Pleura, in which it was document-

ed that the MM incidence is increased with cumulative exposure to asbestos, the pulmonary fiber load and the duration of exposure [73]. In the study of the epidemiology of MM, the cumulative exposure is a proxy for the relevant exposure and the duration and intensity of the exposure are independent determinants of the appearance of MM [73]. The same conclusion was reported in the third Italian Consensus Conference on Malignant Mesotheliomas of the Pleura [74, 75]. Furthermore, a recent case-control study [76] explored the relationship between cumulative exposure and pleural MM after non-occupational exposure and studied the risk associated with asbestos materials in residential areas, with a cumulative exposure index for estimate exposure frequency, duration and intensity. The study showed a relationship between pleural MM risk and cumulative exposure after non-occupational environmental exposure (OR = 2.0 95% CI 1.2-3.2) and confirmed the quantitative relationship between MM incidence and cumulative exposure to asbestos, even at low levels of exposure due to non-occupational exposure (OR = 3.8 95% CI 1.3-11.1). The assessment of environmental exposure was based on the distance between the residence and the source of pollution.

ReNaM has documented that 10.2% of MM cases are due to unprofessional exposure to asbestos [77]. In particular, in our regional register, 10.9% of cases are due to environmental exposure [78]. These data confirm the difficulty in recognizing and attributing non-occupational exposure to asbestos even though this type of exposure is becoming increasingly common among new mesothelioma cases. Coherently with Armstrong, Driscoll [79], this can be defined as exposure to the "third wave". Indeed, Armstrong [76] defined "third wave exposure" as both occupational and non-occupational exposure to asbestos following repairs, renovations, demolition of buildings and environmental exposure to asbestos.

The history of these military barracks shows that the deterioration of asbestos in situ, the removal of asbestos and its exposure require careful control of the concentrations of asbestos fibers in urban air and in areas close to situations considered to be particularly dangerous, such as the renovation or demolition of houses and buildings constructed with asbestos cement products.

Conclusion

In view of the official data available, the sources of asbestos pollution in the city of Bari and the impact on human health coming from asbestos exposure were highlighted.

The presence of the asbestos cement factory and military barracks in urban area is correlated with the increased risk of MM in resident population. The lack of recover and decontaminate of the two areas within the city was a serious public health problem.

According to the second government conference [80] and the national asbestos plan [81], environmental exposure to asbestos and risk of malignant mesothelioma are research priority for ReNaM and COR; the regions will have to commit the regional COR or other competent structures to investigate the degree of risk of mesothelioma related to non-professional exposure (environmental or para-occupational). Moreover, the scientific research, the establishment of the regional registry and the continuous confirmation of the effects of environmental exposure to asbestos have increased the risk awareness among the citizens of Bari and have led the authorities to plan the reclamation of contaminated sites to safeguard health public.

Epidemiological studies has shown that the risk of mesothelioma increases with the increase in exposure to asbestos fibers. There are no doubts regarding the proportional relationship between cumulative dose, and frequency of mesothelioma [73, 74, 82]. Furthermore, the most recent exposures have a lower weight, not a zero weight [83]. As with all carcinogens, however, there is no safety "threshold" below which the risk is zero [74].

Epidemiological surveillance of incident cases of mesothelioma is important to understand the damage to health due to exposure to asbestos, to identify exposure situations still present in the territory and to assess the evolution of asbestos exposure. In agreement with most of the data in the literature, both workers and their families should wear specific personal protective equipment devices to reduce the risk of adverse health effects [84-86].

The registration of mesothelioma cases is essential to epidemiological knowledge develop and to support research activities. It is an instrument of control and prevention of risks, an indicator to guide the choices and organization of health services in terms of public health and population needs [80, 87].

The risk associated with residual non-occupational and environmental exposure after the asbestos ban should not be underestimated. For environmental (residential) exposures the duration of exposure is the duration of the residence period and is a proxy for the cumulative dose to which residents have been exposed. It should be emphasized that in the work of Ferrante et al. [76], an increase in the risk of MM is observed with the increase in the cumulative exposure to asbestos. This increase is also observed when only those subjects who have had non-working exposure to asbestos are considered, as well as for the exposures to asbestos artefacts, including in particular the coverings, flooring materials and other asbestos cement materials in work (in situ). After the cessation of processing, the danger to public health is the presence of both large quantities of materials containing asbestos in a friable matrix, in civil and industrial buildings, in systems and means of transport (e.g naval), and in significant quantities of materials containing asbestos in a compact matrix whose progressive deterioration can cause the release of fibers and the consequent risk to health. Interventions are therefore required to remove even the residual environmental exposure [88] as demonstrated also by the data on the cases discussed here.

The experience of the city of Bari also shows that, being environmental exposures and taking into account the interest of citizens for the protection of public health, the timely identification of the most appropriate recovery

decisions is essential in the risk management process. reclamation as well as the elaboration of specific communication interventions. Particularly important are the psychological support interventions in mesothelioma-affected communities [89]. The asbestos emergency in the city of Bari, which in this chapter was summarily reconstructed, also highlights the importance of EHL (Environmental Health Literacy), literacy on environmental health, which can be defined as the ability to search, understand , evaluate and use information on public health and the environment to encourage the adoption of informed choices, the reduction of health risks, the improvement of the quality of life and the environment. For this reason it is necessary to adopt a communication strategy that involves different stakeholders, health professionals, authorities, local communities, media, presenting results of environmental epidemiology research useful for health interventions and health promotion activities that actively involve the whole community in a participatory process [90, 91].
Public health can directly pursue the public good in terms of the maximum advantage for the greatest number of subjects, or it can have a privileged consideration for the worst situations [92]. It appears essential, in the light of current scientific knowledge, to adopt the precautionary principle to pursue the best solutions with respect to local priorities and the specific needs associated with reducing the health impact of involuntary exposure to asbestos through timely remediation, rehabilitation and surveillance on which the international scientific community agrees.

Acknowledgements

Funding sources: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

LV, DC and GS developed and designed the study. FM and ESSC wrote the manuscript. LV, DC and AC revised it. LDM and MCD entered data. All authors read and approved the final manuscript.

References

[1] Vimercati L, Carrus A, Bisceglia L, Tatò I, Bellotta MR, Russo A, Martina G, Daprile C, Di Leo E, Nettis E, Assennato G. Biological monitoring and allergic sensitization in traffic police officers exposed to urban air pollution. Int J Immunopathol Pharmacol 2006;19(4 Suppl):57-60. Available at: https://www.ncbi.nlm.nih.gov/pubmed/17291408 (Accessed on 18 November 2019).

[2] Zhao Y, Hu J, Tan Z, Liu T, Zeng W, Li X, Huang C, Wang S, Huang Z, Ma W. Ambient carbon monoxide and increased risk of daily hospital outpatient visits for respiratory diseases in Dongguan, China. Sci Total Environ 2019;668:254-60. https://doi.org/10.1016/j.scitotenv.2019.02.333

[3] Campo L, Vimercati L, Carrus A, Bisceglia L, Pesatori AC, Bertazzi PA, Assennato G, Fustinoni S. Environmental and biological monitoring of PAHs exposure in coke-oven workers at the Taranto plant compared to two groups from the general population of Apulia, Italy. Med Lav 2012;103:347-60. Available at: https://mattioli1885journals.com/index.php/lamedicinadellavoro/article/view/2047 (Accessed on 18 November 2019).

[4] Jiang Y, Hu X, Yves UJ, Zhan H, Wu Y. Status, source and health risk assessment of polycyclic aromatic hydrocarbons in street dust of an industrial city NW China. Ecotoxicol Environ Saf 2014;106:11-8. https://doi.org/10.1016/j.ecoenv.2014.04.031

[5] Vimercati L. Traffic related air pollution and respiratory morbidity. LungIndia 2011;28:238. https://doi.org/10.4103/0970-2113.85682

[6] Vimercati L, Baldassarre A, Gatti M.F, Gagliardi T, Serinelli M, De Maria L, Caputi A, Dirodi AA, Galise I, Cuccaro F, Assennato G. Non-occupational exposure to heavy metals of the residents of an industrial area and biomonitoring. Environ Monit Assess 2016;188:673. https://doi.org/10.1007/s10661-016-5693-5

[7] Røe OD, Stella GM. Malignant pleural mesothelioma: history, controversy and future of a manmade epidemic. Eur Respir Rev 2015;135:115-31. https://doi.org/10.1183/09059180.00007014

[8] Visonà S.D. Villani S, Manzoni F, Chen Y, Ardissino G, Russo F, Moretti M; Javan GT, Osculati A. Impact of asbestos on public health: a retrospective study on a series of subjects with occupational and non-occupational exposure to asbestos during the activity of Fibronit plant (Broni, Italy). J Public Health Res 2018;7:1519. https://doi.org/10.4081/jphr.2018.1519

[9] Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. Br J Ind Med 1960;17:260-71. https://doi.org/10.1136/oem.17.4.260

[10] Fowler PB, Sloper JC, Warner EC. Exposure to asbestos and mesothelioma of the pleura. Br Med J 1964;2:211-3. https://doi.org/10.1136/bmj.2.5403.211

[11] Greenberg M. Biological effects of asbestos: New York Academy of Sciences 1964. Am J Ind Med 2003;43:543-52. https://doi.org/10.1002/ajim.10192

[12] International Agency for Research on Cancer. Some inorganic and organometallic compounds. In Monographs on the Evaluation of the carcinogenic risk of chemicals to man. WHO/IARC: Lyon, France, 1973;2:17-47. Available at: https://monographs.iarc.fr/wp-content/uploads/2018/06/mono87.pdf (Accessed on 18 November 2019).

[13] Vimercati L, Carrus A, Martino T, Galise I, Minunni V, Caputo F, Dell'Erba A, Assennato G. Formaldehyde exposure and irritative effects on medical examiners, pathologic anatomy post-graduate students and technicians. Iran J Public Health 2010;39:26-34. Available at: https://www.academia.edu/21676695/Formaldehyde_exposure_and_irritative_effects_on_medical_examiners_pathologic_anatomy_post-graduate_students_and_technicians (Accessed on 18 November 2019).

[14] Mensi C, Romano A, Dallari B, Freddo M, Trinco R, Consonni D, Riboldi L. (2017) "Tumore naso-sinusale ed esposizione recente a polveri di legno / Sinonasal cancer and recent exposure to wood dust". La Medicina del Lavoro 2017;108:233-5. Available at: https://mattioli1885journals.com/index.php/lamedicinadellavoro/article/view/6540 (Accessed on 18 November 2019).

[15] Awan KH, Hegde R, Cheever VJ, Carroll W, Khan S, Patil S, Warnakulasuriya S. Oral and pharyngeal cancer risk associated with occupational carcinogenic substances: Systematic review. Head Neck 2018;40:2724-32. https://doi.org/10.1002/hed.25486

[16] Pira E, Donato F, Maida L, Discalzi G. Exposure to asbestos: past, present and future. J Thorac Dis 2018;10(Suppl 2):237-45. https://doi.org/10.21037/jtd.2017.10.126

[17] Carbone M, Ly BH, Dodson RF, Pagano I, Morris PT, Dogan UA, Gazdar AF, Pass HI, Yang H. Malignant mesothelioma: facts, myths, and hypotheses. J Cell Physiol 2012;227:44-58. https://doi.org/10.1002/jcp.22724

[18] Loomis D, Richardson DB, Elliott L. Quantitative relationships of exposure to chrysotile asbestos and mesothelioma mortality. Am J Ind Med 2019;62:471-7. https://doi.org/10.1002/ajim.22985

[19] Bianchi C, Giarelli L, Grandi G, Brollo A, Ramani L, Zuch C. Latency periods in asbestos-related mesothelioma of the pleura. Eur J Cancer Prev 1997;6:162-6. Available at: https://www.ncbi.nlm.nih.gov/pubmed/9237066 (Accessed on 18 November 2019).

[20] Pan XL, Day HW, Wang W, Beckett LA, Schenker MB. Residential proximity to naturally occurring asbestos and mesothelioma risk in California. Am J Respir Crit. Care Med 2005;172:1019-25. https://doi.org/10.1164/rccm.200412-1731OC

[21] Baumann F, Buck BJ, Metcalf RV, McLaurin BT, Merkler DJ, Carbone M. The presence of asbestos in the natural environment is likely related to mesothelioma in young individuals and women from Southern Nevada. J,Thorac Oncol 2015;10:731-7. https://doi.org/10.1097/JTO.0000000000000506

[22] Magnani C, Terracini B, Ivaldi C, Botta M, Mancini A, Andrion A. Pleural malignant mesothelioma and non-occupational exposure to asbestos in Casale Monferrato. Italy. Occup Environ Med 1995;52:362-7. https://doi.org/10.1023/A:1007691003600

[23] Marsh GM, Riordan A, Keeton KA, Benson S. Non-occupational exposure to asbestos and risk of pleural mesothelioma: review and meta-analysis. Occup Environ Med 2017;74:838-46. https://doi.org/10.1097/CEJ.0b013e32832f9bee

[24] Liu B, van Gerwen M, Bonassi S, Taioli E. International Association for the Study of Lung Cancer Mesothelioma Task Force. Epidemiology of Environmental Exposure And Malignant Mesothelioma. J Thorac Oncol 2017;12:1031-45. https://doi.org/10.1016/j.jtho.2017.04.002

[25] Pasetto R, Comba P, Marconi A. Mesothelioma associated with environmental exposures. Med Lav 2005;96:330-7. Available at: https://www.ncbi.nlm.nih.gov/pubmed/16457429 (accessed 18 November 2019).

[26] Marinaccio A, Binazzi A, Marzio DD, Scarselli A, Verardo M, Mirabelli D, Gennaro V, Mensi C, Riboldi L. Merler E, Zotti R.D, Romanelli A, Chellini E, Silvestri S, Pascucci C, Romeo E, Menegozzo S, Musti M, Cavone D, Cauzillo G, Tumino R, Nicita C, Melis M, Iavicoli S. ReNaM Working Group. Pleural malignant mesothelioma epidemic: incidence, modalities of asbestos exposure and occupations involved from the Italian National Register. Int J Cancer 2012;130:2146-54. https://doi.org/10.1002/ijc.26229

[27] Directive 2003/18/EC of the European Parliament and of the Council of 27 March 2003 amending Council Directive 83/477/EEC on the protection of workers from the risks related to exposure to asbestos at work. Official Journal of the European Union. Available at: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:097:0048:0052:EN:PDF (Accessed on 13 June 2019).

[28] Legge 27 marzo 1992, n. 257 - Norme relative alla cessazione dell'impiego dell'amianto - Suppl.Ord. alla Gazzetta Ufficiale n. 87 del 13 aprile 1992. Available at: https://www.gazzettaufficiale.it/eli/id/1992/04/13/092G0295/sg (Accessed on 13 June 2019).

[29] Ministero della Salute. Stato dell'arte e prospettive in materia di contrasto alle patologie asbesto-correlate. Quaderni del Ministero della salute 2012. http://www.quotidianosanita.it/allegati/allegato6269534.pdf (Accessed on 13 June 2019).

[30] World Health Organization. Regional Office for Europe. Contaminated sites and health: Report of two WHO workshops: Syracuse, Italy, 18 November 2011 & Catania, Italy, 21-22 June 2012. WHO: Copenhagen, Denmark, 2013. Available at: https://apps.who.int/iris/bitstream/handle/10665/108623/e96843.pdf?sequence=1&isAllowed=y (Accessed on 18 November 2019).

[31] Documento sulle bonifiche dei siti contaminati (archivio 1998 - 2003). Available at: https://lexambiente.it/materie/danno-ambientale/19-ceag-legambiente19/788 (Accessed on 13 June 2019).

[32] Coviello V, Carbonara M, Bisceglia L, Di Pierri C, Ferri GM, Lo Izzo A, Porro A, Sivo D, Assennato G. Mortality in a cohort of asbestos cement workers in Bari. Epidemiol Prev 2002;26:65-70. Available at: https://www.ncbi.nlm.nih.gov/pubmed/12125387 (Accessed on 18 November 2019).

[33] Musti M, Pollice A, Cavone D, Dragonieri S, Bilancia M. The relationship between malignant mesothelioma and an asbestos cement plant environmental risk: a spatial case-control study in the city of Bari (Italy). Int Arch Occup Environ Health 2009;82:489-97. https://doi.org/10.1007/s00420-008-0358-5

[34] Napoli S. Asbestos pollution: criticism on methods and experiences in the city of Bari. Lav Um 1975;27(5):148-55. Available at: https://www.ncbi.nlm.nih.gov/pubmed/1219263 (Accessed on 18 November 2019).

[35] The Contaminated Land Rehabilitation Network. Clarinet. Available at: https://www.umweltbundesamt.at/en/clarinet (Accessed on 13 June 2019).

[36] Losito G. Analisi delle modificazioni al territorio connesse ai siti inquinati in territori urbani (brownfields): il caso dell'ex-Fibronit di Bari. Available at http://cdca.it/wp-content/uploads/2017/05/Giuseppe_Losito.pdf (Accessed on 13 June 2019).

[37] Giua R, Bonanno V, Gagliardi N. Inquinamento 2006;79:38-42. Available at: http://energia-plus.it/wp-content/uploads/sites/5/2009/06/20060201025_11.pdf (Accessed on 13 June 2019).

[38] Signorile N. Diario Rossani. Caratterimobili. 2014.

[39] Pirastu R, Iavarone I, Pasetto R, Zona A, Comba P. SENTIERI - Studio Epidemiologico Nazionale dei Territori e degli Insediamenti Esposti a Rischio da Inquinamento: Risultati. Epidemiol Prev 2011;35(5-6 Suppl.4):1-204. Available at: http://www.epiprev.it/materiali/2011/SENTIERI/EP2011Sentieri2_lr_full.pdf (Accessed on 18 November 2019).

[40] Binazzi A, Zona A, Marinaccio A, Bruno C, Corfiati M, Fazzo L, Menegozzo S, Nicita C, Pasetto R, Pirastu R, De Santisi M, Comba P. GdL SENTIERI-ReNaM.[SENTIERI-ReNaM: Results]. Epidemiol Prev 2016;40(5Suppl1):19-98. https://doi.org/10.19191/EP16.5S1.P001.097

[41] Belli S, Bruno C, Comba P, Grignoli M. Mortalità per causa specifica dei lavoratori del cemento-amianto di Bari titolari di rendita per asbestosi. Epidemiol Prev 1998;22:8-11. Available at: http://old.iss.it/dspace/handle/2198/-7934 (Accessed on 18 November 2019).

[42] Bisceglia L, Musti M, Giua R, Assennato G. The asbestos crisis in an urban area: the Bari experience. Epidemiol Prev 2007;31(1 Suppl 2):54-8. Available at [italian]: http://www.epidemiologiaeprevenzione.it/materiali/ARCHIVIO_PDF/Suppl/2007/EP_V31I1S2_54-58.pdf (Accessed on 18 November 2019).

[43] Nannavecchia AN, Cuccaro F, Bisceglia L, Coviello E, Baldassarre A, Caputo E, Assennato G. Gruppo di lavoro del Centro di coordinamento Registro Tumori Puglia. Mortalità in una coorte di lavoratori del cemento-amianto a Bari: aggiornamento al 2012. Not Ist Super Sanità 2016;29(7-8). Available at: https://www.epicentro.iss.it/ben/2016/luglio-agosto/1 (Accessed on 18 November 2019).

[44] Bilancia M, Cavone D, Pollice A, Musti M. Assessment of risk of mesothelioma: the case of an asbestos-cement production plant in the city of Bari. Epidemiol Prev 2003;27:277-84. Available at: https://www.ncbi.nlm.nih.gov/pubmed/14735839 (Accessed on 18 November 2019).

[45] Barbieri PG, Mirabelli D, Somigliana A, Cavone D, Merler E.

Asbestos fibre burden in the lungs of patients with mesothelioma who lived near asbestos-cement factories. Ann Occup Hyg 2012;5:660-70. https://doi.org/10.1093/annhyg/mer126

[46] Tossavainen A. Asbestos, asbestosis, and cancer: the Helsinki criteria for diagnosis and attribution. Scand J Work Environ Health 1997;23:311-6. https://doi.org/10.5271/sjweh.226

[47] Musti M, Cavone D, Aalto Y, Scattone A, Serio G, Knuutila S. A cluster of familial malignant mesothelioma with del (9p) as the sole chromosomal anomaly. Cancer Genet Cytogenet 2002;138:73-6. https://doi.org/10.1016/S0165-4608(02)00575-7

[48] Ascoli V, Cavone D, Merler E, Barbieri P.G, Romeo L, Nardi F, Musti M. Mesothelioma in blood related subjects: report of 11 clusters among 1954 Italy cases and review of the literature. Am J Ind Med 2007;50:357-69. https://doi.org/10.1002/ajim.20451

[49] Vimercati L, Cavone D, Lovreglio P, De Maria L, Caputi A, Ferri GM, Serio G. Environmental asbestos exposure and mesothelioma cases in Bari, Apulia region, southern Italy: a national interest site for land reclamation. Environ Sci Pollut Res Int 2018;25:15692-701.https://doi.org/10.1007/s11356-018-1618-x

[50] Serio G, Pezzuto F, Marzullo A, Scattone A, Cavone D, Punzi A, Vimercati L. Peritoneal mesothelioma with residential asbestos exposure. report of a case with long survival (seventeen years) analyzed by Cgh-array. Int J Mol Sci 2017;18:1818. https://doi.org/10.3390/ijms18081818

[51] International Agency for Research on Cancer. Arsenic, metals, fibres, and dusts. In Monographs on the Evaluation of carcinogenic risks to humans. WHO/IARC: Lyon, France, 2012; Volume 100C;pp. 11–465. Available at: https://monographs.iarc.fr/wp-content/uploads/2018/06/mono100C.pdf (Accessed on 18 November 2019).

[52] Espina C, Straif K, Friis S, Kogevinas M, Saracci R, Vainio H, Schüz J. European code against cancer 4th Edition: Environment, occupation and cancer. Cancer Epidemiol 2015;39:84-92.

[53] Imai M, Hino O. Environmental carcinogenesis - 100th anniversary of creating cancer. Cancer Sci 2015;106:1483-5. https://doi.org/ 10.1111/cas.12798

[54] Baumann F, Carbone M. Environmental risk of mesothelioma in the United States: An emerging concern-epidemiological issues. J Toxicol Environ Health B Crit Rev 2016;19:231-49. https://doi.org/10.1080/10937404.2016.1195322

[55] Ray M, Kindler HL. Malignant pleural mesothelioma: an update on biomarkers and treatment. Chest 2009;136:888-96. https://doi.org/10.1378/chest.08-2665

[56] Dipalma N, Luisi V, Di Serio F, Fontana A, Maggiolini P, Licchelli B, Mera E, Bisceglia L, Galise I, Loizzi M, Pizzigallo M.A, Molinini R, Vimercati L. Biomarkers in malignant mesothelioma: diagnostic and prognostic role of soluble mesothelin-related peptide. Int J Biol Markers 2011;26:160-5. https://doi.org/10.18632/oncotarget.17436

[57] Mensi C, Riboldi L, De Matteis S, Bertazzi P.A, Consonni D. Impact of an asbestos cement factory on mesothelioma incidence: global assessment of effects of occupational, familial, and environmental exposure. Environ Int 2015;74:191-9. https://doi.org/10.1016/j.envint.2014.10.016

[58] Fazzo L, Menegozzo S, Soggiu ME, De Santis M, Santoro M, Cozza V, Brangi A, Menegozzo M, Comba P. Mesothelioma incidence in the neighbourhood of an asbestos-cement plant located in a national priority contaminated site. Ann Ist Super Sanita 2014;50:322-7. https://doi.org/10.4415/ANN_14_04_05

[59] Orenstein M.R, Schenker M.B. Environmental asbestos exposure and mesothelioma. Curr Opin Pulm Med 2000;6:371-7. https://doi.org/10.1097/00063198-200007000-00020

[60] Maule MM, Magnani C, Dalmasso P, Mirabelli D, Merletti F, Biggeri A. Modeling mesothelioma risk associated with environmental asbestos exposure. Environ Health Perspect 2007;115:1066-71. https://doi.org/10.1289/ehp.9900

[61] Spurny K.R. On the release of asbestos fibers from weathered and corroded asbestos cement products. Environ Res 1989;48:100-16. https://doi.org/10.1016/s0013-9351(89)80089-1

[62] Hodgson JT, Darnton A. The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. Ann Occup Hyg 2000;44:565-601. Available at: https://pdfs.semanticscholar.org/16b8/0f0045ecbe5de992e04a9f8bcb5a16fda086.pdf (Accessed on 18 November 2019).

[63] Chirac P, Maillet D, Leprêtre F, Isaac S, Glehen O, Figeac M, Villeneuve L, Péron J, Gibson F, Galateau-Sallé F, Gilly F.N, Brevet M. Genomic copy number alterations in 33 malignant peritoneal mesothelioma analyzed by comparative genomic hybridization array. Hum Pathol 2016;55:72-82. https://doi.org/10.1016/j.humpath.2016.04.015

[64] Serio G, Vimercati L, Pennella A, Gentile M, Cavone D, Buonadonna A.L, Scattone A, Fortarezza F, De Palma A, Marzullo A. Genomic changes of chromosomes 8p23.1 and 1q21: novel mutations in malignant mesothelioma. Lung Cancer 2018;126:106-11. https://doi.org/10.1016/j.lungcan.2018.10.012

[65] Welch LS, Acherman YI, Haile E, Sokas RK, Sugarbaker PH. Asbestos and peritoneal mesothelioma among college-educated men. Int J Occup Environ Health 2005;11:254-258. https://doi.org/10.1179/107735205800245975

[66] Dragon J, Thompson J, MacPherson M, Shukla A. Differential susceptibility of human pleural and peritoneal mesothelial cells to asbestos exposure. J Cell Biochem 2015;116:1540-52. https://doi.org/10.1002/jcb.25095

[67] World Health Organization Regional Office for Europe. Air Quality Guidelines for Europe, 2nd ed, WHO Regional Publications, European Series, No. 91, 2000. Available at: http://www.euro.who.int/__data/assets/pdf_file/0005/74732/E71922.pdf (Accessed on 18 November 2019).

[68] Laamane A, Noro L, Raunio V. Observations on atmospheric air pollution caused by asbestos. Ann N Y Acad Sci 1965;132:240-54. https://doi.org/10.1111/j.1749-6632.1965.tb41105.x

[69] Abakay A, Tanrikulu AC, Ayhan M, Imamoglu MS, Taylan M, Kaplan MA, Abakay O. High-risk mesothelioma relation to meteorological and geological condition and distance from naturally occurring asbestos. Environ Health Prev Med 2016;21:82-90. https://doi.org/10.1007/s12199-015-0501-3

[70] Kurumatani N, Kumagai S. Mapping the risk of mesothelioma due to neighborhood asbestos exposure. Am J Respir Crit Care Med 2008;178:624-9. https://doi.org/10.1164/rccm.200801-063OC

[71] Tarrés J, Albertí C, Martínez-Artés X, Abós-Herràndiz R, Rosell-Murphy M, García-Allas I, Krier I, Cantarell G, Gallego M, Canela-Soler J, Orriols R. Pleural mesothelioma in relation to meteorological conditions and residential distance from an industrial source of asbestos. Occup Environ Med 2013;70:588-90. https://doi.org/10.1136/oemed-2012-101198

[72] Fazzo L, Carere M, Tisano F, Bruno C, Cernigliaro A, Cicero MR, Comba P, Contrino ML, De Santis M, Falleni F, Ingallinella V, Madeddu A, Marcello I, Regalbuto C, Sciacca G, Soggiu ME, Zona A. Cancer incidence in Priolo, Sicily: a spatial approach for estimation of industrial air pollution impact. Geospat Health 2016;11:320. https://doi.org/10.4081/gh.2016.320

[73] Pinto C, Novello S, Torri V, Ardizzoni A, Betta PG, Bertazzi PA, Casalini GA, Fava C, Fubini B, Magnani C, Mirabelli D, Papotti M, Ricardi U, Rocco G, Pastorino U, Tassi G, Trodella L, Zompatori M, Scagliotti G. Second Italian consensus conference on malignant pleural mesothelioma: state of the art and recommendations. Cancer Treat Rev 2013;39:328-39. https://doi.org/10.1016/j.ctrv.2012.11.004

[74] Magnani C, Bianchi C, Chellini E, Consonni D, Fubini B, Gennaro V, Marinaccio A, Menegozzo M, Mirabelli D, Merler E, Merletti F, Musti M, Oddone E, Romanelli A, Terracini B, Zona A, Zocchetti C, Alessi M, Baldassarre A, Dianzani I, Maule M, Mensi C, Silvestri S. III Italian Consensus Conference on Malignant Mesothelioma of the Pleura. Epidemiology, Public Health and Occupational Medicine related issues. Med Lav 2015;106:325-32. Available at: https://mattioli1885journals.

com/index.php/lamedicinadellavoro/article/view/4513/3368 (Accessed on 18 November 2019).

[75] Novello S, Pinto C, Torri V, Porcu L, Di Maio M, Tiseo M, Ceresoli G, Magnani C, Silvestri S, Veltri A, Papotti M, Rossi G, Ricardi U, Trodella L, Rea F, Facciolo F, Granieri A, Zagonel V, Scagliotti G. The Third Italian Consensus Conference for Malignant Pleural Mesothelioma: state of the art and recommendations. Crit Rev Oncol Hematol 2016;104:9-20. https://doi.org/10.1016/j.critrevonc.2016.05.004

[76] Ferrante D, Mirabelli D, Tunesi S, Terracini B, Magnani C. Pleural mesothelioma and occupational and non-occupational asbestos exposure: a case-control study with quantitative risk assessment. Occup Environ Med 2016;73:147-53. https://doi.org/10.1136/oemed-2015-102803

[77] Marinaccio A, Binazzi A, Bonafede M, Corfiati M, Di Marzio D, Scarselli A, Verardo M, Mirabelli D, Gennaro V, Mensi C, Schallembreg G, Merler E, Negro C, Romanelli A, Chellini E, Silvestri S, Cocchioni M, Pascucci C, Stracci F, Ascoli V, Trafficante L, Angelillo I, Musti M, Cavone D, Cauzillo G, Tallarigo F, Tumino R, Melis M, ReNaM working group. Malignant mesothelioma due to non-occupational asbestos exposure from the Italian national surveillance system (ReNaM): epidemiology and public health issues. Occup Environ Med 2015;72:648-55. https://doi.org/10.1136/oemed-2014-102297

[78] Marinaccio A, Binazzi A, Bonafede M, Branchi C, Corfiati M, Di Marzio D, Pirino F, Scarselli A, Iavicoli S, Verardo M, Mirabelli D, Gennaro V, Mensi C, Schallenberg G, Merler E, Negro. C, Romanelli A, Chellini E, Cocchioni M, Stracci F, Forastiere F, Trafficante L, Angelillo I, Musti M, Cauzillo G, Tallarigo F, Tumino R, Melis M, Mazzoleni G, Carrozza F. Gruppo di lavoro ReNaM. V Rapporto. Ed. 2015. INAIL. Tipolitografia INAIL - Milano, novembre 2015. Available at: https://www.inail.it/cs/internet/docs/allegato_renam_v_rapporto.pdf (Accessed on 13 June 2019).

[79] Armstrong B, Driscoll T. Mesothelioma in Australia: cresting the third wave. Public Health Res Pract 2016;26(2). https://doi.org/ 10.17061/phrp2621614

[80] Ministero della Salute. Atti della II Conferenza governativa sull'amianto e le patologie asbesto-correlate, Venezia, Italia, Fondazione Cini, 22-24 novembre 2012. Roma, Italia, Ministero della salute, 2012. Available at: http://www.ausl.mo.it/flex/cm/pages/ServeAttachment.php/L/IT/D/7%252Fd%252Fe%252FD.7838b1e5fca7fe72ae23/P/BLOB%3AID%3D28623/E/pdf (Accessed on 18 November 2019).

[81] Piano Nazionale Amianto. Linee di intervento per un'azione coordinata delle amministrazioni statali e territoriali. Marzo 2013. Available at: http://www.salute.gov.it/imgs/C_17_pubblicazioni_1945_allegato.pdf (Accessed on 13 June 2019).

[82] Magnani C, Fubini B, Mirabelli D, Bertazzi PA, Bianchi C, Chellini E, Gennaro V, Marinaccio A, Menegozzo M, Merler E, Merletti F, Musti M, Pira E, Romanelli A, Terracini B, Zona A. Pleural mesothelioma: epidemiological and public health issues. Report from the Second Italian Consensus Conference on Pleural Mesothelioma. Med Lav 2013;104:191-202. Available at: https://mattioli1885journals.com/index.php/lamedicinadellavoro/article/view/2423/2133 (Accessed on 18 November 2019).

[83] Collegium Ramazzini. Comments on the causation of malignant mesothelioma: rebutting the false concept that recent exposures to asbestos do not contribute to causation of mesothelioma. Industrial Health 2016;54:92-3. https://doi.org/10.2486/indhealth.cr02

[84] Beyene Gebrezgiabher B, Tetemke D, Yetum T. Awareness of occupational hazards and utilization of safety measures among welders in Aksum and Adwa Towns, Tigray Region, Ethiopia, 2013. J Environ Public Health 2019;2019:7. https://doi.org/10.1155/2019/4174085

[85] Vimercati L, Baldassarre A, Gatti MF, De Maria L, Caputi A, Dirodi AA, Cuccaro F, Bellino RM. Respiratory health in waste collection and disposal workers. Int J Environ Res Public Health 2016;13:631. https://doi.org/10.3390/ijerph13070631

[86] Luong Thanh B.Y, Laopaiboon M, Koh D, Sakunkoo P, Moe H. Behavioural interventions to promote workers' use of respiratory protective equipment. Cochrane Database Syst Rev 2016;12:CD010157. https://doi.org/10.1002/14651858.CD010157.pub2

[87] Marinaccio A, Scarselli A, Merler E, Iavicoli S. Mesothelioma incidence surveillance systems and claims for workers' compensation. Epidemiological evidence and prospects for an integrated framework. BMC Public Health 2012;12:314. https://doi.org/10.1186/1471-2458-12-314

[88] Stayner LT. Para-occupational exposures to asbestos: lessons learned from Casale Monferrato, Italy. Occup Environ Med 2015;73:145-6. https://doi.org/10.1136/oemed-2015-103233

[89] Granieri A. Community exposure to asbestos in Casale Monferrato: from research on psychological impact to a community needs-centered healthcare organization. Ann Ist Super Sanita 2015;51:336-41. https://doi.org/10.4415/ANN_15_04_14

[90] Marsili D, Comba P, De Castro P. Environmental health literacy within the Italian Asbestos Project: experience in Italy and Latin American contexts. Ann Ist Super Sanità 2015;51:180-2. https://doi.org/10.4415/ANN_15_03_02

[91] Sørensen K, Van den Broucke S, Fullam J, Doyle G, Pelikan J, Slonska Z, Brand H. (HLS-EU) Consortium Health Literacy Project European. Health literacy and public health: a systematic review and integration of definitions and models. BMC Public Health 2012:12(80). https://doi.org/10.1186/1471-2458-12-80

[92] Comba P, Martuzzi M, Botti C. The precautionary principle in decision-making: the ethical values. In: Martuzzi M, Tickner JA, eds. The precautionary principle: protecting public health, the environment and the future of our children. World Health Organization 2004: pp. 85-92.https://doi.org/10.1093/phe/phy005

Received on June 18, 2019. Accepted on November 7, 2019.

Correspondence: Luigi Vimercati, Department of Biomedical Science and Human Oncology, University of Bari Medical School, Giulio Cesare Square 11, 70124 Bari, Italy - Tel. +39 080 5478216 - E-mail: luigi.vimercati@uniba.it

How to cite this article: Vimercati L, Cavone D, Mansi F, Cannone ESS, De Maria L, Caputi A, Delfino MC, Serio G. Health impact of exposure to asbestos in polluted area of Southern Italy. J Prev Med Hyg 2019;60:E407-E418. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1330

ORIGINAL ARTICLE

Risk factors of occupation related back pain and neck pain among patients attending tertiary care hospital, Ahmedabad, India

V.R. DAVE, H.J. KHANPARA, R.P. SHUKLA, K.N. SONALIYA, J. TOLANI, R. PATEL
GCS Medical College Hospital & Research Centre, Opp DRM office, Naroda Road, Ahmedabad, Gujarat, India

Keywords

Back pain • Neck pain • Occupation • Risk factors

Summary

Introduction. *Neck/back pain is one of the common health problems associated with significant impact on health resulting in sickness absenteeism. Neck/back pain is one of important causes of disability adjusted life years worldwide. The objectives of study were: To identify various occupations related risk factors and their possible role in occurrence of back pain/neck pain and visual analogue scale(VAS) assessment of their perceived pain.*
Methods. *The study was conducted at one of the tertiary care hospital at Ahmedabad city, India. All patients above age of 18 years attending physiotherapy department for treatment of back pain/neck pain and gave consent were taken as study participants.Information about certain body postures in their lifestyle or at workplace which can have effects on back pain/neck pain were asked. VAS for perceived pain was anchored by "no pain" (score 0) and "pain as bad as it could be" (score 100). Data were entered in MS Excel and analyzed by frequency, contingency coefficient and Goodman and Kruskal's Gamma test.*
Results and Conclusion. *Total of 512 participants were included in study, among which(10.3%)and 392 (76.6%) participants had neck pain and back pain alone, respectively, while 67 (13.1%) participants had both neck and back pain. Age, marital status, socioeconomic class, body mass index and type of occupation revealed statistically significant association with severity of pain. Among participants with prolonged computer usage, back rest fitting to natural back curve and adjustable height of chair were significant factors for occurrence of neck pain.Various body postures like prolonged sitting/ standing, frequent bending at waist/ knee, pulling/pushing heavy objects, frequent weight lifting > 10 kg and repetitive movements of back/neck revealed as statistically significant risk factors for back/neck pain.*

Introduction

It has been estimated that between 70% and 85% of the population will have a back problem of some kind in their lifetime, and while many of these problems may be short term, some develop into a chronic condition with serious ramifications [1]. One similar research with meta-analysis suggested that pooled estimate for the occurrence of work absence in workers with back pain was 15.5% [2]. Occupational low back pain is the largest single health problem related to work and absenteeism at most common cause of incapacity among workers aged less than 45, it primarily affects young adults and is responsible for approximately one quarter of all cases of premature invalidity [3]. It has been estimated that 5-10% of cases of spinal pain become chronic [4, 5] and one fifth lead to pain-related disability one year after the first pain episode [6]. The global point prevalence of neck pain is estimated to be 4.9% with Disability-adjusted life years to 33.6 million in 2010. Out of all 291 conditions studied in the global burden of disease 2010 study, neck pain ranked 4th highest in terms of disability as measured by years lived with disability [7]. Low-back pain and neck pain are the biggest and fourth biggest causes of years of life with disability worldwide, respectively [8]. Back pain is one of the most frequent reasons for visiting a general practitioner or physiotherapists [9].
Risk indicator for back pain includes age, gender, education level, weight, height, right or left handed, number of children, smoking habits, body mass index, regular physical exercise, driving time, job, duration of work time, work time a week, manual lifting of heavy weights and uncomfortable static and awkward working positions, heavy physical work, night shifts, bending, twisting, pulling, and pushing and psychosocial factors like Perceived high pressure on time and workload, low job control, job dissatisfaction, monotonous work, and low support from co-workers and management [10, 11].
Studies suggest that between 60% and 90% of people will suffer from low back disorders at some point in their life and that at any one time between 15% and 42% of people are suffering (depending on the study population and the and the definition of back pain used). Data from the European survey on working conditions reveal that 30% of European workers suffer from back pain, which tops the list of all reported work related disorders [12]. Pain, discomfort and loss of function in the back, neck and extremities are common among working people [13].
The problems of back pain or neck pain are commonly neglected in initial stages by community mainly due to

its nonfatal course until it becomes excruciating. Even upon request of detailed investigation for diagnosis by treating doctors, patients are compelling to limit the treatment with painkillers or self-treat themselves. This behaviour has adverse impact on their health later on including sensory or motor disturbances of affected limb.

Objectives of the study were: 1) To identify various occupations related risk factors and their possible role in occurrence of back pain/neck pain and 2) To appreciate role of computer job work on occurrence of back pain/neck pain.

Methods

The present study was conducted at one of the tertiary care hospital attached with medical college at Ahmedabad city, Gujarat, India. All patients above age of 18 years attending physiotherapy department of the institute for treatment of back pain/neck pain and willing to give informed oral consent were taken as study participants. The study was conducted from August 2017 to March 2018 and following convenient sampling size, all patients who gave oral informed consent were included in study. In present study, a total of 512 participants were included. After necessary permission, pilot study was conducted among 30 patients and then questionnaire was finalized. It included sociodemographic details, assessment of various occupation related risk factors, information regarding current/past episodes of back pain/neck pain and treatment being undertaken.

Socio-economic classification was based on Modified Prasad Classification. Modified Prasad Classification is calculated using latest available All India-Consumer Pricing Index (Industrial Worker) [14] and correction factor of 1981 and 2001. Formula to obtain multiplication factor for month of May 2018 is:

MF= AICPI of May 2018 * 4.63 * 4.93 / 100

The multiplication factor that was obtained with above formula is multiplied with baseline classification values. Hence, the latest classification was revealed.

Information about certain body postures in their lifestyle or at workplace which can have effects on back pain/neck pain were asked. Details are given in Table I.

Visual Analogue Scale (VAS) for perceived pain was included in proforma. For pain intensity, the scale is most commonly anchored by "no pain" (score of 0) and "pain as bad as it could be" or "worst imaginable pain" (score of 100 [100-mm scale]) [20]. Pain score was further divided in 2 and 3 categories : Not Severe (1-6) and Severe (7-10) and Mild (1-3), Moderate (4-6) and Severe (7-10). Necessary approval from institutional ethical committee was obtained. Personal interview of all participants were carried out at physiotherapy department of institute. Data were entered in MS Excel and analyzed using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. For statistical analysis of data, tests like contingency coefficient, Cramer's V, Odds ratio and Goodman and Kruskal's Gamma test are applied.

Results

Total of 512 participants were included in present study. General characteristics of participants are given in Table II.

When inquired amongst all the participants, 53 (10.3%) participants had Neck pain, 392 (76.6%) participants had back pain and 67 (13.0%) participants were suffering from both neck pain and back pain. Upon asking details of referred pain: amongst the 120 participants with complaint of neck pain, 19 (15.8%) had shoulder pain, 102 (85.0%) had radiating pain in upper limb, out of which 54 (52.9%), 38 (37.3%) and 10 (9.8%) had radiating pain in right upper limb, left upper limb and bilateral upper limb respectively. In same subset of patients, Tingling (92, 76.7%) and numbness (88, 73.3%) in upper limb complaint were also reported. In 459 participants with complaint of back pain, radiating pain in lower limb was present in 326 (71.0%) participants. Tingling and numbness in lower limb was presented by 105 (22.9%) and 136 (29.6%) patients, respectively. Onset of pain in 207 (40.4%) participants was sudden, while in 305 (59.6%) participants it was gradual and pain increased over period of time. 380 (74.2%) participants had continuous pain which remained almost throughout the day, while 132 (25.8%) participants had intermittent pain. On inquiring about past medical history related to back pain, it was found that out of 512 participants, 70 (13.7%) had received spinal anesthesia, 171 (33.4%) had suffered from trauma at back and 2 (0.4%) had congenital deformity of spine. Lifting heavy weights as a part of job which may be on flat surface, on slop or on staircase was informed by 191 (37.30%) participants.

Tab. I. Information about various body postures and their ideal positions in lifestyle.

Various body postures	Ideal position
Ideal method to use pillow during sleep	Push down pillow next to shoulders but not lay shoulders on pillow. Head should be level when it's on pillow. The pillow should not be so thick that chin is tucked into chest, or not so flat that chin is elevated in air. Pillow should maintain natural curvature of spine [15-17]
Position of armrest while using computer	Proper position of armrest is below elbow, when elbow are at right angle to shoulder. Ideally elbow should not bear weight on armrest while using computer [18]
Height of chair while using computer	Proper height of chair is one in which monitor of computer is at level of eye or few inches above, feet are flat on the floor and knees in line or slightly lower than hips [19]

Tab. II. General characteristics of study participants.

Variable	Sub-category	n = 512 (%)
Age (in completed years)	18-25	35 (6.8%)
	26-35	127 (24.8%)
	36-45	142 (27.7%)
	46-55	111 (21.7%)
	> 55	97 (18.9%)
Gender	Male	192 (37.5%)
	Female	320 (62.5%)
Education status	Illiterate	74 (14.5%)
	Primary	111 (21.7%)
	Secondary	118 (23.0%)
	Higher Secondary	90 (17.6%)
	Graduate and above	119 (23.2%)
Marital status	Unmarried	49 (9.6%)
	Married	403 (78.7%)
	Divorced/widowed/separated	60 (11.7%)
Occupation	Non-worker	52 (10.2%)
	Household work	172 (33.6%)
	Business	53 (10.4%)
	Labourer	78 (15.2%)
	Service	86 (16.8%)
	Tailor	63 (12.3%)
	Others	8 (1.5%)
Socio-economic class (modified Prasad classification)*	I	66 (12.9%)
	II	190 (37.1%)
	III	124 (24.2%)
	IV	104 (20.3%)
	V	28 (5.5%)
BMI	Normal or underweight	293 (57.2%)
	Overweight	156 (30.5%)
	Obese (Class I, II, III)	63 (12.3%)

* AICPI – Industrial worker for month of May 2018-289 [14].

On asking about continuous neck position during work (more than 4 hours without rest in between of at least 30 minutes), 114 (22.3%) participants told that their neck was flexed, 72 (14.1%) told that it was straight, 312 (61.0%) told that it was either flexed or straight most of time, 5 (1.0%) told it was extended while 9 (1.6%) told that it was flexed or rotated most of time. Same way, upon in terms of continuous position of back (more than 4 hours without rest in between of at least 30 minutes) during work, 81 (15.8%) participants told that position was flexed, 152 (29.7%) told it was straight, 264 (51.6%) told it was flexed or straight and 15 (2.9%) told it was flexed or straight or rotated most of time. Upon asking about maintaining ideal body posture of forward head and shoulder position at work, 67 (13.1%) participants told that they were able to follow the ideal posture. The mode of conveyance to workplace revealed: Auto rickshaw (n = 232), Two wheeler (n = 141) and public transport (n = 127) were commonly used as modes of transportation by participants besides bicycle (n = 63) and car (n = 40).

Out of total 512 study participants, 269 (52.5%) participants waited for 1 month or more before consulting a specialist for pain. 416 (81.3%) participants underwent 1-3 consultation with doctors in last one year. 348 (68%) participants were still undiagnosed for the root cause of their pain. From all, 343 (67.0%) participants informed that their pain never subsided even on using some remedy for it. Majority participants - 454 (88.7%) used non steroidal anti inflammatory drugs (NSAIDs) alone or in combination with other drug for relieving pain. Of all the participants taking medication, 409 (79.9%) participants regularly consumed medication for relieving pain. Of total, 33 (6.4%) participants were advised surgical intervention for their spinal problem but they refused, while 2 (0.4%) underwent surgical intervention for their spinal problem. 498 (97.3%) participants took Out-patient service for their problem and had no history of hospitalization due to back pain or neck pain. The participants who were prescribed lumbar belt for back pain were 170 (37.0%) while, 4 (3.3%) were prescribed cervical collar for their neck pain, which was mostly worn during working hours. As a treatment part, 175 (34.2%) participants were advised exercise at home, out of which 155 (88.6%) participants did it regularly. Out of total, 311 (60.7%) participants used some home remedy method to relieve pain. The various house-hold

methods utilized by these participants were: electric heating pad - 39 (12.5%), hot water bag - 269 (86.5%) and vibrating massager - 3 (1.0%).

Participants, who suffered from restriction in some activities due to their pain, were 466 (91.0%). Of total, 99 (19.3%) participants suffered from loss of working days in last year due to their pain. Preventive measure in terms of avoiding specific posture which aggravated the pain was applied by 454 (88.7%) participants. Out of 120 participants suffering from neck pain, 28 (23.3%) participants had habit of using hard and thick pillow during sleep, while 92 (76.7%) used soft and thin pillow. Only 19 (15.8%) participants knew about correct method of using pillow.

On inquiring about prolonged use of computer at workplace, 50 (9.8%) participants used computer at work place for more than 4 hours duration. Out of 50 participants, 32 (64.0%) participants informed that monitor of computer was at the level of eye. 20 (40.0%) participants told that backrest was according to natural curve of the body. 26 (52.0%) participant had chair whose height could be adjusted according to user. The participants using reclining chair were 25 (50.0%) with 42 (84.0%) having armrest of chair proper.

Pain score of all participants categorized as per Visual Analogue Scale (VAS) as described in methodology. Out of total, 89 (17.4%) participants were included in Mild (1-3) pain score category while 286 (55.9%) and 137 (26.7%) participants were included in Moderate (4-6) and Severe (7-10) pain score category, respectively.

Association between general characteristic of study participants and pain score categories was analysed using Gamma test of significance and Contingency coefficient. The characteristics which found to have statistical significance were: age, marital status, socio-economic class, BMI and occupation of participants. No statistical significance was revealed in association between gender, education and severity of pain score (Tab. III). No

Tab. III. Association between general characteristic of study participants and pain score categories.

Variable		Pain score			Total N = 512 (100%)	Gamma (p value)
		Mild (1-3) N = 89 (17.4%)	Moderate (4-6) N = 286 (55.9%)	Severe (7-10) N = 137 (26.7%)		
Age	18-25	10 (11.2%)	12 (4.2%)	13 (9.5%)	35 (6.8%)	0.155 (0.003)
	25-35	29 (32.6%)	62 (21.7%)	36 (26.3%)	127 (24.8%)	
	35-45	18 (20.2%)	86 (30.1%)	38 (27.7%)	142 (27.7%)	
	45-55	17 (19.1%)	55 (19.2%)	39 (28.5%)	111 (21.8%)	
	≥ 55	15 (16.9%)	71 (24.8%)	11 (8.0%)	97 (18.9%)	
Gender	Male	63 (70.8%)	65 (22.7%)	64 (46.7%)	192 (37.5%)	4.789 (0.091)
	Female	26 (19.2%)	221 (77.3%)	73 (53.3%)	320 (62.5%)	
Education	Illiterate	5 (5.6%)	46 (16.1%)	23 (16.8%)	74 (14.5%)	0.081 (0.152)
	Primary	24 (26.9%)	60 (21.0%)	27 (19.7%)	111 (21.7%)	
	Secondary	17 (3.3%)	60 (21.0%)	41 (29.9%)	118 (23.0%)	
	Higher secondary	24 (26.9%)	53 (18.5%)	13 (9.5%)	90 (17.6%)	
	Graduate and above	19 (21.3%)	67 (23.4%)	33 (24.1%)	119 (23.2%)	
Marital status	Unmarried	0(0.0%)	28 (9.8%)	21(15.3%)	49 (9.6%)	0.329 (0.000)
	Married	68(76.4%)	235(82.2%)	100(73.0%)	403 (78.7%)	
	Divorced/widowed/ separated	21 (23.6%)	23 (8.0%)	16 (11.7%)	60 (11.7%)	
Socio economic class	I	9 (10.1%)	46 (16.1%)	11 (8.0%)	66 (12.9%)	0.128 (0.026)
	II	45 (50.6%)	91 (31.8%)	54 (39.4%)	190 (37.1%)	
	III	16 (18.0%)	83 (29.0%)	25 (18.3%)	124 (24.2%)	
	IV	14 (15.7%)	54 (18.9%)	36 (26.3%)	104 (20.3%)	
	V	5 (5.6%)	12 (4.2%)	11 (8.0%)	28 (5.5%)	
BMI	Normal or less than normal	61 (68.5%)	156 (54.5%)	76 (55.5%)	293 (57.2%)	0.141 (0.039)
	Overweight	25 (28.1%)	90 (31.5%)	41 (29.9%)	156 (30.5%)	
	Obese	3 (3.4%)	40 (14.0%)	20 (14.6%)	63 (12.3%)	
Occupation	Business	8 (9.1%)	30 (10.5%)	15 (10.9%)	53 (10.4%)	0.269* (0.000)
	Service	10 (11.2%)	64 (22.4%)	12 (8.8%)	86 (16.8%)	
	Labourer	16 (18.0%)	35 (12.2%)	27 (19.7%)	78 (15.2%)	
	Household work	46 (51.7%)	82 (28.7%)	44 (32.1%)	172 (33.6%)	
	Non workers	5 (5.6%)	33 (11.5%)	14 (10.2%)	52 (10.2%)	
	Tailor	2 (2.2%)	39 (13.7%)	22 (16.1%)	63 (12.3%)	
	Other	2 (2.2%)	3 (1.0%)	3 (2.2%)	8 (1.5%)	

* contingency coefficient value

statistical significance was found between lifting heavy weights at work and severity of pain (5.105; p = 0.078). Statistical significance was found between computer use and pain score severity (0.149; p = 0.003) on applying contingency coefficient test. Statistical significance was found between various risk factors associated with prolonged computer use at work place which can cause back/neck pain. (Tab. IV).

Various body postures like prolonged sitting, prolonged standing, frequent bending waist and knee, pulling/pushing heavy objects, frequent heavy weight lifting > 10 kg (for back pain only), prolonged walking and/or standing and repetitive movement of back/neck (for neck pain only) were found to be statistically significant risk factors for occurrence of back/neck pain. (Tab. V).

Discussion

In present study, it is quite evident that there is relation between occupations related risk factors and back pain and neck pain. Majority of participants attending physiotherapy clinic for spinal problem belonged to working age group of 26-55 years which is statistically significant with pain score. Some other studies in physiotherapists also showed that initial onset of work related back pain occurred before the age of 30 years [21-23]. No statistical association was obtained in the present study between gender, education of participant and severity of pain score. However some studies have reported association between gender and spinal problems, in which it was more common problem among females [24-28].

In case of Body Mass Index, severity of pain was more among obese people compared to other two groups, which is statistically significant. This supports various studies showing that spinal problem is more among obese people compared to normal or overweight people [29, 30]. Yue et al. [31] in their study at China among teachers, found that severity of neck and shoulder pain was more severe among females compared to males. In another study global burden of lower back pain (LBP) showed that LBP was more common among males than females [32]. However in present study, no such significance was obtained between severity of neck and/or back pain and gender. In the same study Yue et al. [31], revealed that neck and shoulder pain was associated with physical exercise, prolonged standing, prolonged sitting and static posture, while lower back pain was associated with twisting posture, uncomfortable back support, prolonged sitting and static posture. Similarly in current study, prolonged sitting and standing, frequent bending at waist and knee, pulling and pushing of heavy objects, prolonged walking and/or standing, prolonged neck bending and repetitive movements and lifting heavy weights were found to be significant factors associated with neck/back pain (Tab. V).

In present study, it was revealed that participants with prolonged computer usage had more severe pain compared to others. All 50 patients using computer, had either moderate or severe back/neck pain. None of them had mild pain. No significant association was found between position of monitor at level of eyes, back rest whether reclining or non-reclining, armrest proper or not, height of chair proper or not and score of severity of pain. This possibly may suggest that unless moni-

Tab. IV. Association between risk factors associated with "computer use" at workplace and pain score categories.

Variable			Pain score			Total N = 50 (100%)	Contingency coefficient value (p value)
			Mild (1-3) N = 0 (0.0%)	Moderate (4-6) N = 34 (68.0%)	Severe (7-10) N = 16 (32.0%)		
Risk factors associated with Computer use	Screen of Monitor at level of eye	Yes	0 (0.0%)	20 (58.8%)	12 (75.0%)	32 (64.0%)	0.155 (0.351)
		No	0 (0.0%)	14 (41.2%)	4 (25.0%)	18 (36.0%)	
	Back rest fitting to natural back curve	Yes	0 (0.0%)	10 (29.4%)	10 (62.5%)	20 (40.0%)	0.301 (0.034)
		No	0 (0.0%)	24 (70.6%)	6 (37.5%)	30 (60.0%)	
	Adjustable height of chair	Yes	0 (0.0%)	14 (41.2%)	12 (75.0%)	26 (52.0%)	0.301 (0.035)
		No	0 (0.0%)	20 (58.8%)	4 (25.0%)	24 (48.0%)	
	Back rest	Reclining	0 (0.0%)	15 (44.1%)	10 (62.5%)	25 (50.0%)	0.169 (0.364)
		Non-reclining	0 (0.0%)	19 (55.9%)	6 (37.5%)	25 (50.0%)	
	Arm rest	Proper	0 (0.0%)	31 (91.2%)	11 (68.8%)	42 (84.0%)	0.274 (0.092)
		Improper	0 (0.0%)	3 (8.8%)	5 (31.2%)	8 (16.0%)	
	Height of chair	Proper	0 (0.0%)	18 (52.9%)	11 (68.8%)	29 (58.0%)	0.148 (0.365)
		Improper	0 (0.0%)	16 (47.1%)	5 (31.2%)	21 (42.0%)	

Tab. V. Association between different body postures at variety of occupations and complaint of back/neck pain.

Body posture	Complaint		Business	House-hold work	Labourer	Non worker	Service	Tailor	Others	Total	p-value
Prolonged sitting (n = 381)	Neck pain	No	32 (10.8%)	103 (34.8%)	32 (10.8%)	23 (7.8%)	63 (21.3%)	37 (12.5%)	6 (2.0%)	296	0.000
		Yes	7 (8.2%)	32 (37.6%)	11 (12.9%)	0 (0.0%)	8 (9.5%)	26 (30.6%)	1 (1.2%)	85	
	Back pain	No	1 (3.8%)	15 (57.7%)	1 3.8%)	0 (0.0%)	0 (0.0%)	9 (34.6%)	0 (0.0%)	26	0.003
		Yes	38 (10.7%)	120 (33.8%)	42 (11.8%)	23 (6.5%)	71 (20.0%)	54 (15.2%)	7 (2.0%)	355	
Prolonged standing (n = 291)	Neck pain	No	27 (12.8%)	97 (45.5%)	41 (19.2%)	3 (1.4%)	24 (11.3%)	15 (7.0%)	6 (2.8%)	213	0.028#
		Yes	16 (20.5%)	28 (35.9%)	11 (14.1%)	6 (7.7%)	7 (9.0%)	9 (11.5%)	1 (1.3%)	78	
	Back pain	No	3 (9.1%)	13 (39.4%)	4 (12.1%)	6 (18.2%)	7 (21.2%)	0 (0.0%)	0 (0.0%)	33	0.000
		Yes	40 (15.5%)	112 (43.4%)	48 (18.6%)	3 (1.2%)	24 (9.3%)	24 (9.3%)	7 (2.7%)	258	
Frequent bending waist knee (n = 217)	Neck pain	No	5 (3.1%)	74 (46.5%)	48 (30.2%)	4 (2.5%)	5 (3.1%)	16 (10.1%)	7 (4.5%)	159	0.002#
		Yes	1 (1.7%)	30 (51.8%)	11 (19.0%)	6 (10.3%)	8 (13.8%)	1 (1.7%)	1 (1.7%)	58	
	Back pain	No	1 (5.0%)	9 (45.0%)	4 (20.0%)	6 (30.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	20	0.001
		Yes	5 (2.6%)	95 (48.2%)	55 (27.9%)	4 (2.0%)	13 (6.6%)	17 (8.6%)	8 (4.1%)	197	
Pulling/ pushing heavy objects (n = 32)	Neck pain	No	N.A.	8 (32.0%)	11 (44.0%)	0 (0.0%)	N.A.	N.A.	6 (24.0%)	25	0.000
		Yes	N.A.	0 (0.0%)	0 (0.0%)	6 (85.7%)	N.A.	N.A.	1 (14.3%)	7	
	Back pain	No	N.A.	0 (0.0%)	0 (0.0%)	6 (100%)	N.A.	N.A.	0 (0.0%)	6	0.000
		Yes	N.A.	8 (30.8%)	11 (42.3%)	0 (0.0%)	N.A.	N.A.	7 (26.9%)	26	
Frequent heavy weight lifting (n = 195)	Neck pain	No	8 (6.2%)	45 (34.9%)	38 (29.5%)	4 (3.1%)	8 (6.2%)	19 (14.7%)	7 (5.4%)	129	0.260#
		Yes	2 (3.0%)	29 (43.9%)	14 (21.3%)	6 (9.1%)	5 (7.6%)	9 (13.6%)	1 (1.5%)	66	
	Back pain	No	1 (5.0%)	9 (45.0%)	4 (20.0%)	6 (30.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	20	0.000
		Yes	9 (5.1%)	65 (37.1%)	48 (27.4%)	4 (2.3%)	13 (7.4%)	28 (16.1%)	8 (4.6%)	175	
Prolonged walking and/ or sting (n = 122)	Neck pain	No	4 (4.5%)	62 (69.7%)	12 (13.5%)	0 (0.0%)	1 (1.1%)	4 (4.5%)	6 (6.7%)	89	0.000
		Yes	3 (9.1%)	9 (27.3%)	3 (9.1%)	6 (18.2%)	5 (15.1%)	6 (18.2%)	1 (3.0%)	33	
	Back pain	No	1 (10.0%)	0 (0.0%)	3 (30.0%)	6 (60.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	10	0.000
		Yes	6 (5.3%)	71 (63.4%)	12 (10.7%)	0 (0.0%)	6 (5.4%)	10 (8.9%)	7 (6.3%)	112	

continues

Tab. V. *follows.*

Body posture	Complaint		Business	House-hold work	Labourer	Non worker	Service	Tailor	Others	Total	p-value
Prolonged squatting (n = 182)	Neck pain	No	0 (0.0%)	91 (68.9%)	9 (6.8%)	4 (3.0%)	3 (2.3%)	18 (13.6%)	7 (5.3%)	132	0.332
		Yes	1 (2.0%)	33 (66.0%)	6 (12.0%)	0 (0.0%)	0 (0.0%)	9 (18.0%)	1 (2.0%)	50	
	Back pain	No	0 (0.0%)	13 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	13	0.427
		Yes	1 (0.6%)	111 (65.6%)	15 (8.9%)	4 (2.4%)	3 (1.8%)	27 (16.0%)	8 (4.7%)	169	
Neck bending (n = 34)	Neck pain	No	N.A.	9 (40.1%)	3 (13.6%)	3 (13.6%)	1 (4.5%)	0 (0.0%)	6 (27.2%)	22	0.001
		Yes	N.A.	3 (25.0%)	0 (0.0%)	0 (0.0%)	5 (41.7%)	3 (25.0%)	1 (8.3%)	12	
	Back pain	No	N.A.	3 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3	0.001
		Yes	N.A.	9 (29.0%)	3 (9.7%)	3 (9.7%)	6 (19.3%)	3 (9.7%)	7 (22.6%)	31	
Repetitive movement of back/neck (n = 13)	Neck pain	No	N.A.	4 (57.1%)	N.A.	0 (%)	2 (28.6%)	N.A.	1 (14.3%)	7	0.604
		Yes	N.A.	0 (0.0%)	N.A.	6 (100%)	0 (0.0%)	N.A.	0 (0.0%)	6	
	Back pain	No	N.A.	4 (57.1%)	N.A.	0 (0.0%)	2 (28.6%)	N.A.	1 (14.3%)	7	0.604
		Yes	N.A.	0 (0.0%)	N.A.	6 (100%)	0 (0.0%)	N.A.	0 (0.0%)	6	

Contingency coefficient test was applied. Other all variables were assessed by Fisher's Exact test.

tor display is in front and not on either side, pain is not severe. Such conclusion was also made in study conducted by Ye et al. [32] at China. In current research, significant association was obtained between computer use, back rest fitting to natural curve of body, adjustable height of chair and severity of pain score. Severity of pain was more among participants who didn't have proper backrest in chair and amongst those whose chair height couldn't be adjusted. Certain studies have found relation between visual display unit and musculoskeletal disorders and advice for appropriate design of workstation [33, 34] similar findings were revealed in present study.

Conclusion

In age group more than 25 years, distribution of occurrence of neck/back pain has more preponderance for females. Household work has significant contribution in occurrence of neck/back pain. Majority of the participants suffered from restriction in some activities due to their pain, while some participants suffered from loss of working days in last year due to their pain. Age, marital status, socioeconomic class, BMI and type of occupation were found to have statistically significant association with severity of pain. Among participants with prolonged computer usage, back rest fitting to natural back curve and adjustable height of chair were significant factors for occurrence of neck pain. Various body postures like prolonged sitting, prolonged standing, frequent bending waist and knee, pulling/pushing heavy objects, frequent heavy weight lifting > 10 kg (for back pain only), prolonged walking and/or standing and repetitive movement of back/neck (for neck pain only) were found to be statistically significant risk factors for occurrence of back/neck pain.

Acknowledgements

Authors are very much thankful to the staff of Physiotherapy Department, GCS Medical College Hospital and Research Centre for their support throughout the study.

Funding sources: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

VRD had conceptualized the topic and study design, VRD, HJK and KNS prepared the questionnaire, all authors have contributed for data collection, HJK, RPS and JT performed data analyses, VRD and RPS wrote the manuscript and the same was reviewed by all authors.

References

[1] Andersson GB. Epidemiological features of chronic low-back pain. Lancet [Internet] 1999;354:581-5. https://doi.org/10.1016/S0140-6736(99)01312-4

[2] Wynne-Jones G, Cowen J, Jordan JL, Uthman O, Main CJ, Glozier N, van der Windt. Absence from work and return to work in people with back pain: A systematic review and meta-analysis. Occup Environ Med 2014;71:448-58. https://doi.org/10.1136/oemed-2013-101571

[3] Marras WS. Occupational low back disorder causation and control. Ergonomics 2000;43:880-902. https://doi.org/10.1080/001401300409080

[4] Cassidy JD, Carroll LJ, Côté P. The Saskatchewan health and back pain survey. The prevalence of low back pain and related disability in Saskatchewan adults. Spine (Phila Pa 1976). 1998;23:1860-6. https://doi.org/10.1097/00007632-199809010-00012

[5] Côté P, Cassidy JD, Carroll L. The Saskatchewan Health and Back Pain Survey. The prevalence of neck pain and related disability in Saskatchewan adults. Spine (Phila Pa 1976). 1998;23:1689-98. https://doi.org/10.1097/00007632-199808010-00015

[6] Von Korff M. Studying the natural history of back pain. Spine (Phila Pa 1976) 1994;19(18 Suppl):2041S-2046S. https://doi.org/10.1097/00007632-199409151-00005

[7] Hoy D, March L, Woolf A, Blyth F, Brooks P, Smith E, Vos T, Barendregt J,Blore J, Murray C, Burstein R, Buchbinder R.The global burden of neck pain: estimates from the global burden of disease 2010 study. Ann Rheum Dis 2014;73:1309-15. https://doi.org/10.1136/annrheumdis-2013-204431

[8] Murray CJL, Lopez AD. Measuring the Global Burden Of Disease. N Engl J Med 2013;369:448-57. https://doi.org/10.1056/NEJMra1201534

[9] Kerssens JJ, Sluijs EM, Verhaak PF, Knibbe HJ, Hermans IM. Back care instructions in physical therapy: a trend analysis of individualized back care programs. PhysTher 1999;79:286-95.

[10] Riihimaki H. Low-back pain, its origin and risk indicators. Scand J Work Environ Heal 1991;17:81-90.

[11] Latza U. Cohort study of occupational risk factors of low back pain in construction workers. Occup Environ Med 2000;57:28-34.

[12] Op De Beek R, Hermans V. Research on work-related low back disorders. 2000. 422 p.

[13] Podniece Z, Pinder A, Yeomans L, van den Heuvel S, Blatter B, Martheverjans KM. Work-related musculoskeletal disorders: back to work report. 2007. 100 p.

[14] Consumer Price Index Numbers for Industrial Workers Base 2001=100. Available at: http://labourbureaunew.gov.in/All_India_gp_subgp_indices_May_2018.pdf.

[15] Liu SF, Lee YL, Liang JC. Shape design of an optimal comfortable pillow based on the analytic hierarchy process method. J Chiropr Med 2011;10:229-39. https://doi.org/10.1016/j.jcm.2011.04.002

[16] The correct way to lay on your pillow | Health | wahpetondailynews.com. Available at: https://www.wahpetondailynews.com/health/the-correct-way-to-lay-on-your-pillow/article_21c43f88-dec9-11e4-bf9f-93871dee96f5.html (cited 2018 Jul 31).

[17] Sleeping position and choosing a pillow: what's best for me? Available at: https://hullopillow.com/sleeping-position-and-choosing-a-pillow/ (Accessed on 2018 Jul 31).

[18] Ergoanswers : elbow and forearm. Available at: https://www.rsiguard.com/help/ErgoAnswers/elbow.html. (Accessed on 2018 Jul 31).

[19] Proper sitting posture at a computer | office chair posture. Available at: https://www.conceptseating.com/how-to-properly-sit-at-a-computer (Accessed on 2018 Jul 31).

[20] Hawker GA, Mian S, Kendzerska T, French M. Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP). Arthritis Care Res 2011;63(Suppl. 11):240-52. https://doi.org/10.1002/acr.20543

[21] Cromie JE, Robertson VJ, Best MO. Work-related musculoskeletal disorders in physical therapists: prevalence, severity, risks, and responses. Phys Ther 2000;80:336-51. https://doi.org/10.1093/ptj/80.4.336

[22] Holder N, Clark H, DiBlasio J, Hughes C, Scherpf J, Harding L. Cause, prevalence and response to occupational musculoskeletal injuries reported by physical therapists and physical therapists assistants. Phys Ther 1999;79:642-52. https://doi.org/10.1093/ptj/79.7.642

[23] Mierzejewski M, Kumaar S. Prevalence of low back pain among physical therapists in Edmonton, Canada. Disabil Rehabil 1997;19:309-17 https://doi.org/10.3109/09638289709166544

[24] Shehab D, Al-Jarallah K, Moussa M, Adham N. Prevalence of low back pain among physical therapists in Kuwait. Med Princ Pract 2003;12:224-30. https://doi.org/10.1159/000072288

[25] King P, Huddleston W, Darragh AR. Work-related musculoskeletal disorders and injuries: differences among older and younger occupational and physical therapists. J Occup Rehabil 2009;19:274-83. https://doi.org/10.1007/s10926-009-9184-1

[26] Bork BE, Cook TM, Rosecrance JC, Engelhardt KA, Thomason MJ, Wauford IJ, Worley RK. Work-related musculoskeletal disorders among physical therapists. Phys Ther 1996;76:827-35. https://doi.org/10.1093/ptj/76.8.827

[27] Adegoke BO, Akodu AK, Oyeyemi AL. Work-related musculoskeletal disorders among Nigerian physiotherapists. BMC Musculoskelet Disord 2008;9:112. https://doi.org/10.1186/1471-2474-9-112

[28] Paksaichol A, Janwantanakul P, Purepong N, Pensri P, van der Beek AJ. Office worker's risk factors for the development of non-specific neck pain: a systematic review of prospective cohort studies. Occup Environ Med 2012;69:610-8. https://doi.org/10.1136/oemed-2011-100459

[29] Sihawong R, Sitthipornvorakul E, Paksaichol A. Predictors for chronic neck and low back pain in office workers: a 1-year prospective cohort study. J Occup Health 2016;58:16-24. https://doi.org/10.1539/joh.15-0168-OA

[30] Punnett L, Sc D, Prüss-ustün A, Ph D, Nelson DI, Ph D. Estimating the global burden of low back pain attributable to combined occupational exposures. Am J Ind Med 2005;48:459-69. https://doi.org/10.1002/ajim.20232

[31] Yue P, Liu F, Li L. Neck/shoulder pain and low back pain among school teachers in China, prevalence and risk factors. BMC Public Health 2012;12:789. https://doi.org/10.1186/1471-2458-12-789

[32] Ye S, Jing Q, Wei C, Lu J. Risk factors of non-specific neck pain and low back pain in computer-using office workers in China: a cross- sectional study. BMJ Open. 2017;7:e014914. https://doi.org/10.1136/bmjopen-2016-014914

[33] Janwantanakul P, Sitthipornvorakul E, Paksaichol A. Risk factors for the onset of nonspecific low back pain in office workers: a systematic review of prospective cohort studies. J Manip Physiol Ther 2012;35:568-77. https://doi.org/10.1016/j.jmpt.2012.07.008

[34] Riccò M, Cattani S, Gualerzi G. Work with visual display units and musculoskeletal disorders: a cross-sectional study. 2016;67:707-19. https://doi.org/10.13075/mp.5893.00471

Received on September 28, 2018. Accepted on July 29, 2019.

Correspondence: Viral R. Dave, Community Medicine Department, GCS Medical College Hospital & Research Centre, Opp DRM Office, Naroda Road, Ahmedabad, Gujarat, India - Tel. +91 079 66048351 - Fax +91 079 22201915 - E-mail: dr.vdave@gmail.com

How to cite this article: Dave VR, Khanpara HJ, Shukla RP, Sonaliya KN, Tolani J, Patel R. Risk factors of occupation related back pain and neck pain among patients attending tertiary care hospital, Ahmedabad, India. J Prev Med Hyg 2019;60:E419-E427. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1069

Letter to the Editor

Master athletes and "1001 miles", the longest and most extreme European randonnée

F. CERIANI[1], L. CHIAPPA[2], A. CRESPI[3], S. DEI[4], M. CAPITANI[4], R. SEVERINI[4], M.G. D'AMATO[4], A. CARTOCCI[5], G. LAZZERI[5], G. TROIANO[5]

[1] U.O. Chirurgia Multimedica Castellanza Varese, Italy; [2] U.O. Cardiologia H. Desio (Monza-Brianza), Italy; [3] Dietologia e Medicina dello Sport Centro Medico Sant'Anna Nerviano (Milano), Italy; [4] Azienda USL Toscana Sud Est, Italy; [5] University of Siena, Italy

Keywords

Cycling • Sport • Physical activity • Italy

Dear Editor,

It is known that the most traditional endurance and ultra-endurance (ultra-endurance competition is defined as events that exceed the 6 hours in duration) sports are swimming, cycling, running, and triathlon as a combination of them. In recent years, several studies reported an increased participation in ultra-endurance performances of six hours or longer [1] such as ultra-running [2, 3], ultra-cycling [4] and ultra-triathlon. In several cases in these ultra-endurance events has been observed an increased participation from master athletes older than 35 years [5].

Several authors investigated the influence of age in triathlon performance [6] and in running performance [7], but only a few studies investigated other endurance disciplines such as swimming or cycling. In particular, cycling as a non-weight-bearing activity represents an interesting model because it can be performed even in older ages [8]. Most of the studies on ultra-endurance race cyclists have been performed in experimental conditions, so the aim of this study was to evaluate "on field" the effects of this activity.

The so-called "1001 miles" is the longest and extreme randonnée of Europe and stretches over a distance of 1600 km to go across in 7 days in a region characterized by high temperatures and high humidity (the race occurs in August, in Italy). Remarkable is also the commitment in terms of slope and altitude changes to deal with [9]. The so-called randonneuring is a sport born in France. During each competition (called randonnée), the participants tackle paths of 200 km and beyond, passing through predetermined control points. The aim is to complete the course within certain time limits, and the order of arrival is irrelevant.

In August 2016 Twenty-one cyclists were voluntary enrolled in the study. This small sample size was due to the limited available economic resources. The study was approved by the Ethical Committee of the race organizer and an informed consent form (in multiple languages) was distributed and signed by the athletes before being enrolled.

A medical evaluation, and the collection of blood and urine samples occurred before the race and at the end of the race. Data were managed in a totally anonymous way. Tests were not technically considered invasive. Samples were collected by a nurse and examined by an external laboratory (in Sesto San Giovanni, Milan). The performed analyses were: blood count, creatinine, urea (Blood Urea Nitrogen - BUN), calcium, sodium, potassium, chlorine, magnesium, bicarbonates, glycemia, creatine phosphokinase (CPK), urine analysis.

Collected data were inserted in a database and exported for statistical analysis. Percentages, means and standard deviations were calculated, followed by the creation of tables for a descriptive purpose. Continuous variables were evaluated using the Kolmogorov-Smirnov test in order to verify the normality hypothesis, and the Bartlett's test for homogeneity of variances between the two groups. For all variables, the assumptions of normality and homogeneity were satisfied. The differences between groups were evaluated with paired t test except for "Urine clarity" that was evaluated using the chi-square test. Statistical analyses were performed using R version 3.3.3. The significance level was set at $p < 0.05$. Athletes who agreed to participate in the study were 3 females (14.3%) and 18 males (85.7%). The mean age was 53.42 years old (Std. Dev. 8.27; range 40-69). 14 (66.6%) were from Italy, 2 (10%) from Germany, 1 (4.7%) from Canada, 1 (4.7%) from Switzerland, 1 (4.7%) from Japan, 1 (4.7%) from Peru, 1 (4.7%) from Spain.

Laboratory results are resumed in Table I. Temperatures recorded during the randonnée are resumed in the Supplementary Table I.

Our investigation showed a statistically significant increase in blood urea concentration (BUN) in cyclists involved in "1001 miles" race. The phenomenon is certainly due to the increase in energy demand by the large striated muscle masses involved in the exercise, whereas the perfusion of body organs such as the kidneys may decrease up to 25% of resting levels [10]. It resulted in a temporary reduction in the glomerular filtration rate (eGFR) with a consequent increase in the values of metabolic wastes such as urea [11, 12]. Not statistically significant differences were observed between men and women.

https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1370

Tab. I. Laboratory analysis.

Blood	Beginning (mean, Std. dev.)	End (mean, Std. dev.)
Urea* (mg/dL)	39.24 ± 6.50	52.62 ± 11.68
Creatinine* (mg/dL)	0.86 ± 0.12	0.81 ± 0.09
Calcium (mmol/L)	2.37 ± 0.10	2.15 ± 0.08
Sodium (mEq/L)	144.67 ± 1.59	138.76 ± 2.79
Potassium (mEq/L)	4.29 ± 0.31	4.10 ± 0.46
Chlorine (mEq/L)	105.19 ± 1.72	105.05 ± 2.87
Magnesium (mmol/L)	0.64 ± 0.07	0.85 ± 0.05
Bicarbonates (mmol/L)	25.60 ± 2.30	21.21 ± 2.34
Glucose (mg/dL)	97.10 ± 24.17	94.81 ± 21.77
Urine	**Beginning**	**End**
Urine clarity*		
Clear (%)	21 (100%)	6 (28.57%)
Turbid (%)	0 (0%)	15 (71.43%)
PH (mean, Std. dev.)	5.71 ± 0.89	5.43 ± 0.18
Specific gravity (mean, Std. dev.)	1.02 ± 0.004	1.03 ± 0.002
Blood* (mean, Std. dev.)	13.24 ± 40.61	16.95 ± 38.34
Leucocytes (mean, Std. dev.)	5 ± 5.5	10.14 ± 18.92

* statistically significant difference (p value < 0.05)

Concluding, in master athletes, it is important to have an adequate water-saline reintegration during ultra endurance races: this could lead to a good perfusion pressure of the internal organs and, especially some functional sport drinks (particularly those containing Glucose-Fructose and sodium) can improve athletic performance by sustaining metabolism and optimizing water absorption [13]. It is also important an adequate diet during the whole preparation phase and the race, aimed at enriching the hepatic and muscular glycogen reserves as much as possible, in order to contain protein catabolism and the synthesis of nitrogen compounds [14].

Acknowledgements

Funding sources: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

FC had the idea of the study. LC helped conceptualize ideas, provided support and suggestions. AC helped conceptualize ideas, provided support and suggestions. SD provided helped conceptualize ideas, support and suggestions. MC helped conceptualize ideas, provided support and suggestions. RS helped conceptualize ideas, provided support and suggestions. MGD helped conceptualize ideas, provided support and suggestions. AC performed statistical analysis. GL helped conceptualize ideas, provided support and suggestions. GT provided support and wrote the article.

References

[1] Zaryski C, Smith DJ. Training principles and issues for ultra-endurance athletes. Curr Sports Med Rep 2005;4:165-70. https://doi.org/10.1097/01.csmr.0000306201.49315.73

[2] Hoffman MD, Ong JC, Wang G. Historical analysis of participation in 161 km ultramarathons in North America. Int J Hist Sport 2010;27:1877-91. https://doi.org/10.1080/09523367.2010.494385

[3] Rüst CA, Knechtle B, Rosemann T, Lepers R. Analysis of performance and age of the fastest 100-mile ultra-marathoners worldwide. Clinics (Sao Paulo) 2013;68:605-11 https://doi.org/10.6061/clinics/2013(05)05

[4] Shoak MA, Knechtle B, Knechtle P, Rüst CA, Rosemann T, Lepers R. Participation and performance trends in ultracycling. Open Access J Sports Med 2013;4:41-51. https://doi.org/0.2147/OAJSM.S40142

[5] Reaburn P, Dascombe B, Endurance performance in master athletes. Eur Rev Aging Phys Act 2008;5:31-42. https://doi.org/10.1007/s11556-008-0029-2

[6] Lepers R, Knechtle B, Stapley PJ. Trends in triathlon performance: effects of sex and age. Sports Med 2013;43:851-63. https://doi.org/10.1007/s40279-013-0067-4

[7] Lehto N. Effects of age on marathon finishing time among male amateur runners in Stockholm Marathon 1979-2014. J Sport Health Sci 2016;5:349-54. https://doi.org/10.1016/j.jshs.2015.01.008

[8] Pozzi L, Knechtle B, Knechtle P, Rosemann T, Lepers R, Rüst CA. Sex and age-related differences in performance in a 24-hour ultra-cycling draft-legal event - a cross-sectional data analysis. BMC Sports Sci Med Rehabil 2014;6:19. https://doi.org/10.1186/2052-1847-6-19

[9] MigliaItalia. 20/07/2019. Available at https://www.1001migliaitalia.it/

[10] Poortmans JR Vanderstraeten J. Kidney function during exercise in healthy and diseased humans. An update. Sports Med 1994;18:419-37. https://doi.org/10.2165/00007256-199418060-00006

[11] Lemon PW, Deutsch DT, Payne WR. Urea production during prolonged swimming. J Sports Sci 1989;7:241-6. https://doi.org/10.1080/02640418908729844

[12] Bongers CCWG, Alsady M, Nijenhuis T, Tulp ADM, Eijsvogels TMH, Deen PMT, Hopman MTE. Impact of acute versus prolonged exercise and dehydration on kidney function and injury. Physiol Rep 2018;6: e13734. https://doi.org/10.14814/phy2.13734

[13] Orrù S, Imperlini E, Nigro E, Alfieri A, Cevenini A, Polito R, Daniele A, Buono P, Mancini A. Role of functional beverages on sport performance and recovery. Nutrients 2018;10(10). https://doi.org/10.3390/nu10101470

[14] Pramukova B, Szabadosova V, Soltesova A. Current knowledge about sports nutrition. Australas Med J, 2011. 4(3): p. 107-10 doi: 10.4066/AMJ.2011.520.

Tab. S1. Temperature recorded in each 1001 miles stop (in Celsius degrees).

Day/place	Temp. med.	Min. temp.	Max. temp.	Humid. med.	Wind
I Colorno	27°	19°	32°	47%	19 km/h
II Lugo	25°	20°	30°	64%	15 km/h
III Todi	25°	17°	30°	61%	7 km/h
IV Staffoli	24°	20°	29°	85%	9 km/h
V Castellania	23°	21°	24°	85 % (rainy)	6 km/h
VI Nerviano	24°	21°	29°	67%	7 km/h

Received on August 13, 2019. Accepted on August 21, 2019.

Correspondence: Gianmarco Troiano, University of Siena, via A. Moro 2, 53100 Siena, Italy - E-mail:gianmarco-89@hotmail.it

How to cite this article: Ceriani F, Chiappa L, Crespi A, Dei S, Capitani M, Severini R, D'Amato MG, Cartocci A, Lazzeri G, Troiano G. Master athletes and "1001 miles", the longest and most extreme European randonnée. J Prev Med Hyg 2019;60:E428-E430. https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1370


MSD
INVENTING FOR LIFE


Pfizer
Vaccines


Seqirus
A CSL Company


SANOFI PASTEUR


VISMEDERI
ANALYSES FOR LIFE IMPROVEMENT


emergent
biosolutions
Emergent Italy S.r.L.