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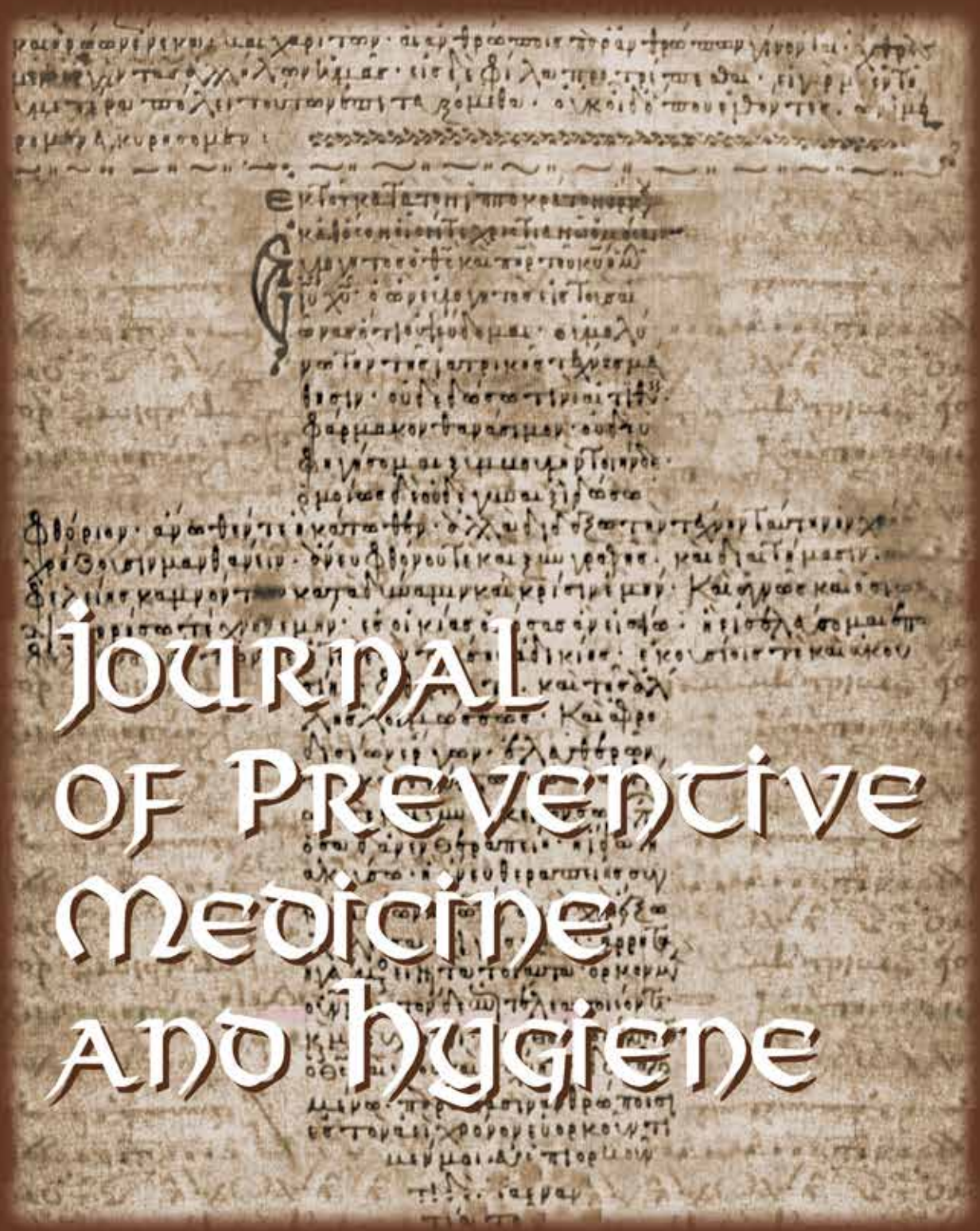

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CONTENTS

Original articles

ORIGINAL ARTICLE

Impact of erythrocyte species on assays for influenza serology

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Keywords

Avian and mammalian erythrocytes • Sialic acid receptors • Haemagglutination assay • Haemagglutination inhibition assay

Summary

The influenza viruses have the ability to agglutinate erythrocytes by binding to sialic acid receptors on the host cell. Human influenza viruses preferentially bind to sialic acid linked to galactose by α 2.6 linkage, while avian influenza viruses preferentially bind to sialic acid linked to Gal by α 2.3 linkage. There is a close correlation between the ability of influenza A viruses to agglutinate erythrocytes from different animal species and their receptor specificity. The haemagglutination and haemagglutination inhibition assays are influenced by the species of erythrocytes. To provide an overview of the expression of sialic acid receptors on different erythrocytes, avian (turkey, chicken, pigeon) and mammalian (sheep, horse, human) species have been analysed by flow cytometry. Chicken, turkey and human erythrocytes display both types of linkages. Horse and sheep erythrocytes show almost exclusively α 2.3 Gal linkages, while pigeon erythrocytes express almost exclusively α 2.6 Gal linkages. The erythrocytes from the same avian and mammalian species have been evaluated by haemagglutination and haemagglutination inhibition assays with seasonal and avian strains. Chicken and turkey erythrocytes seem to be the most appropriate for both assays with seasonal influenza strains, in addition to pigeon erythrocytes, particularly for the B strains. In the case of the avian strain, chicken erythrocytes are suitable for haemagglutination assay and horse erythrocytes for haemagglutination inhibition assay. The choice of erythrocytes has a significant impact on the titres measured by both assays.

Introduction

The influenza viruses have the ability to agglutinate erythrocytes by binding to sialic acid (SA) receptors on the host cell [1, 2]. Human influenza viruses preferentially bind to SA linked to galactose (Gal) by α 2.6 linkage, while avian influenza viruses preferentially bind to SA linked to Gal by α 2.3 linkage [3].

There is a close correlation between the ability of influenza A viruses to agglutinate erythrocytes from different animal species and their receptor specificity. The agglutination of cells by viruses with specific linkage preferences depends on the amount of SA α 2.3 and α 2.6 Gal linkages on the erythrocytes surface [4]. Several studies have shown that human, chicken, pig and guinea pig erythrocytes express both linkages; pig erythrocytes present a high proportion of SA α 2.6 Gal linkage, while chicken erythrocytes display greater SA α 2.3 Gal linkage than those of guinea pigs and humans. Sheep, mouse erythrocytes display SA α 2.3 Gal linkage only; horse and cow mainly exhibit this linkage, while rabbit shows low amounts of SA α 2.6 Gal linkage [1, 4-6].

The haemagglutination assay (HA) and haemagglutination inhibition assay (HI) are influenced by the species of erythrocytes. The HI assay is currently the most widely used to measure immune responses to influenza vaccines, and is considered the gold standard as a correlate of protection [7]. However, the assay has limitations including differences in the sensitivity of erythrocytes from individual animals of the same species, a high degree of variability among laboratories owing to many factors (including the source of erythrocytes) and low sensitivity to B strains [8-10]. Traditionally the assay is performed with turkey erythrocytes, which are small, nucleated cells, displaying rapid sedimentation [11]. However, these erythrocytes could significantly underestimate antibody responses to avian viruses, as they express a mixture of SA α 2.3 and α 2.6 Gal linkages, while avian viruses prefer the SA α 2.3 Gal linkage [6, 11, 12]. The use of horse erythrocytes has improved the detection of H5 antibody responses, owing to their higher proportion of SA α 2.3 Gal linkage [6, 11, 13, 14] and are routinely used to measure HI responses to avian viruses. Furthermore, goose and guinea pig erythrocytes can enhance the sensitivity of the assay for avian strains [8].

The aim of this study was to provide an overview of the expression of SA receptors on avian (turkey, chicken, pigeon) and mammalian (sheep, horse, human) species and possible intra-species variation for chicken and horse erythrocytes. The erythrocytes from the same avi-

an and mammalian species have been analysed by HA and HI assays with seasonal and avian strains.

Methods

Influenza viruses, serum samples and erythrocytes

The influenza viruses were the live, seasonal and pandemic, strains from NIBSC and CBER: A/California/07/2009 (H1N1, 15/252), A/Victoria/361/2011 (H3N2, 11/226) B/Wisconsin/01/2010 (Yamagata lineage) (B, 12/198) and A/Indonesia/05/2005 (H5N1, H5-Ag-0904). The live viruses were propagated in 10-days-old embryonated chicken eggs and stored at – 80°C until use.
The human serum samples from the serum bank of the University of Siena had been drawn from adults aged 17-59 years, in compliance with Italian ethics law.
All serum samples were pre-treated with receptor destroying enzyme (RDE) from Vibrio Cholerae at 1:5 ratio (Sigma Aldrich, Italy) for 18 h at 37°C in a water bath and then heat-inactivated for 1 h at 56°C in a water bath with 8% sodium citrate at 1:4 ratio before testing in the HI assay.
The sources of erythrocytes for HA and HI assays and flow cytometry were: turkey, chickens (different batches), sheep, horses (different breeds: Italian (IT), Argentinian (AG), German Mecklenburger Kaltblut (GM), French Trait Percheron (TP F) and French Cheval de Trait (CT F), pigeon (Emozoo S.N.C., Italy) and humans (group 0) (Tab. I).

Haemagglutination assay

Erythrocytes were used at a 0.35% (for chicken, pigeon and turkey - seasonal strains), 0.50% (for humans, horses, sheep - seasonal strains and turkey - avian strain), 0.75% (sheep - avian strain) concentrations suspended in saline solution (0.9%).
The HA assay was performed as previously described [15].
The HA titre was expressed as the reciprocal of the highest influenza virus dilution showing complete agglutination [15].

Tab. I. The erythrocytes used for the HA, HI assays and flow cytometry (FC). Argentinian horse (AG), French horse (Cheval de Trait (CT F), German horse (Mecklenburger Kaltblut GM), Italian Horse (IT), French horse (Trait Percheron (TP F).

Erythrocytes	HA assay	HI assay	FC analysis
Chicken	X	X	X
AG horse	X	X	X
CT F horse			X
GM horse			X
IT horse	X	X	X
TP F horse			X
Human	X	X	X
Sheep	X	X	X
Pigeon	X	X	X
Turkey	X	X	X

The HA assay was performed in triplicate. Geometric mean titres (GMTs) of seasonal and avian strains were calculated in order to compare the different erythrocytes analyzed.

Haemagglutination inhibition assay

Erythrocytes were used at the same concentration of the HA assay.
The HI assay was performed as previously described [15].
The antibody titre was expressed as the reciprocal of the highest serum dilution showing complete inhibition of agglutination. Since the starting dilution was 1:10, the lower limit of detectable antibody titre was 10. When the titre was below the detectable threshold, the results were conventionally expressed as 5 for calculation purposes, half the lowest detection threshold.
The HI assay was performed in triplicate with human serum samples. GMTs of seasonal and avian strains were calculated in order to compare the different erythrocytes analyzed.

Flow cytometry

The expression pattern of SA α 2.3 and 2.6 Gal linkages on erythrocytes was evaluated by flow cytometry using digoxigenin-conjugated specific lectins Sambucus nigra (SNA) and Maackia amurensis (MAA), which recognize the α 2.6 and the α 2.3 Gal linkages, respectively (Digoxigenin Glycan Differentiation Kit, Roche) as previously described [5]. Sheep anti-digoxigenin fluorescein antibody was used to detect the lectins. Negative controls without lectins were evaluated. Flow cytometry was carried out on a GUAVA EasyCyte 6-2L flow cytometer (Millipore) and the data were analyzed and plotted by means of FlowJo software (TreeStar Inc).

Statistics

Geometric Coefficient of Variation was calculated from the SD per sample across erythrocytes species. GCV = 100 x Sqrt (2SDlog2-1). Ratio's between HI titres for different erythrocytes combinations were calculated per sample. The geometric mean ratio with 95% CI is presented in the ratio graphs.

Results

The expression of SA α 2.3 and α 2.6 Gal linkages was investigated by flow cytometry in avian species (chicken, turkey, pigeon) and mammalian species (sheep, horse and human). Chicken, turkey and human erythrocytes displayed both types of linkages. Notably, chicken erythrocytes expressed higher amounts of SA α 2.3 Gal linkage, and turkeys higher amounts of SA α 2.6 Gal linkage; human erythrocytes expressed both linkages in similar proportions. Horses showed almost exclusively SA α 2.3 Gal linkage, while pigeon displayed almost exclusively SA α 2.6 Gal linkage. Sheep erythrocytes expressed only α 2.3 Gal linkage (Fig. 1).

Fig. 1. Expression of SA α 2,3 and α 2,6 Gal linkages in erythrocytes from avian (turkey, chicken, pigeon) and mammalian (sheep, horse, humans) species. Data originated from replicates of the same samples. a) Erythrocytes were incubated with DIG-labeled SNA (α 2,6 Gal linkage), DIG-labelled MAA (α 2,3 Gal linkage) or without lectins (-) and subsequently incubated with anti-digoxigenin antibody conjugated with fluorescein. Cells were analysed by flow cytometry and the results were expressed as the log10 relative fluorescence versus number of cells. The graphs show the Mean Fluorescence Intensity (MFI). Data are presented as mean ± SD (n ≥ 2). Representative histograms are shown. **b)** Distribution of α 2,3 and α 2,6 Gal linkages among animal species. The graphs show the MFI. Data are presented as mean ± SD (n ≥ 2).

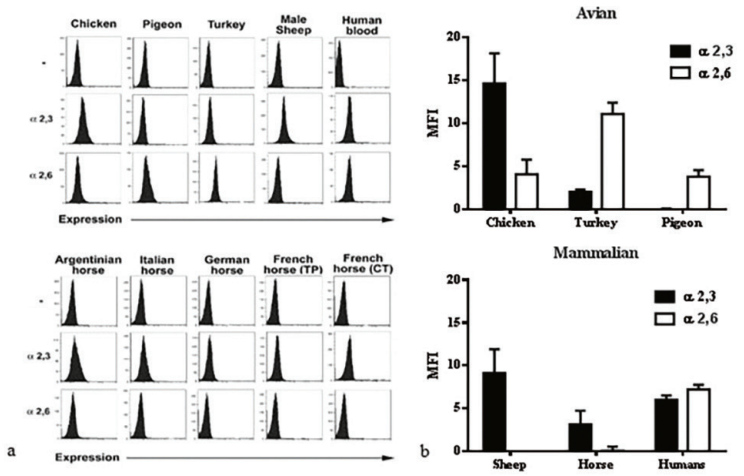
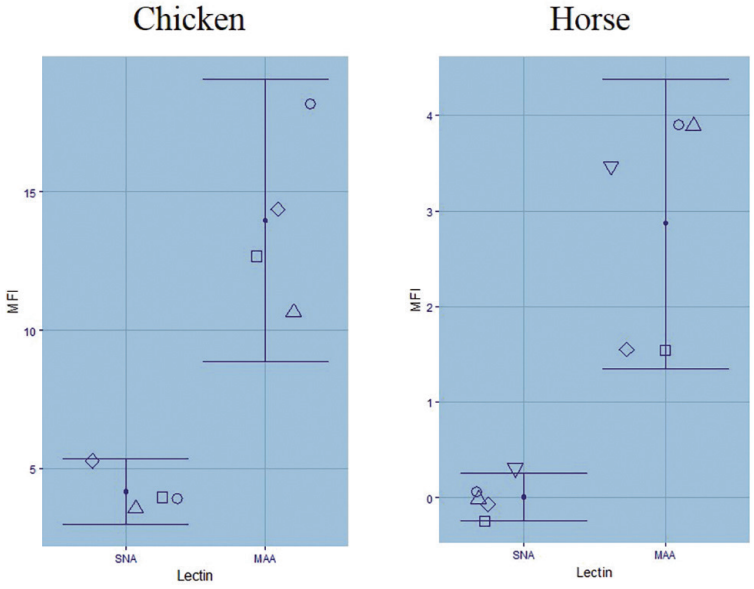


Fig. 2. Variation of SA α 2,3 (MAA) and α 2,6 (SNA) Gal linkages within different chickens and horses (Mean MFI with 95% confidence intervals). Data are presented as mean SD (n ≥ 4). Different symbols indicate chickens (4 individuals) and horses (circle = Cheval de Trait – French –; square = Mecklenburger Kaltblut (German); diamond = Percheron (French); up triangle = Italian horse and down triangle = Argentinian horse).



To assess intra-species variation, erythrocytes from different batches of chickens and different breeds of horses – Italian, Argentinian, French (two breeds) and German – were tested (Fig. 2).

Chicken and horses erythrocytes showed much higher α 2.3 Gal linkage than α 2.6 Gal linkage with variation in the expression among the same species.

In addition to the flow cytometry, the HA and HI assays were performed with erythrocytes from chicken, turkey, sheep, pigeon, humans and horses for both, seasonal (A/California/07/2009 H1N1, A/Victoria/361/2011 H3N2, B/Wisconsin/01/2010 - Yamagata lineage) and avian (A/Indonesia/05/2005 H5N1) strains to investigate the influence of different erythrocytes on seasonal and avian titres. Seasonal viruses did not agglutinate horse and sheep erythrocytes. Regarding the A/California/07/2009 H1N1 strain, the highest geometric mean (GM) HA titre was displayed by chicken erythrocytes (2016), followed by turkey (1600) and pigeon (1270). Turkey erythrocytes (1600) were more sensitive to the A/Victoria/361/2011

H3N2 strain than those from pigeons (1008), chickens (800) and humans (716). In the case of the B/Wisconsin/01/2010 strain, turkey erythrocytes (716) yielded the highest HA titre, whereas other erythrocytes species displayed much lower HA titres. Human erythrocytes showed relatively low HA titres for all three seasonal strains (Tab. II).
The avian H5N1 strain elicited the highest HA titres in chicken erythrocytes (12800) (Tab. II).
Considerable variations in HI titres were detected when using different sources of erythrocytes for seasonal and avian strains. Overall, chicken erythrocytes displayed the highest titres for all the seasonal strains. Horse and sheep erythrocytes added much variation for H5N1 strain and horse erythrocytes yielded the highest titres (Fig. 3).
Regarding the H1N1 and H3N2 strains, the highest HI titres were displayed by chicken erythrocytes followed by pigeon and turkey erythrocytes showing the same titres. The only statistically significant difference is between chicken and human erythrocytes for H1N1 strain. For the H3N2 strain, turkey and human yield lower HI titres than chicken and human gives lower titres than turkey (Fig. 4). Human erythrocytes showed low HI titres for both strains (Fig. 4). For the B strain turkey yields lower titres than chicken.
Samples 1-4 yielded positive titres with chicken erythrocytes while all other erythrocytes were negative.
The avian strain showed the highest HI titres when horses erythrocytes were used. In particular, Italian horse gave significantly higher titres than Argentinian horse. Turkey and chicken displayed similar HI titres but lower than the other species. The HI titres with sheep erythrocytes were lower than human and horse and higher than chicken, turkey and pigeon (Fig. 5).

Discussion

The agglutination of cells by viruses with specific linkage preferences depends on the amount of SA α 2.3 and α 2.6 Gal linkages on the erythrocytes surface. The human influenza viruses preferentially bind to SA linked to Gal by α 2.6 linkage, while avian influenza viruses prefer SA linked to Gal by α 2.3 linkage [13].
The HA and HI assays are based on the ability of influenza viruses to agglutinate erythrocytes and are influenced by the erythrocytes used, resulting in different HA and HI titres; these may explain some of the variation observed in the titres of both assays between different laboratories [8, 16-18].

Tab. II. HA GMTs of influenza seasonal and avian strains with different erythrocytes.

Influenza strain	Erythrocytes						
A/California/07/2009 H1N1	Chicken 2016	Turkey 1600	Sheep no HA titer	Pigeon 1270	Human 800	IT horse no HA titer	AG horse no HA titer
A/Victoria/361/2011 H3N2	Chicken 800	Turkey 1600	Sheep no HA titer	Pigeon 1008	Human 716	IT horse no HA titer	AG horse no HA titer
B/Wisconsin/01/2010	Chicken 358	Turkey 716	Sheep no HA titer	Pigeon 179	Human 89	IT horse no HA titer	AG horse no HA titer
A/Indonesia/05/2005 H5N1	Chicken 12800	Turkey 8063	Sheep 800	Pigeon 6400	Human 1600	IT horse 6400	AG horse 4032

Fig. 3. HI titres of human serum samples with different erythrocytes.

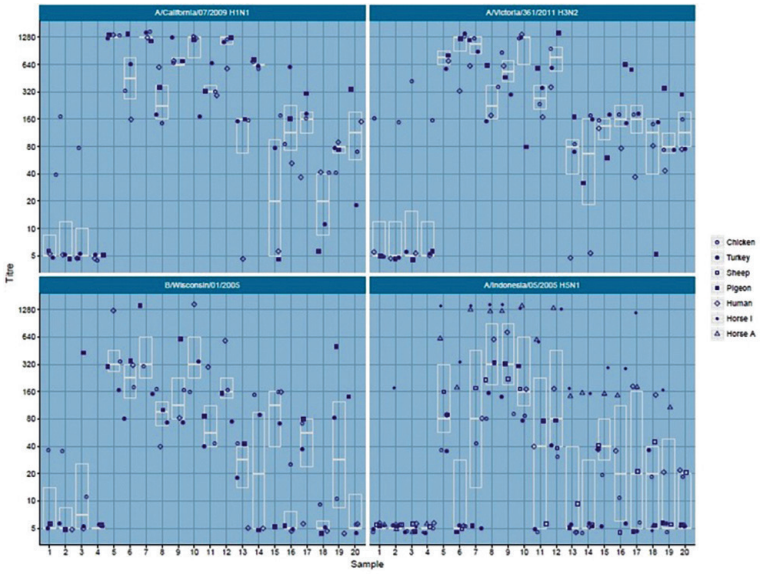


Fig. 4. HI titre ratio's for erythrocytes comparisons (a ratio of 1 indicates equality, when the 95% CI do not include 1 the titre ratio is statistically significant p < 0.05) with seasonal influenza strains.

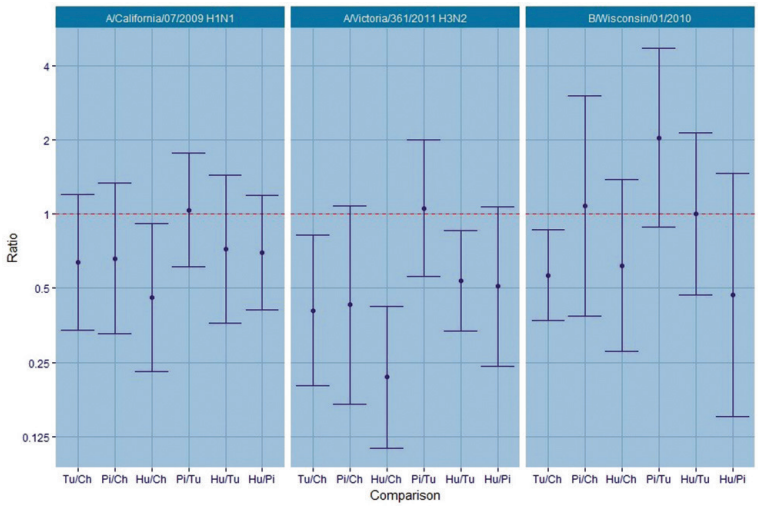
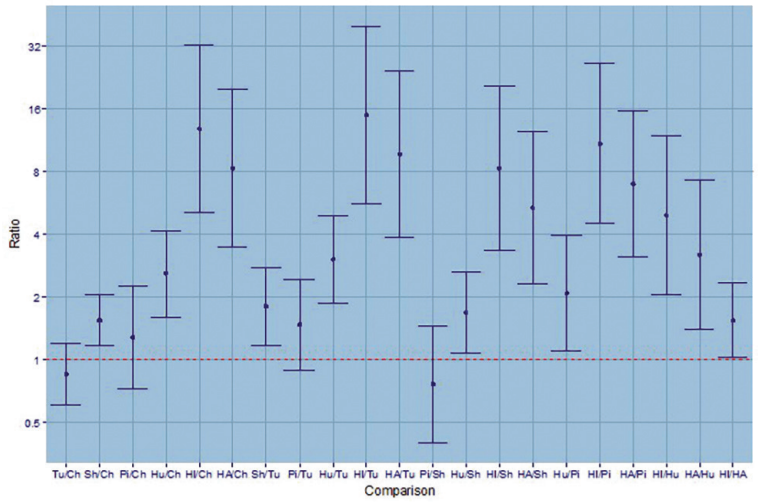


Fig. 5. HI titre ratio's for erythrocytes comparisons with avian H5N1 strain.



This study provides a comprehensive investigation of avian (turkey, chicken, pigeon) and mammalian (sheep, horse, humans) erythrocytes analysed by flow cytometry in order to investigate the expression of SA receptors. Moreover, the HA and HI titres with seasonal and avian strains have been evaluated to investigate the influence of different erythrocytes on both assays.

As reported in previous studies [1, 4-6], the results confirm that chicken erythrocytes showed higher amounts of SA α 2.3 Gal linkage, while turkey SA α 2.6 Gal linkage, sheep SA α 2.3 Gal linkage only and humans both linkages. In addition, a deeper investigation has been performed with the analysis of different batches of chickens' erythrocytes. The results provide evidence of the variability in the expression of SA α 2.3 Gal linkages. This finding is of relevance as chicken with turkey erythrocytes are the most commonly used in HA and HI assays. It would therefore be interesting to analyse different batches of chicken in both assays and different batches of turkey by flow cytometry and HA/HI assays in order to evaluate possible intra-species variability. The data seem to support that chicken and turkey erythrocytes, which are characterized by both linkages, are suitable for use in the HA assays with seasonal strains. For the avian strain, the chicken erythrocytes seem to be the best choice. The results are in good agreement with those of other studies, which have demonstrated the advantage of goose, guinea pig and turkey erythrocytes for avian strain in both assays and turkey erythrocytes for the HI assay with the human pandemic virus H1N1 [8, 16, 18, 19].

As expected, sheep erythrocytes were not agglutinated by seasonal influenza strains due to the lack of SA α 2.6 Gal linkage. This is the only species analysed in this study that expresses exclusively SA α 2.3 Gal linkage.

Nevertheless, they did not show high HA and HI titres for the avian strain.
Human erythrocytes seem not to be very useful for both assays with seasonal and avian strains despite expressing both linkages.
The novelty of the study is the characterization of pigeon erythrocytes displaying almost exclusively SA α 2.6 Gal linkage. As for the chicken and turkey, which show appreciable expression level of SA α 2.6 Gal linkage, pigeon erythrocytes yielded good HA titres with seasonal strains. Unexpected results were obtained with the avian strain where an appreciable HA titre has been observed. The HA data from sheep and pigeon erythrocytes may suggest that other mechanisms, in addition to a preference for SA α 2.3 or 2.6 Gal linkages, could be involved in the assay and need to be further investigated. The pigeon erythrocytes resulted in appreciable HI titres with H1N1 and H3N2 strains, comparable to those of turkey, and a high titre with influenza B strain. These data suggest that this animal species may constitute an interesting alternative, particularly for the B strains, and additional data should be gathered on pigeon erythrocytes.
Horse erythrocytes showed almost exclusively SA α 2.3 Gal linkages and therefore were not agglutinated by seasonal strains. A deeper investigation has been performed analysing different breeds of horse' erythrocytes. The data displayed diverse expression of SA α 2.3 Gal linkages among the breeds. It would be interesting to perform the HA and HI assays with different breeds of horses in order to evaluate possible differences in the titres.
The HA titre of the horses was lower than that seen in erythrocytes from other species with the avian strain. These results are in line with those of a previous study, in which horse erythrocytes were the least sensitive with the HA assay but provided the highest antibody titre in the HI assay [17]. The use of horse erythrocytes significantly improved the sensitivity of the HI assay for the detection of antibodies against avian H5N1 virus, thus overcoming the initial drawback of using avian strains and supporting the HI assay as an effective means of evaluating pandemic vaccines [11, 13, 14, 20, 21].

Conclusions

The data from the present study provide evidence that the choice of erythrocytes has large impact on the HI titres for seasonal and avian strains. Collectively, chicken and turkey erythrocytes seem to be the most appropriate for both assays with seasonal influenza strains, in addition to pigeon erythrocytes, particularly for the B strains. In the case of the avian strain, chicken erythrocytes are suitable for HA assay and horse erythrocytes for HI assay. Especially the horse erythrocytes showed a deep impact for the HI titres. However, the differences in HA and HI titres highlight the need to harmonize the choice of erythrocytes for both assays, in order to define the most suitable species and conditions for assays involving seasonal and avian strains. The choice of erythrocytes has a significant impact on the titres measured by both assays. The selection and standardization of appropriate erythrocytes could significantly increase the sensitivity and reproducibility of the assays. The cooperation among regulatory authorities, academia, public health institutions and manufacturers is aimed at standardizing assays such as HI [22].

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The authors declare that there is no conflict of interest.

Authors' contributions

EM and CMT conceived and designed the experiments; CMT, CU, CC, DP, GP, SM performed the experiments; CMT, EJR and SR analysed the data; CMT wrote the paper; RJC and EJR revised the paper.

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ORIGINAL ARTICLE

Measles outbreak from February to August 2017 in Messina, Italy

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Keywords

Measles • Outbreak • Vaccination coverage

Summary

Introduction. *Measles continues to be a major public health issue worldwide, with high morbidity and mortality rates. The disease remains endemic in 14 European countries, including Italy where, from 2013 to 2016, over 5,000 cases have been reported. In 2017, many Italian regions, including Sicily, have reported many cases of measles. In this study, we described the latest measles outbreak in the city of Messina, from 1st February to 31st August 2017.*
Methods. *We considered all reported measles cases that came to the "Public Health, Epidemiology and Preventive Medicine" Operative Unit of the Messina Provincial Health Agency Prevention Department, which receives all reported cases of measles in the Messina province.*
Results. *From 1st February to 31st August 2017, a total of 59 measles cases were reported, of which 44 were confirmed, nine were classified as possible, four were probable and two cases were discarded. Of the 57 possible, probable and confirmed cases, 31 (54%) were males and 26 (46%) were females. Moreover, 54 (95%) had not been previously vaccinated while the remaining cases had documented evidence of one (two cases) or two doses (one case). Genotype B3 was identified in 39/44 cases (88,6%) by the regional reference laboratory in Palermo.*
Conclusions. *Despite the development of an effective vaccination, unfortunately measles continues to threaten the lives of millions of children worldwide each year. The suboptimal immunization level in Italy has led to an increase in the transmission of measles with detrimental effects on both public health and ongoing measles elimination efforts.*

Introduction

Measles continues to be a major public health issue causing substantial outbreaks worldwide, with high morbidity and mortality rates. In 2015, there were 134,200 measles deaths globally – about 367 deaths every day or 15 deaths every hour –. In the same year, approximately 85% of children worldwide received primary vaccines before they are a year old (79% in 2000). Thanks to vaccinations, from 2000 to 2015, the incidence of measles in the world declined by 75%, from 146 to 36 cases per million inhabitants. However, the goal was to reduce, by 2015, the mortality of 95% but this goal has not been achieved [1].
Since the beginning of 2017 and up to 10 September, the Italian Ministry of Health has reported 4,487 cases of measles and three deaths [2]. In this period, measles outbreaks have also been reported in other European countries, such as in Romania, which reported over 7,000 cases and 31 deaths from January 2016 to June 2017, most of them in small children [3]. In Romania, more than half of the cases occurred in children under five years of age, among unvaccinated or incompletely vaccinated individuals; in Italy, the majority of cases occurred among unvaccinated adolescents and young adults. This can be explained by the fact that Romania had very high levels of vaccine uptake (> 95%) up to 2010, so many adults are protected; on the contrary, in Italy, the suboptimal vaccination coverage (< 95%), in particular among young adults born in the 1990s and 2000s, has determined that these were more vulnerable and therefore were more affected by measles [4].
Eliminating measles and rubella is one of the top immunization priorities of the European region, as outlined in the European Vaccine Action Plan 2015-2020, by defining priority action areas, indicators and targets, taking into account the specific needs and challenges of Member States in the European Region [5].
Today, in the WHO European Region, 42 of 53 countries have interrupted endemic transmission of measles [6]. In Italy, measles has been reported since 1934 and an improved surveillance system was introduced in 2007 [7]. From the 1970s to the end of the 1990s, measles showed the typical cyclical trend with very high epidemic peaks. With the increase in vaccine cover since the beginning of the 2000s, the extent of the spikes has decreased considerably, and since 1997 the epidemic period has lengthened. However, from 2013 to 2016, over 5,000 cases of measles have been reported. In January 2017, there was an increase in measles cases compared to previous months and January 2016 [8]. From the beginning of 2017, a measles outbreak occurred in Messina (a city in eastern Sicily) and the surrounding areas. This outbreak was part of the wider epidemic in Italy that started in January 2017 and affected 20 of the 21 Italian adminis-

trative regions [9]. Most cases occurred in Piedmont and Lombardy (northern Italy), Tuscany, Lazio and Abruzzo (central Italy) and Sicily (southern Italy). From January to the beginning of September 2017, Lazio region reported the highest number of cases (n = 1589); Sicily, with 257 reported cases, is the sixth Italian region for number of cases. Measles outbreaks involved family, school and nosocomial spheres [10].

Even though it is a vaccine-preventable disease, measles vaccination coverage is suboptimal in Italy and especially in Sicily, with 87.3% and 81.1%, respectively, about the first dose for those born in 2014 [7]. Smaller coverage was reached for the second dose: 82.2% in Italy and 64,7% in Sicily for those born in 2010 [11]. As a result, the pockets of vulnerable populations have increased. There is an even lower vaccine coverage about MMR vaccine in the city of Messina: 73% about the first dose for those born in 2014 and 45.4% about the first dose for those born in 2010 (data collected from the vaccine center in Messina, Department of Prevention Sanitary Provincial Hospital, Messina, Italy). As a result of the high transmissibility of the measles virus, the herd immunity threshold is very high, and consequently very high coverage (≥ 95%) is necessary to interrupt virus transmission.

The aim of this study was to describe the measles outbreak that affected the city of Messina this year, to conduct a complete and rapid characterization of wild-type measles virus strains circulating and to implement appropriate control measures to limit the spread of cases.

Methods

We analysed the measles outbreak of Messina that occurred from the 1st February to the 31st August 2017. We considered all measles cases reported to the "Public Health, Epidemiology and Preventive Medicine" (SPEM) Operative Unit of the Messina Provincial Health Agency Prevention Department, which covers all measles cases in the Messina province.

The measles case definition used during this outbreak was based on the European Commission case definition [12].

Measles cases were defined as possible, probable or confirmed, depending on clinical, epidemiological and laboratory criteria. A possible case was any person who met clinical criteria, i.e., fever, maculopapular rash and cough/coryza/conjunctivitis; a probable case was any person who met clinical criteria and had an epidemiological link to a confirmed case; a confirmed case was any possible case with laboratory evidence of infection with the measles virus, i.e., detection of viral RNA in a biological sample and/or a positive IgM result in serum, under the indications of the National Plan of Elimination of Measles and Congenital Rubella [13]. Following the WHO criteria, cases were discarded when clinical, epidemiological or laboratory criteria were not met, taking into account vaccination history and risk of measles infection in the community or abroad [14].

Immediately after the notification, our Public Health team started an epidemiological investigation to understand the retrospective identification of each reported case. Then, the case was included in the digital platform "Integrated measles and rubella surveillance" and was notified to health authorities.

Tests for confirmation included: specific IgM antibodies detection by enzyme-linked immunosorbent (ELISA) assay from a capillary or venous blood sample and a sample of saliva collected between 4 and 28 days after the appearance of skin rash (this test was conducted by local laboratories of Messina city); molecular detection and genetic characterization of Morbillivirus (MV) by PCR assay on urine and/or oral fluid samples within 3 days of the appearance of the exanthema and no later than 7 days (carried out by the regional reference laboratory in Palermo, Sicily).

Total RNA was extracted using QIAmp Viral RNA Mini Kit (Qiagen) for saliva samples and RNeasy mini Kit (Qiagen) for urine samples, as per the manufacturer's protocols. Nucleic acid was tested by RT-PCR using a hemi-nested protocol directed to a highly conserved part of the MV RNA, which is located on the N gene (the carboxyl terminus of the nucleoprotein, N-450).

Kit SuperScript One-Step RT-PCR kit with Platinum Taq (Invitrogen) was used for RT-PCR reaction. The sequences were genotyped by comparing the fragment coding for N-450 with that one of the WHO reference strains.

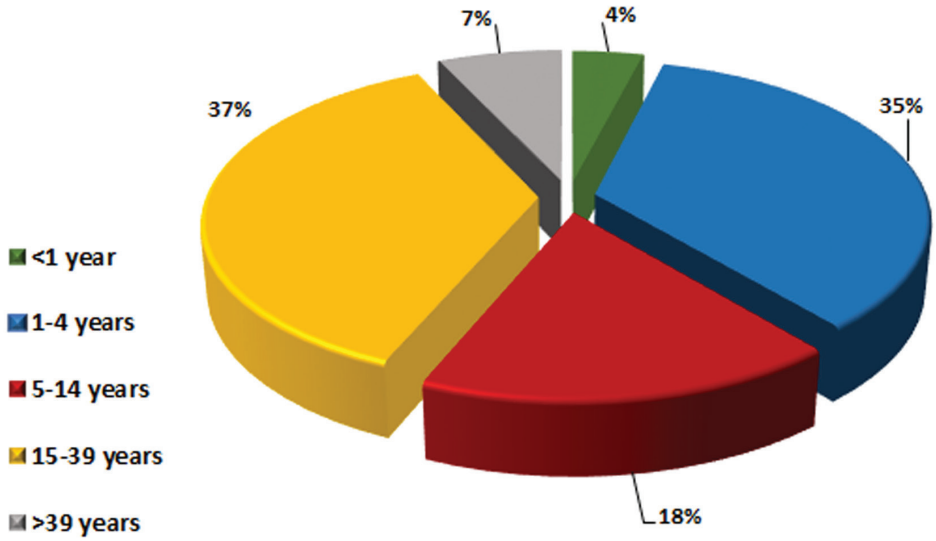
Results

From the 1st February to the 31st August 2017, 59 measles cases were notified in Messina, Sicily. 44 cases were laboratory-confirmed, nine were classified as possible, four were probable and two cases were discarded.

The earliest case was reported on 2nd February, although the symptoms began at the end of January. Of the 57 possible, probable and confirmed cases, 31 (54%) were males and 26 (46%) were females. Concerning the age, the most involved groups were 15-39 years (37%), children between one and four years were 20 (35%); only 2 cases occurred in children under one year. The percentage of all age groups are shown in Figure 1. Two of these cases were doctors and acquired measles in a healthcare setting.

We defined a cluster as having a minimum of three people with measles, for whom an epidemiological link to an identified index case, either directly or by secondary infection, was established. We recorded seven clusters, two clusters in Messina and the others in the rest of the province. The largest cluster involved 7 children who participated in a birthday party of a child with measles at the incubation stage; the other clusters were familiar clusters and involved 4 or 5 people maximum. In a family cluster of Messina city our Public Health team has given measles-mumps-rubella (MMR) vaccine to two close contacts within 72 hours of exposure.

Fig. 1. Percentage of measles cases.



Of the 57 cases, 54 (95%) had not been previously vaccinated while the remaining cases had documented evidence of one (two cases) or two doses (one case). Among the unvaccinated cases, three were infants under a year old and thus too young to be vaccinated; the remaining cases were in adolescents and adults.

Considering the trend of reported cases (Fig. 2), the peak was reached in April with 17 reported cases.

In addition, 37 subjects (65%) were hospitalized; of which two infants (5%) were less than a year old, 11 (30%) were between one and four years, five (13%) were 5-14 years old, 18 (49%) were 15-39 and one (3%) was more than 39 years of age. Of the total cases, nine subjects (16%) had at least one complication; diarrhea was the most frequent complication (n = 5), followed by kerato-conjunctivitis (n = 4), pneumonia (n = 2), stomatitis (n = 2), convulsion (n = 1) and otitis (n = 1) (Fig. 3). More complications were present simultaneously in the same subject.

Finally, genotype B3 was identified in 39 cases (88.6%) by the regional reference laboratory in Palermo; the other 5 confirmed cases remained not-typed.

Discussion and conclusions

Despite the development of an effective vaccination, measles unfortunately continues to threaten the lives of millions of children worldwide each year. Indeed, immunity gaps persist and the accumulation of measles-susceptible population pockets in the context of an increasing number of outbreaks in European countries

Fig. 2. Monthly notification trend of outbreak.

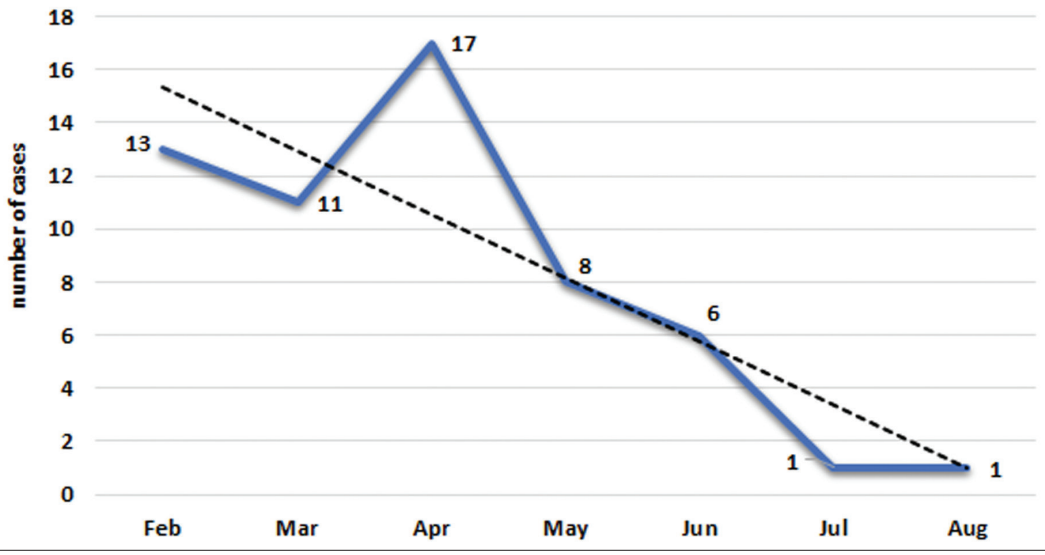
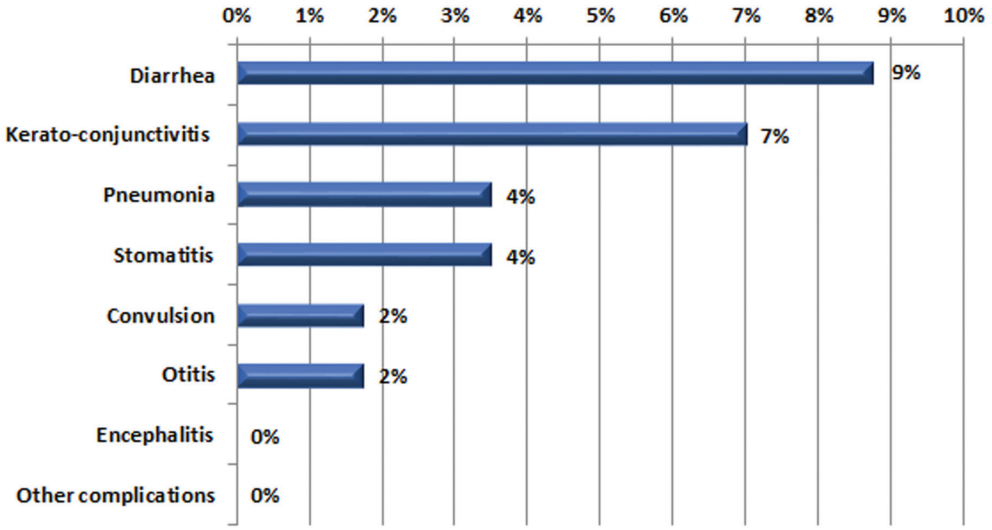


Fig. 3. Complications.



since 2016 could have contributed to this outbreak in our country [15, 16].

The decreased uptake of the measles-mumps-rubella (MMR) vaccine in Italy in recent years is also the result of vaccine hesitancy [17]. The immunization level in Italy for MMR is suboptimal with 87% of vaccination coverage rate for the first dose carried out by those born in 2014 and 79% for the second dose carried out by born in 2000 [7]. In Messina city, immunization level is very low with 73% of vaccination coverage rate for the first dose carried out by born in 2014 and 64% for the second dose carried out by born in 2000 (Department of Prevention Sanitary Provincial Hospital, Messina, Italy). This situation has led to an increase in the transmission of measles with detrimental effects on both Public Health and ongoing measles elimination efforts.

Although the collective picture for European countries in 2016 reflects that the most affected age groups in Romania were infants and young children, more than 50% of cases in Italy and 45% of cases in the United Kingdom were ≥ 20 years of age. On the other hand, a high number of infections continues to be observed in unvaccinated one to four year olds in several countries across Europe [3].

From January 2009 to May 2010, 522 cases of measles occurred in Catania, Sicily, and all but one patients had not been vaccinated. This outbreak has shown that the practice of immunization was and continues to be inadequate in Sicily [18].

In our outbreak, all reported suspected measles cases were investigated and control measures were promptly implemented in order to contain transmission. Our public health team undertook extensive contact tracing for all measles cases. Furthermore, surveillance and control measures included the immediate isolation of suspected cases and administration of measles-mumps-rubella (MMR) vaccine. Epidemiological investigations were complemented with broader Public Health measures that included raising public awareness about the importance of vaccinations, especially in children < 18 years and in healthcare workers, according to the National Vaccine Prevention Plan (PNPV 2017-19).

In our study, only two healthcare workers were involved, but a recent outbreak in Pisa (northern Italy) has involved many healthcare workers and their families [19].

Moreover, our data show all cases of B3 MV strains were spread across Italy and caused several autochthonous cases and clusters. From November 2015 to April 2016, an outbreak of measles B3 variant in the Roma/Sinti population with transmission occurred in the nosocomial setting. Therefore, in addition to healthcare workers, Roma also represent one of several undervaccinated population groups for whom stronger vaccination efforts are needed [20].

Genotype B3 was detected in some cases in the Milan (northern Italy) outbreak occurred from 1st March to 30th June 2017, even if the most common genotype detected was genotype D8 [21]. Genotype B3 was the same as the genotype detected in other outbreaks in Europe in 2017, e.g., in Romania, Belgium, Portugal and the United Kingdom [22-25].

It is also important to remember that, for the most part, hospitalised patients was in the 15-39 age group. This result confirms that at most advanced age there is a higher hospitalization rate [26]. Moreover, we recorded 16% of complications, which were largely diarrhea, pneumonia and kerato-conjunctivitis, according to Italian cases (as detailed in the Measles in Italy weekly bulletin); however, measles can cause more serious complications and sometimes death. Not long ago, a case of fulminant subacute sclerosing panencephalitis has occurred, which led to the death of a five-year-old Italian child [27]. From January to end August 2017, in Italy, three deaths due to respiratory insufficiency occurred among children aged 16 months, 6 years and 9 years respectively. All were unvaccinated and one child was immunocompromised due

to ongoing chemotherapy for a malignancy [9]. In September 2017, the fourth death for measles was recorded: a Sicilian 42-year-old man that was not vaccinated and immunocompromised [10].
The only positive side has been that the outbreak made physicians more aware of the measles diagnosis and subsequent cases were quickly reported. So the problem of underreporting that has existed for many years, at least as far as measles is concerned, has been slightly reduced. Considering the current low coverage of the eligible population of Messina, an Extraordinary Plan for the Improvement of Vaccine Coverage for MMR was launched in accordance with the objectives set by the National Vaccine Prevention Plan (PNPV 2017-2019) and the Regional Prevention Plan [28]. This plan involves pediatricians, general practitioners and other healthcare professionals in Sicily; it aims to make vaccinations more accessible and to raise awareness in order to overcome the widespread distrust of those against vaccinations.
As already stated by the WHO, the elimination of measles and rubella will depend largely on obtaining political commitment, achieving high coverage, closing immunity gaps and implementing high quality, case-based surveillance.

Acknowledgments

The authors declare that there is no conflict of interest.

Authors' contributions

AD and IP designed the study; GDA, FM and GP provided the data; MAP, GV and AF analyzed and interpreted the data; MAP wrote the paper. All authors have read and approved the final version of the manuscript.

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ORIGINAL ARTICLE

"PErCEIVE in Umbria": evaluation of anti-influenza vaccination's perception among Umbrian pharmacists

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Keywords

Anti-flu vaccine • Pharmacist • Vaccine • Knowledge • Vaccine attitude • Italy • Cross sectional study

Summary

Vaccines recommendations are available for both healthcare professionals and the general public, but although the vaccination is the most effective method to prevent infectious diseases, the coverage is still behind the recommended rate. In Italy, according to a recent study, the anti-flu vaccination rate among healthcare worker range between 9% to 30%. The aim of our study was to identify knowledge, attitude and behaviours regarding influenza vaccination among community pharmacists in order to increase the coverage rate among healthcare professional. "PErCEIVE (Pharmacist Perception on Influenza Vaccine) in Umbria" was a cross sectional survey among community pharmacists in Umbria conducted between 16th November 2015 to 29th February 2016. The questionnaire was anonymous, on-line self-administered survey. Statistical analysis were performed using STATA/SE 12 software. The response rate was 28.91% (n = 72/249). Among the studied population 76.39% (n = 55) had never performed influenza vaccine during the previous 5 years. Regarding source of information, only 15.28% of the subjects (n = 11) consulted the scientific publications, vs 52.78% (n = 38) who did not show any kind of interest upon the influenza vaccine. Our results show a low attitude to be vaccinated among pharmacists together with a low grade of awareness regarding the important role that pharmacists might play in order to reduce influenza burden, to promote health literacy among their patients and to decrease the risk of patients infection. Pharmacists might be crucial healthcare workers involved in health promotion, in vaccines' uptake and practices progression.

Introduction

Seasonal influenza is an airborne acute viral infection caused by influenza virus [1]. It is highly contagious, and despite the fact that, in the majority of the case, the progress is mild, influenza is responsible for about 3 to 5 million cases of severe illness worldwide [1], and about 5,000-70,000 death in Europe [2]. In particular, in industrialized countries most deaths occur among people age 65 or older [3]. Vaccines recommendations are available for both healthcare professionals and the general public, but although the vaccination is the most effective method to prevent the disease, also in the high risk group, the coverage is still behind the recommended rate. In Italy, the vaccination coverage for the Anti-flu, has fallen from 68.3 per 100 inhabitants during the 2005-2006 vaccination campaign, to 49.9 per 100 inhabitants in 2015-2016 [4]. It is extremely below the minimum established target (75%), set by the National Immunisation Plan. Thanks to the last National Immunisation Plan (2017-2019), the immunisation schedules are recommended for children, adolescents and adults with the regular update (through life approach), and vaccines are included in the basic levels of care (LEA-Livelli Essenziali di Assistenza) [5]. For example, in Italy, the Anti-flu vaccine is recommended for: i) general population with some specified chronic diseases, responsible for severe complication or death; ii) people aged 65 or more; iii) pregnant women who are in the second or third trimester during the beginning of influenza season; iv) professionals who are exposed to animals responsible for infection; v) professionals of public security interest; vi) family members of at high risk people; and last but not least vii) healthcare professionals [5]. Actually, anti-flu vaccines among healthcare workers is strongly encourage because it can prevent potential flu spread to patients, with unstable health status that can rapidly deteriorate, or to other professionals reducing the work absenteeism [6]. Moreover, the healthcare vaccination might serves as a positive model and promoter in influenza vaccination campaigns.

However, despite the recommendation, the vaccine coverage among healthcare workers is still low. The minimum objectives established in the US by Healthy People 2020 is 90% of influenza vaccination coverage among healthcare personnel [7]. In Italy there are two levels of vaccination coverage: 75% established as minimum rate to reach, and 95% as optimal goal [5]. Although the Anti-flu vaccine coverage rate is high heterogeneous among Europeans Countries, it is significantly under the recommended rate, ranging from 10% to 50% [8]. In Italy, according to a recent study conducted by Ali-

cino et al., the anti-flu vaccination rate among healthcare worker was around 30% among physicians, 11% among nurses and 9% among other clinical personnel during the vaccine campaign 2013/14 [9].
This significant low vaccine coverage seems to have serious consequences both individually and collectively. Even more serious is also the low perception of the importance of influenza vaccination among health care workers. Actually, Verger et al. in a recent National Cross-sectional Survey in Frances evidenced that only 54.5% of General Practices were "very confident" with vaccine utility, and only 26.2% of them were "very confident" with vaccine safety [10].
The aim of our study is to understand the determinants related to influenza vaccination uptake among community pharmacists. Pharmacists' role is not only to dispense drugs, but also they play a key role to provide appropriate consultations to the patients, to address patients' doubts among medications or vaccines [11]. However, community pharmacies are not always included in the vaccine campaign despite many patients receiving their medications and tips. Identification of knowledge, attitude and behaviours regarding influenza vaccination among community pharmacists is important to regulate the efforts need to increase the coverage rate among healthcare professionals and among general population. Moreover, understanding the reasons of vaccine hesitancy among pharmacists could identify the underlying reasons of vaccination campaigns' failure, other than to tailor public health strategies designed to increase the healthcare worker vaccinations' attitude.

Methods

"PErCEIVE (Pharmacist perception on Influenza VaccinE) in Umbria" was a cross sectional survey among community pharmacists in Umbria. The survey was conducted between 16th November 2015 to 29th February 2016 on community pharmacists working in Umbria. The questionnaire was anonymous, on-line self-administered survey. We used the validated questionnaire previously developed by the Medical Residents in Hygiene and Preventive Medicine of the Italian Society of Hygiene, Preventive Medicine and Public Health (S.It.I.-Società Italiana di Igiene, Medicina Preventiva e Sanità Pubblica) [12]. The questionnaire was first designed aimed to investigate the knowledge and attitude of the Italian Medical Residents in Hygiene. We decided to use the same questionnaire because of the similarity of the aims, and due to the correspondence of target groups' characteristics. Actually, both medical residents and pharmacists were Italian, with not cultural and language differences, moreover both this two professions categories are healthcare workers with a trustworthy relationship with the patients. We just replaced the term "medical residency" with "pharmacist" in the questionnaire. We invited the community pharmacists by sending them an email. The enrolment was voluntary base. Data were collected by an electronic survey, administered on-line (Google Moduli®).
The survey, with lasted no more than 20 minutes, contained 21 questions soliciting information about: demographics characteristics; personal experience of influenza; personal influenza immunization history; reasons for getting vaccination and for recommending it to the patients; potential barrier or reasons to refuse influenza vaccination; their recommendation for patients regarding influenza vaccine; main sources of information on anti-flu vaccine; readiness in vaccination campaign participation. The questionnaire had mainly multiple-choice questions. Box for comments was also provided for some section.

Statistical analysis

Statistical analysis were performed using STATA/SE 12 software. Chi-square test (for categorical variables) and Student's t test (for continuous variables) were used for examining statistical significance. Pearson's correlation coefficient was also used to assess linear dependences between variables. We used the two-tailed version of all tests, and the p-value ≤ 0.05 were considered statistically significant.

Ethical approval

All data collected was recorded on a computerized database in an anonymous way; the file was protected by password, known only to researchers. Written informed consent was obtained from all participants. The "PErCEIVE in Umbria" study received ethical approval from the local ethics committee of the University of Perugia (Comitato Universitario di Bioetica), Reference Number: 2015-013.

Results

Characteristics of the population

The population constituted of 249 community pharmacists who received the invitation mail, 72 of which agreed to fill the questionnaire, with a response rate of 28.91%. Out of 72 participants, 40 were female (55.56 %) and 32 were male (44.44 %), with a mean age of 45 ± 12.99 years. The 45.83% of the subjects self-reported a good or discreet knowledge related to influenza vaccine (Tab. I). In the majority of the case, the respondents (n = 46, 63.89%) had contracted the Influenza Like Illness (ILI) at least once during the past 5 years. However, 43.06% (n = 31) did not perceive themselves as a risk subjects, despite the professional exposure. Among the studied population 76.39% (n = 55) had never performed influenza vaccine during the previous 5 years. Among the only 5 pharmacists who performed anti-flu vaccination during the last year, the reason was the awareness to be a risk subject, due to the professional exposure. Inversely, among the subjects who had not performed the vaccination, the main reasons were: they believe that the low risk asso-

Tab. I. Characteristic of studied population. Number and percentage in parenthesis n (%).

Gender	Male	Female
	32/72 (44.44)	40/72 (55.56)
Anti-flu knowledge	Good-discrete	Not sufficient
	33/72 (45.83)	39/72 (54.17)
ILI in the previous 5 years	Yes	No
	46/72 (63.89)	26/72 (63.89)
Get a vaccine last year	Yes	No
	5/72 (6.94)	67/72 (93.06)
Get a vaccine during last 5 years	Yes	No
	17/72 (23.61)	55/72 (76.39)
Perception to be a risk subject	Yes	No
	38/72 (52.78)	34/72 (47.22)
Search information on anti-flu vaccine	Yes	No
	23/72 (31.94)	49/72 (68.06)
Institutional source of information	Yes	No
	15/23 (65.22)	8/23 (34.78)

ciated to the disease do not justify the vaccine need and because they did not consider themselves as a relevant element in the chain contagion both for relatives and for patients. Thirdly, they did not consider vaccination because of their age (younger than 65 years). Regarding source of information, 52.78% (n = 38) who did not show any kind of interest upon the influenza vaccine. Among them who search information (n = 23), 65.22% (15/23) of the subjects consulted institutional sources (scientific publication or institutional reports/web pages). Only 19.44% (n = 14) are intended to update their influenza vaccination during the following campaign. Among them in 50% (n = 7) of the cases, the reason was the awareness to be a high-risk subject, the remaining 50% (n = 7) recognize their responsiveness in contagion chain. Regarding the possibility to recommend the vaccination 25.00% (n = 18) of the subject did not invited people to get influenza vaccine, however the percentage decrease to 18.06% (n = 13) considering the intention to promote vaccine during the next campaign.

Statistical analysis

Had been performed influenza vaccine, during the last 5 years, is statistical significant associated with consider themselves as risk subjects rho di Spearman = 0.33 (p = 0.005). This data was also confirmed considering who performed vaccination during the previous year (rho di Spearman = 0.26 (p = 0.028). At the same time, the awareness to be a risk subjects is statistical associated with the intention to get a vaccine during the following influenza campaign, rho di Spearman = 0.39 (p = 0.0006). The degree of self-reported knowledges, regarding influenza and influenza vaccine, is statistical associated with the source of information. Moreover, the intention to be vaccinated next year is also statistical associated with had performed influenza vaccine during the last 5 years and previous year. Interestingly, who have the intention to be vaccinated next year is also associated with the propensity to recommend vaccine to their patients. Actually, who consult scientific publication or institutional documents/web sites reported a higher degree of knowledges in a statistical significant manner, rho di Spearman = 0.52 (p = 0.012). Furthermore, the high self-reported degree of knowledge is also associated with having received the request for information from general population, rho di Spearman = 0.29 (p = 0.014). Having received the request for information is also associated with the attitude to recommend vaccination, rho di Spearman = 0.39 (p = 0.0006); and to be incline to suggest vaccination for the next campaign, rho di Spearman = 0.25 (p = 0.035). The duration of years of work is statistical associated with the vaccination performed last year, actually who get vaccine the previous year had 33.2 ± 9.73 years (mean ± standard deviation) of work (p = 0.0056) (Tab. II).

Discussion

In Umbria, during the 2011/14 season, influenza vaccine coverage rate in the population aged between 18 and 64 years with at least 1 risk factor was 19.4%. Among individuals over 65 years, the coverage rate was 62.8 per 100 inhabitants in 2015/16 season, the highest rate among the Italian regions. However, the national coverage rate is still 20-points percentage less compared to the 2005/06 season (2005/06 season was the period with the highest coverage rate since the flu-vaccine introduction in Italy, with a coverage rate of 68.3% among people over 65

Tab. II. Correlation between consider themselves as risk subjects and vaccine behaviour and between had received request of information regarding anti-flu vaccination and attitude.

	Be vaccinated last 5 years	Be vaccinated last year	Intention to be vaccinated next year
Consider themselves as risk subjects	Rho = 0.33 p = 0.005	Rho = 0.26 p = 0.028	Rho = 0.39 p = 0.0006
Had received request of information	Degree of influenza knowledges	Attitude to recommend vaccination	Be incline to suggest vaccination next campaign
	Rho = 0.29 p = 0.014	Rho = 0.39 p = 0.0006	Rho = 0.25 p = 0.035

years) [13]. Actually, Ministry of health, in collaboration with Istituto Superiore di Sanità, set up several activities in order to ensure influenza control. In particular the development of InfluNet (National sentinel influenza surveillance system), InfluWeb (community based participatory surveillance system) and Flunews (a report combining information from different sources) [14]. However, despite the high efforts, the low influenza vaccine coverage is probably due to a lack of influenza vaccine confidence after the 2009 pandemic [15]. Our survey confirmed that many healthcare workers, specifically pharmacists, do not consider anti-flu vaccination relevant to prevent patients/relatives' infection.

This is not the first study evaluating the vaccine hesitancy among healthcare professionals; however, the originalities of the present study are the ability to investigate the knowledge and the attitude such as the selection of the studied population. Although the questionnaire was already described in literature, it is for the first time used to assess the behaviour and willingness on influenza vaccine among Italian pharmacists. One of the most important result of "PErCEIVE in Umbria" study is that among pharmacists there is the wrong assumption that pharmacists are not professionals at high risk. Actually 5.56% of the subjects (n = 4) refuse the influenza vaccine, because younger than 65 years. These statements confirm the lack of appropriate knowledge regarding the definition of groups for whom the influenza vaccine is recommended. Previous studies has been demonstrated that people failed to be vaccinated because of the lack of healthcare workers' recommendations [16]. Although 59%-81% of adults in USA used internet to acquire health information [17, 18], healthcare professionals is the most important information source among patients [19]. Moreover, Johnson and colleagues in a recent survey conducted among general population and GPs found that both groups are more likely to discuss about vaccinations during well-care visits [20]. It seems likely that it might be the same also in pharmacies, during drugs' dispensation for mild diseases [21].

Moreover, Johnson et al. also indicated that influenza vaccine is less recommended compared to tetanus and pneumococcal [20]. This may indicate the absence of routinely vaccines' recommendations system that might reduce the missed opportunities to educate patients on immunizations or to update their immunization status. The missed opportunities for flu vaccine is particularly high (confirmed by the low coverage rate reached in every season), and a reminder/recall system for physician can increase GPs awareness on both their patient's vaccination status and vaccine indications.

Although anti-flu vaccination is universally recognized as an essential element of disease prevention, our results show a low propensity to be vaccinated among pharmacists. The consequences of these results are basically the urgent need to identifying the reasons responsible for low vaccine coverage, even in healthcare workers. Furthermore, it is crucial to identify strategies to increase awareness of the risk for general population, high-risk group such as healthcare workers. The results also show the need to propose new training courses for the future, such as our sample suggested (58.33%, n = 42). However, Johnson previously described the healthcare professionals' lack of knowledge that we confirm in our sample. In particular only 2.78% (n = 2) of the subjects consulted the institutional web pages, 6.94% (n = 5) referred to ministerial disposal, and 11.11% (n = 8) read scientific articles. Recently, due to the low influenza coverage rate among Italian healthcare professionals, Alicino et al. conducted an educational project. The study was aimed to increase knowledge on influenza burden, risk of patients' infection from healthcare workers, and the immunization benefits [9]. The intervention was carried on for eight consecutives influenza seasons and was based on several courses, informative materials, and easier access for healthcare workers' immunization. Although during the whole period, the coverage rate had had a discontinuous trend, however the multicomponent intervention resulted in a significant increase of the influenza coverage rate. Recent evidences show as continuing education, incentives after immunization, easier access to vaccination are key strategies in increasing vaccination coverage among health care workers [22-24].

However, the study has some limitations. Firstly the small sample, although we obtained a good response rate compare to previous study, where the respondents were around 3% among general practitioners [20]. Secondly, the questionnaire was a self-administered, and suffer for the classical limitations of the survey such as social desirability bias and recall bias. However, we conducted an on-line survey that is associated with a lower social desirability bias compared with traditional version [25]. Thirdly, the vaccination history was self-reported and we did not check the pharmacists' vaccination status, due to the anonymous version of the survey. Nevertheless, there are indisputable advantages in the use of the questionnaire. It is a cheaper and easy tool, such as manageable for both respondents and researchers. Nonetheless, our study has also some strengths such as the use of previously validated questionnaire and the use of web-based questionnaire. Further, to the best of our knowledge it is the first study aimed to understand knowledge, attitude and behaviour on influenza vaccine among Italian pharmacists.

Conclusions

The aim of our study was to verify the knowledge and attitude of pharmacists on influenza vaccination. The results shown a low grade of awareness regarding the important role that pharmacists might play in order to reduce influenza burden, to promote health literacy among their patients, to decrease the risk of patients infection. In our opinion, pharmacists might be crucial healthcare workers involved in health promotion, in vaccines' uptake and practices progression. However, it is essential to plan a training courses tailored for healthcare workers.

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The authors declare that there is no conflict of interest.

Authors' contributions

TS, VG, OF and the "PErCEIVE in Umbria" study group collaborated with data collection. VG, DN and MV have contributed in study conception and design, data management and statistical analysis. VG in interpretation of results and preparation of the article, and MM for reviewing the last version of the present manuscript.

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ORIGINAL ARTICLE

Perceived barriers to breast cancer screening among Saudi women at primary care setting

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Keywords

Breast cancer • Cancer screening • Perceived barriers • Primary Care • Saudi Arabia

Summary

Introduction. *Screening for breast cancer (BC) is of low rate in Saudi Arabia; although it is provided in the country free of charge to the population. This cross-sectional study aimed at investigating the perceived barriers towards BC screening in Al Hassa, Saudi Arabia.*
Participants and methods. *A total of 816 adult Saudi women aged ≥ 30 years attending for routine primary health services or accompanying patients at the selected primary health care centers (PHCs) were randomly selected from 12 PHCs (8 urban and four rural) using multi-stage sampling method. Participants were invited to personal interview using semi-structured data collection instrument including inquiries about socio-demographics, reproductive history, previous histories of diagnosed breast lesions and breast cancer. The perceived individual barriers towards screening, their attitudes, the reasons for not attending previously held screening campaigns in Al Hassa, were also included.*
Results. *Low utilization of BC screening being significantly positively associated with woman's age, higher educational status, higher family income, using hormonal contraception and positive history of previous breast as shown by the results of the logistic regression model. Exploratory factor analysis showed that personal fears (especially fear of doctors/examiners, fear of hospitals and health facilities and fear of consequences/results) were the major factors that hinder women from utilizing the free of charge BC screening with high loading eigenvalue of 3.335, explaining 30.4% of the barriers.*
Conclusions. *Educational interventions aim at improving breast cancer knowledge and addressing barriers should be incorporated as core component of the screening program in Saudi Arabia.*

Introduction

Breast cancer (BC) is a public health problem globally, and it became the most common cancer among women contributing to a substantial death toll among them worldwide [1].
BC is the most common type of cancer among Saudi females and accounted for more than 25% of all newly diagnosed cancer [2]. It has been estimated that BC is the ninth leading cause of death among females in the Kingdom of Saudi Arabia (KSA) year 2010 [3]. The incidence of BC is expected to increase over the coming decades in Saudi Arabia due to the population's growth and aging [4].
In KSA mammography has been introduced prior to 2002 [5]. In 2007, a nationwide BC screening center was constructed in Riyadh, and 1,215 were screened in the first year [6]. Another regional mammography screening program, targeted women 35-60 years old, was conducted in 2007 in Al Qasim, and preceded by an awareness campaign [7]. Although mammography has been available in all regions of KSA since 2005, the national Saudi Health Interview Survey (SHIS) 2015, had reported a very low rate of breast cancer screening (BCS) where out of 10,735 participants, 1,135 were 50 years or older women, 89% of them reported not having a clinical breast examination (CBE) and 92% never had mammogram in the past year [8].
Early detection of BC plays a crucial role in reducing both its morbidity and mortality. Both mammography and CBE are screening methods for early detection of BC [9]. It has been reported that mammographic screening reduces BC mortality by 23% (Wang et al., 2014). Despite the effectiveness of BCS in reducing mortality, low uptake rates have been reported among Arab women [10].
Many barriers to BCS with the underutilization of services have been studied worldwide [11]. Factors affecting screening compliance can be grouped into patient, health care system, provider, and policy factors [12]. Barriers to access to health services, incomplete information, difficulties in infrastructure, socioeconomic, ethnic and geographical conditions are some of the factors affecting behavior toward screening [13].
In Saudi Arabia, a significant number of women were presented with the advanced stages of disease due to lack of information, knowledge and awareness of early detection measures [14]. It is still unclear with scarcity of literature about the possible predictors responsible for the late presentation of BC among Saudi women despite

the availability of free of charge screening program. Barriers and facilitators that influence women's BCS practices need to be examined in order to effectively promoting BCS programs [5].

Needless to mention the pivotal role of health care providers posted at primary health care centers (PHCs) in promoting the screening programs [15] as they have a major role in screening practice due to their frequent encounter with large population groups.

The objective of this study was to define the perceived personal barriers to BCS among Saudi women aged 30 years or more years and attending the primary care facilities (PHCs) in Al Hassa, Saudi Arabia. Findings from this study can help in formulating and tailoring culturally sensitive educational and other relevant interventions for women in Saudi Arabia in order to promote the uptake of breast cancer screening.

Subjects and methods

Setting and design

A cross-sectional study that was carried out in Al Hassa Governorate, located in Eastern Province of Saudi Arabia; 50 km from the Arabian Gulf, 450 km from the capital Riyadh, and populated by about 1.5 million. Al Hassa is comprised of three regions; urban, populated by about 60% of the total population, rural consisting of 23 villages (35% of the population) and "Hegar" Bedouin scattered communities making up the remaining 5%. The Ministry of Health provides primary care through 54 PHCs, while the rest of the population are provided with similar services through other sectors e.g., National Guard, ARAMCO (oil company), military and others.

Participants and methods

Population and sampling

Adult Saudi women aged ≥ 30 years attending primary health care centers in both urban and rural areas in Al Hassa were targeted for inclusion and they were constituted around 350,000 registered at the PHCs for year 2013 as reported by the local health directorate.

Sample size

Epi-Info ™ version 3.5.3, year 2008 (CDC, Atlanta, GA, U.S.A) [16] was used to calculate the sample size. Assuming the percentage with perceived barriers towards screening among Saudi women (aged ≥ 30 years) of 50%, with a precision of ± 5%, employing a 95% confidence interval, 80% power, and with a design effect of 2.0, the minimal sample size required was accounted for 768 participants. Adding 20% to compensate for potential non-response, the final total sample size was estimated to be 925 women.

Sampling method

An updated list of all primary care centers in Al Hassa distributed by districts from which eight primary health care centers were randomly selected from urban areas (Hofuf and Mubaraz four for each) and four from rural areas (from 15 centers serving the major villages), (PHCs at Hegar 'Bedouin' were excluded due to transportation problem). All Saudis women aged ≥ 30 years or more, attending for routine services at the selected PHCs during the period from January 13th 2015 to July 2nd 2015 were invited to participate through personal approach after receiving proper orientation. Of 1013 women personally approached, 923 agreed to participate.

Data collection

Women agreed to participate were invited to personal interview using semi-structured data collection instrument, the interviews were conducted by trained investigators with medical bachelor degrees. Each woman was interviewed on solicited base in a separate room or clinic within each PHC at the conclusion of their visits. The following information was gathered during the interview:

Socio-demographic and reproductive history: age in years, residence, educational and employment status, marital status, age at marriage, age at first birth, number of living children, intake and duration of hormonal contraception, age at menarche, and age at menopause (if any).

History of previous breast lesions: personal and family history of any breast lesions, their nature and age at diagnosis.

Previous history of breast cancer screening: methods, reasons, who recommended, age at screening and the results (if any).

Ever heard about the previously held breast cancer campaigns in Al Hassa, year 2010, and 2012 respectively, reasons of attendance and reasons for non-attending such campaigns.

Attitudes towards breast cancer screening: three close ended questions (responses ranged from strongly agree, agree, not sure, disagree and strongly disagree) were used to assess their attitudes towards breast cancer screening including:

Early detection of breast cancer is necessary for prevention of complications and mortality.

I am seriously planning to go for breast cancer screening in the near future.

I will go for mammography if it is free, available and comfortable and in the presence of female providers.

Perceived personal barriers to breast cancer screening: possible barriers to breast cancer screening including both clinical breast examination and mammography were evolved from the available literature [8, 17], expert opinions and the results of pilot study. For each barrier multiple options were provided in the form of yes, no and not sure with instructions to the participants to choose all the possible barriers they perceived. Open-ended questions were provided to include the other possible barriers beyond the previously mentioned.

Pilot testing

The provisional data collection form was tested on 41 Saudi women attended for primary health services at a nearby primary center beyond the sample size with the following objectives:
Training on conducting personal interview.
Clarity and Comprehension of the terms and questions.
Absence of ambiguity (if any).
The perceived barriers were initially formulated and listed from the available literature and expert opinions; further addition of the possible barriers was considered after pilot testing.

Data analysis

Of the 923 Saudi women agreed to participate, 56 refused to give responses on items related to their screening history, and another 51 women did not complete the interview, 816 interview sessions were eligible for final analysis with a response rate of 88.4%. There were no difference in relation to the socio-demographics and other characteristics between those responded and the non-respondents. Data analysis was carried out using SPSS 21.0 (SPSS Inc, IBM, U.S.A). For categorical data, frequency, proportions and percentage were used for reporting, Chi square and Fisher Exact were used for comparison. For continuous data; mean, standard deviation, and median were used, t-test, and Mann Whitney tests were used for comparison. Logistic regression model was generated to determine possible predictors for the uptake of breast cancer screening (dependent variable) by inclusion of significant independent variables revealed at univariate analysis reporting Odds ratio and 95% confidence intervals. P value of ≤ 0.05 was considered significant.

Exploratory factor analysis

Principal components analysis with an orthogonal (Varimax) rotation was used to identify the factors underlying the different perceived barriers to the uptake of breast cancer screening among the sampled Saudi women. Eigenvalue of 1 was used for factor inclusion with examination of scree plots to confirm appropriate number of possible factors. The criteria used for item elimination to maintain simple structure included were the primary factor loading below 0.4 and/ or the presence of cross-loading [18]. Following the process of items elimination, the remaining items were included in the factor analysis with examination of their loadings. The retained factors were assessed for reliability using Cronbach's alpha as a measure of internal consistency [19]. The factorability of the included barriers (n = 24) was examined at the outset of the analysis. Criteria [20] employed to determine the factorability of the correlation included: the result of the intercorrelation matrix which showed that 16 (out of 24 items) were correlated (correlation coefficient r = 0.35 with at least one item) suggested reasonable factorability. In addition to the Kaiser-Meyer-Olkin measure of sampling adequacy (0.763) which was above the commonly recommended value of 0.6, with significant the Bartlett's test of Sphericity (Chi square = 855.35, P = 0.001), confirming that each item shared some common variance with other items. Based on the above indicators, principal component analysis was warranted suitable for these 16 items.
The encountered barriers were categorized into the following types:
Personal including: fears of hospitals and health facilities, fears of screening consequences, feeling uneasy (distressed when come close to health providers, breast cancer is not a serious disease, previous bad experience with screening, fears of physicians and health care providers, screening for breast cancer is painful, and lack of time).
Cultural-social barriers including: it is unacceptable touching my breasts, embarrassing to tell people (family and relatives) about it, bad impression about what others might think about it, stigma following the diagnosis, breast examination is considered a taboo by the community and I might feel ashamed to uncover my breasts for examination or mammography.
Health facilities-service barriers including: breast cancer awareness program is deficient, lack of trust in health providers, physician and providers conducting screening are not adequately trained, health facilities offer screening are far with transportation problems, health providers can't conduct clinical breast examination properly, lack of specialized clinics, cost of screening, and it is not right to be examined by male physician or provider.

ETHICAL CONSIDERATIONS

Permissions were obtained from the local Health Authorities and our institution. Participants were provided with full explanation of the study with the emphasis on their right of not to participate. Informed consent forms were obtained and data confidentiality was maintained all though

Results

Socio-demographic characteristics and previous history of breast cancer screening of the study participants were shown in Table I. Mean age of the study sample was 43.8 ± 6.6years, nearly half of them (52.3%) were of the age category between 40- < 50 years, 63.6% were urban. Almost sixty percent had secondary school education or more, 56.1% were housewives, 82.4% were married, out of those having children (n = 558), 48% had more than 4 children, 47.2% had family income ranged from 6000- < 10000 (monthly in Saudi Riyals). Only 7.4% had a previous history of benign breast lesions, 18.9% mentioned having relatives with breast cancer. Out of the total (n = 816), only 16.2% (n = 132) had been ever screened for breast cancer. Among those previously screened (n = 132), methods used for screening was both Mammography and CBE, 46% out of them mentioned that they were advised by the health care providers for screening.
The results of univariate analysis to define the independent variables associated with women's screening status showed that living in the urban region (Odds ratio 'OR' = 1.51; 95% confidence intervals 'CI' = 1.01-2.71;

P = 0.047), those aged ≥ 50 years (OR = 2.55; 95% CI = 1.71-3.83; P = 0.0001), having college education or more (OR = 2.98; 95% CI = 2.05-4.34; P = 0.0001), with monthly family income ≥10000 Saudi Riyals (OR = 1.96; 95% CI = 1.31-2.93; P = 0.009), ever used hormonal contraception (OR = 1.46; 95% CI = 0.99-2.13; P = 0.050) and women previously complained of benign breast lesions (OR = 12.16; 95% CI = 6.89-21.46; P =†0.0001) had higher likelihood of being screened for BC. Whereas working, marital status and history of breast cancer among relatives/family members were not significantly associated with BCS (Tab. I).

The logistic regression model showed that women's age (≥ 50 years), having college education or more, with monthly family income ≥ 10000 Saudi Riyals and having a previous benign breast lesions were the significant positive predictors for the uptake of screening among the included women (Tab. II).

Perceived barriers towards BCS are demonstrated in Table III. Barriers mentioned by women who never screened included efficiency of the health care providers due to lack of training (HCPs), their ability to conduct CBE, they aren't trusted and use scary tools and painful maneuvers.

Tab. I. Socio-demographics, previous screening for breast cancer of the included participants (N = 816).

Characteristics	Number	%
Age in years (mean ± SD)	43.8 ± 6.6	
Age groups categories		
30 < 40	216	26.5
40 < 50	427	52.3
≥ 50	173	21.2
Residence		
Urban	519	63.6
Rural	297	36.4
Educational status		
Illiterate/read & write	54	6.6
Primary/preparatory	194	23.8
Secondary	262	32.1
College or higher	306	37.5
Working status		
Employed	178	21.8
Unemployed but able to work	120	14.7
Housewives	458	56.1
Students	48	5.9
Retired	12	1.5
Marital status		
Married	672	82.4
Single	126	15.4
Divorced/widowed	18	2.2
Number of children: (n = 558)		
< 4	290	52.0
≥ 4	268	48.0
Family income: (monthly in Saudi Riyals)		
< 6000	243	29.8
6000-< 10000	385	47.2
≥ 10000	188	23.0
Had a benign breast lesions	60	7.4
History of breast cancer among relatives/family	154	18.9
Ever screened for breast cancer	132	16.2
Methods used for screening	18/132	2.2
Mammography	18/132	2.2
Clinical Breast examination	54/132	6.6
Both	56/132	6.9
Screening advised by		
Self	52/132	6.4
Family/friends/relatives	34/132	4.2
Health care providers	46/132	5.6

SD = standard deviation.

Tab. II. Predictors of breast cancer screening among the included women (N = 816).

Independent variables	Ever had breast cancer screening (clinical breast examination and mammography): no. (%)					
	Univaraite analysis				Multivariate logistic regression analysis	
	Yes (N = 132)	Never (N = 684)	Odds ratio (95% C.I)	P value	Odd ratio (95% C.I)	P value
Residence						
Rural	38 (28.8)	259 (37.9)	Reference		Reference	
Urban	94 (71.2)	425 (62.1)	1.51 (1.01-2.71)	0.047	1.12 (0.73-1.71)	0.606
Age groups in years						
30-< 40	25 (18.9)	191 (27.9)	Reference		Reference	
40-< 50	59 (44.7)	368 (53.8)	0.69 (0.47-1.01)	0.055	0.67 (0.44-1.01)	0.812
> 50	48 (36.4)	125 (18.3)	2.55 (1.71-3.83)	0.0001	2.82 (1.77-4.51)	0.009
Educational status						
≤ Secondary	58 (43.9)	452 (66.1)	Reference		Reference	
College or higher	74 (56.1)	232 (33.9)	2.98 (2.05-4.34)	0.0001	2.81 (1.99-3.97)	0.001
Working status						
Yes	30 (22.7)	148 (21.6)	1.06 (0.68-1.67)	0.781		
No	102 (77.3)	536 (78.4)	Reference			
Marital status						
Single	20 (15.2)	106 (15.5)	Reference			
Married	106 (80.3)	566 (82.7)	0.85 (0.53-1.36)	0.499		
Divorced/widowed	6 (4.5)	12 (1.8)	2.66(0.80-7.83)	0.093		
Family income is Riyals						
< 6000	21 (15.9)	222 (32.5)	Reference		Reference	
6000-< 10,000	66 (50.0)	319 (46.6)	1.14 (0.79-1.66)	0.478	1.03 (0.70-1.53)	0.661
≥ 10000	45 (34.1)	143 (20.9)	1.96 (1.31-2.93)	0.009	1.79 (1.28-2.50)	0.023
Use hormonal contraception						
Yes	56 (42.4)	228 (33.3)	1.46(0.99-2.13)	0.050		
Never	76 (57.6)	452 (66.7)	Reference			
Previous benign breast lesions						
Yes	38 (28.8)	22 (3.2)	12.16 (6.89-21.46)	0.0001	15.90 (8.57-29.52)	0.0001
Never	94 (71.2)	662 (96.8)	Reference		Reference	
Breast cancer family and relatives						
Yes	32 (24.2)	122 (17.8)	1.47 (0.94-2.29)	0.852		
No	100 (75.8)	562 (82.2)	Reference			

C.I. = Confidence Intervals; % predicted for the logistic regression model was 82.8%, Hosmer-Lemeshow Chi-Square test = 7.225, P = 0.513.

Barriers mentioned also difficulty to communicate with foreign physicians and it is not right to be examined by male physician or provider. Of those women never screened 29.2% considered people's thoughts, 28.9% complained of transportation problems and 21.1% with perceived fears from hospitals and health care facilities. Significantly encountered barriers perceived by those never screened were stigma following the diagnosis of cancer (P = 0.010), shyness (P = 0.020), lack of specialized clinics (P = 0.002), being busy with lack of time for screening (P = 0.001) and being an expensive procedure (P = 0.013). Fear of consequences (P = 0.050) and previous bad experience with HCPs (P = 0.009) were significant perceived barriers among those ever screened. Lack of awareness program was mentioned as a possible barrier more by women who ever screened compared to those never screened (36.4% vs. 30.7%), BC isn't dangerous (6.1% vs. 0.9%), breast screening (mammogram) and examination are painful (33.3% vs. 1.5%) and being a taboo as viewed by the community (6.1% vs. 2.3%) (Tab. III).

Exploratory factor analysis: The three components model explained 75.7% of the variation in the perceived barriers towards BCS among the included Saudi women. A predefined barrier was considered as being loaded on a specific component when its absolute factor loading was < 4. Exploratory factor analysis with three factors solution showed that personal fears (especially fear of consequences/results and fear of hospitals and health facilities) was the major factor that hinder BCS with high loading eigenvalue of 3.335, explaining 30.4% of the barriers of the included sample toward utilization of BCS. The second factor with high eigenvalue of 2.778, and explaining 25.3% of the barriers to BCS was related to cultural and community barriers, including items related to shyness from been uncovered or touched by others, embarrassing from telling people about their disease or to be examined

by male physician, the third factor included health care related barriers mainly difficult in communication with foreign providers, deficiency in awareness programs and lack of specialized clinics (Tab. IV).

The stated reasons for not attending the previously held BCS campaigns included that screening is only for those aged ≥ 50 years (41.7%), not interested (26%), transportation problems (20.1%) and fear of diagnosis results (17.6%) (Tab. V). Attitudes towards breast cancer screening among the participants in relation to their screening status showed that the majority of those not screened before (99.4 %) agreed that early breast cancer detection is the cornerstone for its prevention, 86.5% 10(7.6) were seriously planning to have breast cancer screening in the near future, and 80.4% of them willing to go for mammography if it is free, painless, and the examiner is a female provider (Tab. VI).

Discussion

The results of this study showed that women in Saudi Arabia perceived several types of barriers toward BCS, only 16.2% of the studied participants were ever

Tab. III. Barriers towards breast cancer screening as perceived by participants according to their screening status (N = 816).

Barriers *	Total (N = 816)	Ever screened (N = 132)	Never screened (N = 684)	P value **
	N. (%)	N. (%)	N. (%)	
1. Unacceptable touching to my body	116(14.2)	14(10.6)	102(14.9)	0.194
2. Embarrassing to tell people about	92(11.3)	14(10.6)	78(11.4)	0.790
3. No idea about what other people think	224(27.5)	24(18.2)	200(29.2)	0.791
4. Stigma following the diagnosis of cancer	22(2.7)	2(1.5)	20(2.9)	0.010[!]
5. Taboo as viewed by the community	24(2.9)	8(6.1)	16(2.3)	0.563
6. Ashamed-shy to uncover my breasts	112(13.7)	14(10.6)	98(14.3)	0.020
7. Fear of hospitals and health facilities	162(19.9)	18(13.6)	144(21.1)	0.256
8. Fear of consequences	276(33.8)	54(40.9)	222(32.5)	0.050
9. Felt uneasy-distressed when come close to HCPs	46(5.6)	8(6.1)	38(5.6)	0.060
10. Breast cancer is not dangerous	14(1.7)	8(6.1)	6(0.9)	0.947
11. Previous bad experience with HCPs	14(1.7)	3(2.3)	11(1.6)	0.009[!]
12. Fear of physicians and examiners	92(11.3)	10(7.6)	82(12.0)	0.154
13. Breast screening (mammogram) and examination are painful	54(6.6)	44(33.3)	10(1.5)	0.590
14. Busy, no time to do it	220(27.0)	30(22.7)	190(27.8)	0.001
15. Awareness program are deficient	258(31.6)	48(36.4)	210(30.7)	0.231

HCPs = health care providers; * Not mutually exclusive; ** Chi-square test for independence; [!] Fisher Exact.

Tab. IV. Summary of items and factor loadings for three Factor solution for the perceived barriers to breast cancer screening among the included Saudi women.

Barriers to breast cancer screening*	Factor loadings **			
	1 Personal fears	2 Cultural and community barriers	3 Health care related barriers	Communality
Fear of doctors/examiners	0.787			0.738
Fear of hospitals and health facilities	0.855			0.656
Fear of consequences/results	0.868			0.686
Ashamed/shay to uncover your breast		0.745		0.795
Unacceptable touching to my body		0.793		0.775
Embarrassing to tell people about		0.656		0.528
It is not right to be examined by male physician		0.653		0.627
HCPs are not trustworthy			0.486	0.551
Not easy to communicate with foreign providers			0.713	0.727
Awareness programs are deficient			0.685	0.551
Lack of specialized clinics			0.662	0.598
HCPs are not competent			0.455	0.603
Cronbach's alpha	0.731	0.651	0.503	
Eigenvalue	3.335	2.778	1.911	
% variance explained	30.39	25.25	20.06	

* Not mutually exclusive; ** Principal Component Analysis, Varimax with Kaiser Normalization, Kaiser-Meyer-Olkin for sample adequacy = 0.763, Bartlett's test for sphericity; Chi = 855.35, P = 0.001.

screened for BC in a country where health services are provided free of charge to the population.

Despite the effectiveness of BCS behaviors in reducing mortality [10], finding of this study and others that have been published on BCS practices in Saudi Arabia [5, 6, 8, 21] reported that the pattern of utilization of BCS was low compared to other studies in both developing and developed countries [23, 24].

Findings from the current study as well as Patel et al. [25] indicated the associations between socio-demographic characteristics and BCS behavior. Average age at presentation of BC in Arab countries is 48 years, which is a decade earlier than in the Western countries [26]. The role of age is controversial, finding of the present study coincided with those reported from Villanueva et al., where old age was a significant predictor for BCS [23], in contrast Abolfotouh et al., reported a negative association between age and screening behavior in their study [17].

In the current study, the educational level of women emerged as a significant determining factor for screening uptake. This finding was in agreement with other studies [24, 27] but inconsistent with Agboola et al. [28].

The results of this study as well others [25, 29] found that study participants with low annual household incomes were less likely to have a mammogram compared to those with higher incomes. Our study also showed that women with previous history of breast lesion are more likely to perform screening more frequently, and this was in agreement with other studies from both developed and developing countries [17, 30].

In the present study, personal fears were the main barriers for not practicing BCS; fear of doctors/physicians, fear of consequences/results, fear of hospitals and health facilities are explaining 30.4% of the barriers among the included women towards utilizing screening services as depicted by the results of exploratory factor analysis. These findings are in agreement with the results of previous studies [17, 31]. Of the included women in this study, the significant screening barriers perceived on the part of providers were lack of trust, perceived inadequacy of their training, their ability to conduct CBE and being foreign/male physicians as mentioned by participants, consistently Engelman, Filippi and Khazaeepoo [32-34] reported the similar findings.

Tab. V. Stated reasons for not attending the last breast cancer screening campaign (N = 816).

Reasons *	Number	%
Busy with no time to attend	122	15.0
Not interested	212	26.0
Crowded places for the campaign	52	6.4
Distance-transportation problems	164	20.1
Not needed for my age (it is for those aged 50 or above)	340	41.7
Inconvenient time/place	24	2.9
Fear of the results	144	17.6
Personal/family issues	64	7.8
Do not know where about	48	5.9
Already screened	132	16.2
Sickness/pregnancy	24	2.9

Tab. VI. Attitudes towards breast cancer screening among the participants in relation to their screening status (N = 816).

Items	Total N (%)	Screening for breast cancer: N (%)		P value *
		Yes (N = 132)	None (N = 684)	
Early breast cancer detection is the cornerstone for its prevention:				
Agree	812 (99.5)	132 (100.0)	680 (99.4)	0.378
Disagree	4 (0.5)	0	4 (0.6)	
I am seriously planning to have breast cancer screening in the near future:				
Agree	714 (87.5)	122 (92.4)	592 (86.5)	0.227
Disagree	84 (12.5)	10 (7.6)	74 (13.5)	
I will go for mammography if it is free, painless, and the examiner is a female provider				
Agree	644 (81.1)	94 (71.2)	550 (80.4)	0.005
Disagree	150 (18.9)	36 (28.8	114 (19.6)	

* Chi square for independent samples.

Saudi women are more likely to shy away from preventive medical exams resembling breast examination. All clinics in Saudi Arabia have a female section that is operated by female physicians; however the uptake of screening services showed low rates, El Bcheraoui et al. [8], Amin et al. [21] and Tavafian et al. [35] foundthat traditions, mainly shyness and not wanting to be examined by a male physician were some of the barriers for not seeking CBE. Preparing environmental conditions and proper messages about the availability of screening carried out by female physicians may ease the women's embarrassment and overcome the shyness issue during breast screening for better program uptake.

Saudi women in this study reported that cultural beliefs and the social stigma of cancer limit their participation in BCS. Similar findings have been revealed by several investigators in different cultures [36, 37]. The social stigma of cancer revolved around a misunderstanding of cancer, a fear that BC screening practice would lead to getting the disease, and bring shame to the family [38].

Clinicians should be aware of the culture, traditions, beliefs and practices in different communities and the influence of these factors on their conclusion to contribute in BCS. The health care related barriers reported by participants in the current study included the lack of awareness program, lack of specialized clinics and incompetency of the health care providers, all should be considered both by managers and health professionals in the planning and organization of primary health care education programs. These barriers create not only delays in diagnosis but also in the treatment implementation [39, 40].

Women empowerment is a crucial and necessary component for improving woman's health. Unfortunately, most information on breast screening comes from screening campaigns. Although these campaigns are currently widespread in Saudi Arabia, the knowledge about the disease and the existence of this campaigns is still very low among women [41]. Advances in technology and messaging should be used to reach women everywhere. Moreover, awareness campaigns based solely on marketing are not enough to produce mass screening and increase mammography in other Middle Eastern countries [42]. Women should be involved and given a voice to gather other around health and society issues to reduce the burden of disease.

It has been reported that breast carcinoma occurs in relatively younger age groups among Saudi patients than in Western patients [26, 43]. This could be due to the demographic characteristic of the Saudi population, which is characterized by a dominance of a younger population (more than 60% of the population is under 18 years) [44]. Despite of this fact, being young and aged less than 50 years was one of the reasons mentioned by the study participants for not attending the last breast cancer screening campaigns in Al Hassa, year 2010, and *2012* respectively [45], this was also mentioned by Elobaid et al. in their study [24]. The American Cancer Society recommends that women get a mammogram and CBE yearly after the age of 40 years [46].

Many women in this study and others [25, 34] did not perceive that BC screening as a health priority, 15% of the studied women mentioned that they were too busy to attend the last breast cancer screening campaign [45] and 26% were not interested. This can be partially explained by their lower perception for being at risk for BC, the daily life norms, high responsibilities towards their families and lack of time to attend BCS campaigns. It has been reported that the multi-responsibilities of working women and shortage of time forced the working women to postpone their own affairs for the sake of other family members [47], although this was not similar to our case as the majority of the studied women were not working. The results of our study are consistent to those reported by Patel et al. [25] who found that women reported several reasons for not attending BCS campaigns; some issues related to transportation, lack of information about where to go for screening, and fear of having the diagnosis of cancer. However promising attitude toward screening have been noticed among studied participants, they agreed that, early breast cancer detection is the cornerstone for its prevention, seriously planning to have breast cancer screening in the near future and willing for mammography if it is free, painless, and the examiner is a female provider.

In conclusion, the screening rate for breast cancer among women in Al Hassa, Saudi Arabia is low, the most commonly perceived barriers to BC screening included personal fears, the main barriers for not practicing BCS; fear of doctors/physicians, fear of consequences/results, fear of hospitals and health facilities. Such fears should be addressed during launching and implementing BC screening programs, community based awareness plans and intensive educational campaign for women based on socio-cultural contexts and culturally sensitive educational materials targeting their influences and stressing the importance of early detection benefits needed to be developed in Saudi Arabia to promote breast cancer screening.

Study limitations

The results of this study can viewed in the presence of the following limitations: Results of the study cannot be generalized as the study included women from Al Hassa, not from all Saudi Arabia regions. The design is a cross-sectional study design with the possibilities of recall-bias, social desirability and interviewer bias. Lack of qualitative component with more in depth elaboration of personally perceived barriers especially the psychological and socio-cultural.

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Authors' contributions

TTA conceived, designed and coordinated the research, MBA-G and AIA collected data, AA-R performed the data quality control, MA-H and MB-M optimized the informatics database, LBA-H and EHA performed the statistical analyses, SBA-A evaluated the results and wrote the manuscript. All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Awareness and knowledge about cervical cancer prevention methods among Tunisian women

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Keywords

Cervical cancer prevention• Anti-HPV vaccine • Women • Tunisia

Summary

Introduction. *Epidemiological and biological arguments put papillomavirus infection (HPV) as a determining factor in the etiology of cervical cancer. The main objective of this study is to assess the knowledge, attitudes and practices related to HPV prevention and cervical cancer screening among women living in the city of Sousse, Tunisia.*
Methods. *Five hundred Tunisian women were interviewed face to face between May and June 2016. The questionnaire consisted in 14 questions relating participants' socio-demographic information, their awareness level, attitudes and practices regarding HPV and cervical cancer, including their understanding of the underlying cervical cancer etiology and preventive actions such as the Pap Smear test, and finally their acceptability and willingness to receive the anti-HPV vaccine under certain circumstances. Multivariate analyses were conducted to identify predictive factors of good acceptability of cervical cancer prevention methods.*
Results. *Four hundred fifty-two questionnaires were completed and included in the analysis. The awareness of a sexually transmitted infection as an etiological agent for cervical cancer was expressed by 175 (38.7%) participants, the correct finality of the Pap Smear test by 308 (68%) and its recommended frequency by 176 (38.9%) respondents. Among all participants, 125 (27.7%) had undertaken the Pap Smear test at least once in their lifetime, 363 (80.3%) were interested in receiving the anti-HPV vaccine for themselves, 387 (86%) for their daughters and 405 (90%) approved the introduction of the anti-HPV vaccine in the Tunisian national program of vaccination.*
Conclusions. *The knowledge and awareness of HPV infection and cervical cancer among Tunisian women was found to be moderate (around 40%) and the acceptability of the anti-HPV vaccine was found to be high (over 80%). These results are concordant with the results of other studies conducted in other MENA countries.*

Introduction

Given the conservative cultural context of the Middle East - North Africa (MENA), the prevalence of sexually transmitted infections (including human papillomavirus [HPV]) has previously been reported to be low in this specific region compared to the rest of the world [1]. Knowing this context, and given the rapid changes in the lifestyle induced by globalization, sexual behavior, especially among younger generations, is changing towards more liberal practices compared to the previous decades. These changes may induce an increase of the prevalence of sexually transmitted diseases among youth population of the MENA region [2].
Knowing the scientifically proved etiologic link between HPV infection and cervical cancer, similar findings were reported regarding the cervical cancer incidence in this area [3, 4].
However, despite the current relatively low incidence rates, cervical cancer is considered a public health issue in the MENA region [5]. This fact may be explained by the lack of governmental willing to establish a national cervical cancer prevention program in the MENA region and to include the anti-HPV vaccine within national vaccination programs [5]. This position has often been justified by cultural and religious sensitivities that may limit the success of sexually behavior related vaccination such as the anti-HPV vaccine [6].
That said, several studies investigated the acceptability of the anti-HPV vaccine in various MENA countries (Lebanon, Iran, Morocco, Saudi Arabia, Turkey, etc). These studies showed that despite the low level of HPV and cervical cancer knowledge among women in this area [7-10], all findings revealed moderate to high acceptability of the anti-HPV vaccine [11-14]. Since no study has been yet focusing on the specific case of Tunisia, we conducted the current study to assess whether trends reported for the rest of the region were also valid in the Tunisian context. These results would be useful for the design of an efficient and effective Tunisian national anti-HPV vaccination program.

Methods

This cross-sectional study involved the administration of 500 questionnaires to randomly selected women from different age groups in the general population of the city of Sousse, Tunisia. The questionnaires were administered through direct interview of patients visiting outpatient clinics in "Farhat Hached Hospital" or visiting the waiting area of the National Health Insurance Fund - CNAM

(la Caisse Nationale de l'Assurance Maladie) between May and June 2016. Participants answered a questionnaire that assessed their knowledge, attitudes and practices regarding HPV and cervical cancer screening.
The designed questionnaire is semi-structured and composed of 14 questions. The first 4 questions gather sociodemographic information of the participants (age, professional activity, marital status, health sector). The next 6 questions assess participants' knowledge, attitudes and practices regarding HPV and cervical cancer, including their understanding of the underlying cervical cancer etiology and preventive actions such as the Pap smear test. Finally, the last 4 questions assess the acceptability and willingness of participants to receive the anti-HPV vaccine under certain circumstances.
All respondents were informed of the voluntary and anonymous nature of the study. A research assistant assisted each of the participants when filling the questionnaire to make sure that all the questions were well-understood. Fully completed questionnaires were obtained from 452 respondents, representing a final participating rate of 90.4% of our conventional sample.

Statistical analysis

Data was entered to the computer software Statistical Package for Social Sciences (SPSS Inc., Chicago, IL., USA) version 20 for the purpose of analysis.
Descriptive statistics were reported using mean ± standard deviation (SD) for participants' age, frequency and percentage for all the rest categorical variables.
In addition to the descriptive analysis, multivariate analyzes were conducted to assess the association between participants' demographics (age, work status, marital status, and health sector) and the Pap Smear test history, then second the acceptability of the anti-HPV vaccine. The acceptability of the vaccine was assessed through several proxies such as the willingness to receive the vaccine, the willingness to get daughter vaccinated and being favourable to the inclusion of the anti-HPV vaccine in the Tunisian national vaccination program. Multivariate logistic regression analyses were performed for the following associations:

- the association between main participants' demographics [age (continuous), marital status (categorical: single/married/divorced or widow), professional status (categorical: none/employee/student) and health sector (categorical: private/public/private and public)] as independent variables and the Pap Smear test history as the dependent variable (categorical: yes/no);
- the associations between Pap Smear test history (categorical: yes/no) and main participants' demographics [age (continuous), marital status (categorical: single/married/divorced or widow), professional status (categorical: none/employee/student) and health sector (categorical: private/public/private and public)] as independent variables and a) the awareness of the existence of an anti-HPV vaccine, b) the interest in receiving the vaccine, c) the interest in giving the anti-HPV vaccine to daughters and d) the acceptability of including the anti-HPV vaccine within the national vaccination program as dependent variables (categorical: yes / no);
- logistic analyses results are presented in Table I and all association with statistical p-values of less than 0.05 were considered as significant.

Results

The results are grouped under first socio-demographic data, second the participants' knowledge and awareness level, third the acceptability of cervical cancer prevention methods and finally the associations between participants sociodemographic determinants and proxies of the acceptability of cervical cancer prevention methods.

Participants' socio-demographic profile

452 completed questionnaires were suitable for analysis. The respondents consisted in Tunisian women with an overall mean age of 30.8 years old (± SD 6.9 years), ranging between 18 and 62 years old.
Among the 452 participants, 193 (43%) were single, 242 (53%) married and 17 (4%) divorced or widow. Two hundred and sixty-three (58%) respondents were professionally active, 115 (25%) students and 74 (16%) housewives. Three hundred and thirty-one (73 %) participants usually consulted in the private sector, 99 (22%) in the public sector and the remaining 22 (5%) participants declared consulting in both sectors. The demographic information is shown in Table II.

Participants' knowledge and awareness regarding cervical cancer prevention methods

The awareness of the Pap Smear test recommended frequency, cervical cancer risk factors, HPV infection spread ways and the awareness of the existence of an anti-HPV vaccine are shown in Table III.
Only 125 (28%) respondents had already received the Pap Smear test at least once in their lifetime and 188 (42%) were aware of existence of the anti-HPV vaccine. Regarding the recommended frequency of Pap smear test, 176 (39%) women were aware that the correct frequency is 3 to 5 years.
With regards to the Pap smear test finality, 109 (24%) participants declared knowing or assuming that it is for sexually transmitted infections screening, 308 (68%) for cervical cancer screening, 7 (2%) for cervical cancer treatment and 93 (21%) women declared having no idea.
Concerning the cervical cancer risk factors, the "genetic dimension" was selected by 227 (50%), "sexually transmitted infection" by 175 (39%), "multiple partners" by 146 (35%), "partner having multiples partners" by 107 (24%), "immunity issues" by 153 (34%), "smoking" by 125 (28%), "no idea" by 69 (15%) women among all the participants.
Regarding the HPV spread ways, the correct options are: "unprotected sex" selected by 242 (54%), "protected sex" selected by 5 (1%) participants. Regarding incor-

Tab. I. Analysis of association between demographics and acceptability of Pap Smear test and anti-HPV vaccine.

	Age		Work status		Health sector		Marital status		Pap Smear test history	
	OR-IC (95%)	P-value	OR-IC (95%)	P-value	OR-IC (95%)	P-value	OR-IC (95%)	P-value	OR-IC (95%)	P-value
Pap Smear test history	1.10 (1.06-1.15)	0.00	0.63 (0.40-0.98)	0.04	1.93 (1.10-3.40)	0.22	4.37 (2.43-7.86)	0.00	-	-
Aware of the existence of the anti-HPV vaccine	0.96 (0.89-1.03)	0.25	0.69 (0.35-1.34)	0.27	2.30 (1.02-5.20)	0.04	0.94 (0.40-2.20)	0.89	0.58 (0.24-1.42)	0.24
Interested in receiving the anti-HPV vaccine	0.99 (0.95- 1.03)	0.54	1.03 (0.68-1.57)	0.89	0.97 (0.60-1.56)	0.89	0.94 (0.56-1.60)	0.83	0.67 (0.36-1.25)	0.21
Interested in giving the anti-HPV vaccine to their daughters	0.99 (0.94-1.03)	0.62	0.83 (0.51-1.34)	0.44	1.40 (0.81-2.41)	0.23	0.85 (0.46-1.56)	0.60	1.04 (0.53-2.04)	0.91
Interested in including the anti-HPV vaccine within the vaccination national program	0.98 (0.93-1.04)	0.51	1.01 (0.59-1.75)	0.96	0.94 (0.51-1.73)	0.84	1.22 (0.61-2.46)	0.57	0.49 (0.20-1.19)	0.12

rect options, "blood" was selected by 71 (16%), "public toilets" by 69 (15%) women and 157 (35%) participants reporting having no idea.

Participants' acceptability of cervical cancer prevention methods

Concerning the willingness to receive the anti-HPV vaccine, the results are presented in Table III: 363 (80%) participants were or would be interested in receiving the anti-HPV vaccine if recommended by their doctors, 387 (86%) were or would be interested in giving their (future) girl the anti-HPV vaccine if recommended by their doctors, and finally 405 (90%) were favourable to the inclusion of the anti-HPV vaccine in the national vaccine program as it would be free and systematic for all Tunisian girls.

Tab. II. Socio-demographic information.

	Total sample (452)	%
Profession		
None	74	16,4%
Employee	263	58,2%
Student	115	25,4%
Health sector		
Private	331	73,2%
Public	99	21,9%
Private and public	22	4,9%
Marital status		
Single	193	42,7%
Married	242	53,5%
Divorced/Widow	17	3,8%

Associations between participants socio-demographic determinants and proxies of the acceptability of cervical cancer prevention methods

Multivariate logistic regression analyses results presented in Table I are showing statistically significant associations between Pap Smear test history and the four considered demographics: age 1.10 CI (1.06-1.15), working status 0.63 CI (0.40-0.98), marital status 4.37 CI (2.43-7.86) and the health sector 1.93 CI (1.10-3.41). However, a unique statistically significant association was found between the awareness of the existence of an anti-HPV vaccine and the health sector 2.30 CI (1.02-5.20). No further statistically significant associations were found between participants' demographics and the interest in receiving the vaccine, giving it to daughters or including the anti-HPV vaccine within the national vaccination program.

Discussion

Cervical cancer is a preventable disease and one of the key aspects of its prevention is the early detection of the premalignant lesion through the cervical screening or by the anti-HPV vaccine prior to any sexual relationships.

Major findings of the present study showed that 28% of the respondents had already received the Pap smear test at least once during their lifetime. This result is concordant with Hammas-Hlaili study that revealed that 22.6% (17.6-27.6) of the women from east-center of Tunisia had already received the Pap smear test at least once in a lifetime [15]. However, these results are not valid for es-

Tab. III. Level of Cervical Cancer and HPV knowledge and anti-HPV vaccine acceptability among respondents.

	Total sample (452)	%
Pap Smear test history	**125**	**27.7**
Cervical cancer risk factors		
Genetics	227	50.2
Viral infection	175	38.7
Multiple sexual partners	156	34.5
Partner had multiples sexual partners	107	23.7
Immune system issues	153	33.8
Smoking	125	27.7
No idea	69	15.3
Pap Smear test finality		
Sexually transmitted infection screening (false)	109	24.1
Cervical cancer screening	308	68.1
Cervical cancer treatment (false)	7	1.5
No idea	93	20.6
Recommended Pap Smear test frequency		
Annual (false)	185	40.9
Every 3 to 5 yeas	176	38.9
Every 10 years (false)	1	0.2
No idea	90	19.9
Routes of HPV spread		
Unprotected sex	242	53.5
Protected sex	5	1.1
Blood (false)	71	15.7
Public toilets (false)	69	15.3
No idea	157	34.7
Already heard about anti-HPV vaccine	188	41.6
(Would be) interested in receiving anti-HPV vaccine if suggested by their doctor	363	80.3
Favourable to the administration of the anti-HPV vaccine to their (future) daughters if suggested by the doctor	387	85.6
Supports the introduction of anti-HPV vaccine in the national immunization program (systematic and free)	405	89.6

pecially the rural region of Tunisia where only between 11% and 13% had received the Pap smear test [15].

This study also revealed some concerning findings: first, regarding cervical cancer risk factors, less than the half of all participants identified sexually transmitted infections as a risk factor for cervical cancer. Second, regarding HPV spread ways, participants were not properly aware especially of the possibility of HPV spread through unprotected as much as through protected sex. Third, regarding the recommended frequency of the screening, we notice that 20% of participants reported having no idea about the recommended periodicity. All these results represent real opportunities for improving HPV and cervical cancer awareness among Tunisian women and confirm findings reported in other studies regarding the low to moderate knowledge level about HPV infection and prevention from the cervical cancer.

Regarding the acceptability of the anti-HPV vaccine, participants were highly willing to receive the anti-HPV vaccine if it was relevant for them and to agree that their daughters or future daughters receive it. They were also extremely favourable (> 90%) to the inclusion of the anti-HPV vaccine in the national vaccination program in order to make it systematic and free for all Tunisian girls. This finding also confirms the trend reported throughout the region of moderate to high acceptability of the HPV vaccine among women in the region despite the low level of knowledge about HPV and cervical cancer [16].

Regarding the assessment of the association between participants demographics and the acceptability of cervical cancer prevention methods, results showed that history of Pap Smear test is statistically associated to participants' working status, health sector in which participants are used to consult and their marital status.

However, these associations were not valid for the acceptability of anti-HPV vaccine. These results can be explained by the fact the anti-HPV vaccine is relatively new in comparison to the Pap Smear; therefore, women from all ranges of demographics are still much less informed and sensitive to this primary prevention method. This situation may explain the absence of trends or statistically associations between participants demographics and the proxy variables of the acceptability of the anti-HPV vaccination.

Limitations

This study is the first in Tunisia to analyze the awareness and acceptability of Tunisian women of two prevention methods of cervical cancer. Among the main limitations of this study, we first mention the potential recall bias: women may not accurately recall if yes or no they undertook a Pap Smear test. The second limitation of the study is the potential selection bias associated to the participants' selection that included women only from care centers or healthcare administrative services; this selection of a non-representative conventional sample may limit the external validity of the study and the generalizability of our findings to the rest of Tunisian women. Finally, a final limitation of this study is the potential bias of social desirability since women responses may not reflect the reality of their opinions and may be formulated in a way to not be judged by the interviewer.

Conclusions

In conclusion, the knowledge and awareness levels of cervical cancer prevention methods through screening and anti-HPV vaccination among Tunisian women are similar to the trend found in other MENA countries. However, these levels are considered low compared to developed countries. Therefore, a national cervical cancer prevention program is still needed to be implemented in Tunisia. In this sense, regional cervical screening and inclusion of the anti-HPV vaccine in the national vaccination program should be encouraged. All involved health care professionals (gynaecologists, nurses, midwives etc.) should benefit from educational initiatives in order to contribute to the enhancement of the usage of cervical cancer prevention methods available for Tunisian women.

In addition to that, it is important to promote awareness among women with risk factors of cervical cancer, to emphasize the importance of Pap smear test on a regular basis and to guarantee them adequate follow-up and emotional support when needed. It is also important to sensitize parents about the advantages the anti-HPV vaccine would give to their daughters and to reassure them about the vaccination safety. These are all urgent measures essential to undertake in order to reduce morbidity and mortality associated to cervical cancer in Tunisia.

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The author declares that there is no conflict of interest.

Author's contributions

RG was responsible of all the phases of this study: study design, data collection, descriptive analysis and manuscript writing. She was supervised by Pr Soltani who unfortunately passed away in 2016 and was not able to give his explicit approval on the final draft of this study.

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ORIGINAL ARTICLE

Current data in Greek children indicate decreasing trends of obesity in the transition from childhood to adolescence; results from the National Action for Children's Health (EYZHN) program

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Keywords

Obesity • Total • Central • Children • Adolescents

Summary

Introduction. *The aim of the study is to present the most recent estimates of obesity (total and central) prevalence in Greek children and associated risk factors.*
Methods. *Population data are derived from a yearly, school-based health survey polled in 2015 on 336,014 (51% boys) children aged 4 to 17 years old from almost 40% of all schools of primary and secondary education in Greece. Anthropometric and physical fitness measurements were obtained by trained investigators. Dietary habits, physical activity status, sedentary activities and sleeping hours were assessed through self-completed questionnaires. The gender and age-specific Body Mass Index (BMI) cut-off points were used in order to define BMI groups.*
Results. *The prevalence of overweight and obesity in the whole population was 22.2% and 9.0% in boys and 21.6% and 7.5% in girls, respectively. Obesity presented decreasing trends in the transition from childhood to adolescence. Central obesity was diagnosed in 95.3% and 93.5% of the simple obese boys and girls, respectively, in almost two to three of overweight children (68.6% of boys and 64.3% of girls), and in 12% of normal weight children. Age, physical fitness, low adherence to Mediterranean diet, insufficient sleeping hours, inadequate physical activity levels and increased screen time were all associated with higher odds of total and central obesity.*
Conclusions. *Serious and urgent actions need to be taken from public health policy makers in order not only to prevent a further increase in obesity rates but, more important, to treat obesity and/or the obesity associated co-morbidities.*

Introduction

In today's society the prevalence of obesity in children and adolescents has been recognized as a global epidemic [1]. In the European Union the number of overweight children is expected to rise by 1.3 million per year, with more than 300.000 of them becoming obese each year [2]. Greece is among the European countries with the highest levels of childhood obesity [3]. It is estimated that the prevalence of overweight and obesity is markedly different among children and adolescents in Greece and elsewhere [4, 5]. Childhood obesity has been associated with the development of cardiovascular diseases, diabetes, metabolic syndrome, and excess weight status in adult life [6], and moreover, it influences social and psychological functioning of children [7]. Central obesity (i.e., abdominal subcutaneous and visceral adiposity) is an important risk factor for insulin resistance and an identifier of cardio-metabolic disorders and cardiovascular disease in later life [8]. Waist Circumference (WC) and Waist-to-Height Ratio (WtHR) are measurement methods for evaluation of central obesity. It is estimated that they predict better than Body Mass Index (BMI) cardio-metabolic risks [9]. The causes of childhood obesity are multi-factorial, including genetic predisposition, perinatal factors, and lifestyle, environmental and socioeconomic variables [6]. In the last decade, the Greek government in collaboration with several independent authorities has significantly forced public health efforts aiming to reduce childhood obesity. EYZHN (National Action for Children's Health) is an intertemporal program aiming to record health- and lifestyle-related parameters of the total student population of Greece.

To our knowledge, only a few studies [4, 10-12] have currently estimated the prevalence of total or abdominal obesity in Greek children and adolescents based on a national representative sample; most of them have drawn conclusions from selected geographic areas or from a deteriorated age range [10-12]. Accordingly, the aim of the present study is: (a) to examine the prevalence of total and central obesity groups among 4- to 17-y-old children and adolescents as a basis for effective prevention strategies and (b) to investigate whether there is an association between several anthropometric and lifestyle factors and total/central obesity.

Methods

Participants

Population-based, representative data were derived from a nation-wide, school-based survey under the auspices of the Ministry of Education. Specifically, anthropometric, physical activity, sedentary 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 and 49% girls) children aged 4 to 17 years old from pre-elementary (4- to 5-y-old), elementary (6- to 11-y-old) and middle (12- to 17-y-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). The participation rates assured the proportional enrollment of children based on the urban/rural areas student population distribution.

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, weight and waist circumference were measured in the morning, using a standardized procedure. Data collection activity in each school was completed in one working day. Children wear little clothing and stand with feet close together, arms at the side and body weight evenly distributed. 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). Waist circumference was measured at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest, using a flexible measure to the nearest 0.1 cm. Underweight (3 grades), normal weight, overweight and obese children were classified using the International Obesity Taskforce age- and gender-specific BMI cut-off criteria [13, 14], as the most proper for epidemiologic studies [15]. Central obesity was defined as waist circumference (cm) to height (cm) ratio (WtHR) ≥ 0.5, given that this specific cut-off point has been established suitable for the prediction of obesity-related cardiometabolic abnormalities in children and adolescents [16].

All measurements were repeated; if the measurements were within 1 cm of one another, the average was calculated. All anthropometric measurements were performed by trained professionals (teachers of physical education). Specifically, measurements were performed by one teacher of physical education in each class. All physical education professionals were instructed through a detailed and extended manual of operations and followed a standardized procedure of measurements in order to minimize the potential inter-rate variability among schools. The physical education teachers were first trained by school advisor of physical education for accurate anatomical landmarks, subject positioning and measurement techniques. Verbal informed consent for the child to participate in the measurements was taken from physical education teachers. As the measurements were included in an obligatory school program, verbal informed consent was considered sufficient.

Assessment of physical fitness levels

The Euro-fit PF test battery was used to evaluate children's PF levels [17], initially proposed by the Council of Europe and used systematically from many European countries during the last decades. 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) from a standing start to evaluate speed and agility; (c) a sit-ups test in 30 seconds (SUs), in which the student lies on the mat with the knees bent at right angles, feet flat on the floor and held down by a partner, to measure the endurance of the abdominal and hip-flexor muscles; (d) a standing long jump (SLJ), where the children are asked to bend their knees with their arms in front of them, parallel to the ground, then swing both arms and push off vigorously and jump as far as possible, trying to land with their feet together and stay upright, to evaluate lower body explosive power; and (e) a sit and reach (SR) test that involves sitting on the floor with legs stretched out straight ahead without shoes to measure flexibility. Two trials were allowed for the SLJ, SR, SUs, and 10 × 5 m SRT, with the best performance of each recorded. All five fitness tests were administered during the physical education class by physical education professionals, who were instructed through a detailed manual of operations and followed a standardized procedure of measurements in order to minimize the inter-rate variability among schools.

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, all previously provided with specific written guidelines for its proper completion. This was done in order to provide an accurate reflection of their habits and for a standardized evaluation protocol to be implemented among all participating schools. Regarding students' dietary habits, these were assessed through the KIDMED (Mediterranean Diet Quality Index for children and adolescents), developed by Serra-Majem et al. (2004) [18]. The KIDMED index was developed in an attempt to combine the MD guidelines for adults with the general dietary guidelines for children in a single index. The index comprises 16 yes or no questions, including dietary habits that are in accordance with

the principles of the Mediterranean dietary pattern and the general dietary guidelines for youth (e.g. consumption of at least one fruit at a daily basis, consumption of fish 2-3 per week, use of olive oil as the main culinary fat in salad and cooking etc.) and other habits that undermine them (e.g. breakfast skipping, daily consumption of sweets, frequent consumption of fast food etc.). Questions denoting a negative connotation with respect to a high-quality diet are assigned a value of – 1, while those with a positive aspect are assigned a value of + 1. Thus, the total KIDMED score ranges from – 4 to 12 and is classified into three levels: ≥ 8, suggesting an optimal adherence to the 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

With regard to physical activity habits, patterns of physical activity were also self-reported. The questionnaire has been previously used in children in other large-scale epidemiological studies [19], and included simple closed-type questions regarding children's frequency, time and intensity of participation in (i) school-related physical activity (including activity during physical education classes; (ii) organized sports activities and (iii) physical activities during leisure time. For the current analysis, a student's weekly frequency of participation in organized sports activities, physical activities during leisure time and school-related physical activity (range 0-7, i.e. from rare to daily participation), had an average duration (in minutes) per bout of engaging in the above physical activities. The average duration per bout of engaging, if it caused them "to breathe hard or feel tired", (providing a subjective estimation of moderate to vigorous intensity) was calculated. The frequency of all reported activities 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 physical activity [20].

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 (via multiplying the weekly frequency of participation with the duration per bout of participation in sedentary activities, and then dividing by 7). Using the threshold of two hours per day proposed by current scientific evidence and guidelines [21, 22], 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.

Moreover, daily time in sleeping hours was assessed through self reported recordings. Based on the Consensus Statement of the American Academy of Sleep Medicine, we classified as meeting the recommendations of sufficient sleep those children (aged 6 to 12-y-old) who were sleeping at least nine hours daily and those adolescents (aged 13 to 17-y-old) 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 [23].

Ethical approval

Ethical approval for the health survey was graded by the Ethical Review Board of the Ministry of Education and the Ethical Committee of Harokopio University.

Data analysis

Normality of the distributions regarding continuous variables was verified through the Shapiro-Wilk test, despite the fact that the large sample allows for the assumption of normality of the data. Descriptive statistics of anthropometric measurements were expressed as means ± standard deviations. Prevalence of thinness, normal weight, overweight and obesity was calculated as the ratio of those children belonging in the corresponding class, based on the proposed cut-off points for BMI by IOTF [13, 14] 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 anthropometric variable (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 ± SE. In order to assess the potential effect of several demographic and lifestyle factors (e.g. age, physical fitness measurements, adherence to Mediterranean diet, sleeping hours, physical activity levels and sedentary activities levels) on the total and central obesity status, 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. All other 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

Anthropometric measurements and weight status

Mean values for anthropometric measurements of children and adolescents by age and gender are presented in Table I. Statistically significant differences were incorporated in all anthropometric measurements with boys having higher mean values than their girl peers (all p-values < 0.01), with the exception of WtHR where this was not a stable finding across all age groups. Weight,

Tab. I. Anthropometric indices (means ± standard deviation) of population by gender and age.

	Boys						Girls					
Age †	N	Height (cm)	Weight (kg)	BMI (kg/m²)	WC (cm)	WHtR	N	Height (cm)	Weight (kg)	BMI (kg/m²)	WC (cm)	WHtR
4	3157	107.9 (5.0)*	18.7 (2.8)*	16.0 (1.9)	54.8 (4.7)	0.58 (0.49)	3091	106.6 (5.1)	18.3 (2.8)	16.0 (1.9)	54.5 (4.6)	0.60 (0.49)
5	4550	113.1 (5.4)*	20.8 (3.8)*	16.3 (2.1)*	56.6 (5.3)*	0.48 (0.50)	4477	111.8 (5.3)	20.2 (3.7)	16.1 (2.2)	56.0 (5.4)	0.49 (0.50)
6	11361	120.0 (5.6)*	24.0 (3.7)*	16.4 (2.4)*	58.4 (6.3)*	0.34 (0.47)	11139	118.8 (5.5)	23.3 (4.6)	16.4 (2.4)	57.7 (6.4)	0.35 (0.48)
7	21034	125.1 (5.8)*	26.6 (5.5)*	16.9 (2.6)*	59.9 (7.1)*	0.29 (0.45)*	21165	124.1 (5.8)	26.0 (5.3)	16.8 (2.6)	59.2 (7.2)	0.30 (0.46)
8	21159	131.4 (6.0)*	30.4 (6.7)*	17.6 (3.0)*	62.9 (8.2)*	0.30 (0.46)	20140	130.0 (6.1)	29.7 (6.5)	17.4 (3.0)	62.0 (8.0)	0.31 (0.46)
9	21387	136.9 (6.4)*	34.5 (8.0)*	18.3 (3.3)*	65.8 (9.0)*	0.33 (0.47)	20524	135.6 (6.5)	33.7 (7.7)	18.1 (3.2)	64.7 (8.9)	0.32 (0.47)
10	21162	142.1 (6.8)*	38.5 (9.0)*	18.9 (3.5)*	68.6 (9.7)*	0.35 (0.48)*	20424	141.8 (7.7)	38.0 (8.9)	18.7 (3.4)	67.2 (9.5)	0.31 (0.46)
11	19875	147.3 (7.1)*	43.2 (10.2)*	19.6 (3.6)*	71.3 (10.2)*	0.36 (0.48)*	18910	148.4 (7.6)	43.0 (9.9)	19.3 (3.6)	70.0 (9.8)	0.30 (0.46)
12	16349	153.7 (7.9)*	47.5 (11.3)*	20.1 (3.8)*	73.4 (10.5)*	0.34 (0.47)*	15465	154.8 (7.4)	47.9 (10.6)	19.9 (3.6)	71.5 (10.0)	0.26 (0.44)
13	8515	160.5 (8.7)*	54.0 (12.9)*	20.7 (3.9)*	75.5 (10.9)*	0.28 (0.45)*	7819	159.2 (6.7)	52.1 (10.8)	20.5 (3.7)	72.2 (9.9)	0.21 (0.41)
14	7635	167.4 (8.5)*	60.5 (13.6)*	21.4 (3.9)*	77.9 (10.9)*	0.25 (0.43)*	6734	162.0 (6.2)	55.6 (10.5)	21.2 (3.6)	73.5 (9.8)	0.20 (0.40)
15	6273	172.5 (7.5)*	65.2 (13.1)*	21.8 (3.8)*	79.0 (10.7)*	0.22 (0.41)	5425	163.2 (6.1)	57.5 (10.2)	21.6 (3.5)	74.2 (9.8)	0.21 (0.40)
16	3695	175.5 (7.0)*	69.3 (13.0)*	22.5 (3.8)*	80.3 (10.3)*	0.20 (0.40)*	3413	164.2 (6.3)	58.9 (10.4)	21.8 (3.5)	74.0 (9.3)	0.20 (0.38)
17	2358	177.0 (7.1)*	72.0 (13.2)*	22.9 (3.7)*	81.4 (10.8)*	0.22 (0.41)	2228	164.7 (6.3)	60.1 (10.8)	22.1 (3.6)	74.8 (10.4)	0.21 (0.40)
P for trend	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001			< 0.001	< 0.001	< 0.001	< 0.001	

† = completed age, e.g., 4 years = 4.00-4.99 years; * = p-value < 0.01 between boys and girls; BMI = body mass index; WC = waist circumference; WHtR = waist to height ratio.

height, BMI and waist circumference increased with age in both genders (all p-values for trend < 0.001). In opposite, WtHR presented a relatively decreasing rate with age in boys and girls (p-values for trend < 0.001).

Prevalence of underweight (three grades), normal weight, overweight, and obesity by age and gender, based on IOTF criteria, is incorporated in Table II. In the whole population (4 to 17-y-old), proportions of underweight decreased with age, in both genders (p-values for trend < 0.001). Normal weight boys ranged between 64.3% in 10-y-old and 79.1% in 4-y-old (p-value for trend = 0.137).In girls, the lower proportion (64.4%) incorporated in 9-y-old and the highest in 16-y-old (p-value for trend = 0.031). Overweight boys showed an increasing trend among 4-y and 17-y-old (p = 0.007), while obese girls decreased between the above age range (p-value for trend < 0.001) and presented their lower proportion at the age of 17-y-old. Overweight (including obesity) in boys ranged from 16.2% in 4-y-old to 34.7% in 10-y-old, (p-value for trend = 0.06) while in girls it varied between 19.3% in 17-y-old and 34.0% in 9-y-old (p-value for trend = 0.12). Given that a significant interaction was observed between BMI categories and age (p = 0.01) and additionally individual previous findings in Greece showed that the prevalence of overweight is significantly lower in adolescence in comparison with childhood [3, 10-11, 24], we decided to stratify the analysis in two groups: childhood and adolescent. Analytically, further analysis was performed, splitting the prevalence of BMI categories in two age periods; early/middle childhood (4- to 11-y-old) and adolescence (12- to 17-y-old). Findings regarding the trend of BMI groups by gender during childhood and adolescence are presenting in Table II. During childhood, rates of overweight (including obesity) increased from 16.2% in boys 4-y-old to 34.7% in 11-y-old (p < 0.001) with an increasing trend per 2.7 ± 0.3% (p < 0.001) per year of birth. For girls, an increase in overweight (including obesity) rates from 21.2% in 4-y-old to 34.0% in 9-y-old and 30% in 11-y-old was evident (p < 0.001), with an annual increasing trend equal to 1.58 ± 0.4% (p = 0.005). In opposite, adolescent boys who belong to the overweight (including obesity) group decreased from 33.5% in 12-y-old to 27.9% in 17-y-old (annual rate: – 1.23 ± 0.14%, p = 0.001). Similarly, for adolescent girls, a decrease in overweight (including obesity) rates from 29.6% to 19.3% was evident between 12-y-old and 17-y-old (p = 0.008), with an annual decreasing trend equal to 2.03 ± 0.42% (p = 0.008). In childhood, normal weight and thinness rates were decreasing with age,

Tab. II. Prevalence of BMI categories according to IOTF definitions, by gender and age, in 4 to 17- y-old Greek children.

	Boys				Girls			
Age †	Thinness (%)	Normal weight (%)	Overweight (%)	Obesity (%)	Thinness (%)	Normal weight (%)	Overweight (%)	Obesity (%)
Children								
4	13,8*	70,0*	10,4*	5,8	10,9	67,9	15,5	5,7
5	12,6*	65,7	13,2*	8,5	10,9	65,0	15,8	8,3
6	9,9	65,4*	15,8*	8,9	9,8	62,5	18,3	9,4
7	8,4*	64,5*	18,0*	9,2	9,3	61,6	19,9	9,2
8	7,0*	62,1*	20,7*	10,2	8,4	59,7	22,2	9,7
9	6,1*	59,8*	23,5*	10,7*	7,6	58,5	25,0	9,0
10	5,4*	59,9	25,7	9,0*	7,8	59,4	25,0	7,8
11	5,5*	60,0*	25,8*	8,8*	8,0	61,4	24,2	6,4
B ± SE per year change	− 1.3 ± 0.15	− 1.4 ± 0.20	2.3 ± 0.13	0.36 ± 0.19	− 0.52 ± 0.08	− 1.0 ± 0.31	1.5 ± 0.19	0.02 ± 0.24
P for trend < 0.001	< 0.001	< 0.001	= 0.116	= 0.001	= 0.014	< 0.001	= 0.937	-
Adolescents								
12	5,3*	61,3*	25,2*	8,2*	8,4	64,0	22,4	5,2
13	5,6*	62,0*	24,4*	8,1*	8,3	67,4	19,9	4,3
14	5,0*	63,2*	23,5*	8,1*	6,7	70,9	18,2	4,3
15	4,4*	66,6*	22,2*	6,8*	7,4	72,2	16,5	3,9
16	4,8*	66,9*	21,6*	6,7*	7,5	74,1	14,7	3,7
17	5,1*	67,0*	21,4*	6,5*	8,7	71,4	15,0	4,3
B ± SE per year change	− 0.11 ± 0.09	1.3 ± 0.23	− 0.82 ± 0.08	− 0.40 ± 0.08	− 0.01 ± 0.20	1.7 ± 0.51	− 1.6 ± 0.20	− 0.19 ± 0.10
P for trend	= 0.293	= 0.004	< 0.001	= 0.008	= 0.979	= 0.031	= 0.001	= 0.012
All								
B ± SE per year change	− 0.62 ± 0.11	− 0.05 ± 0.22	0.78 ± 0.24	− 0.22 ± 0.09	− 0.23 ± 0.06	0.78 ± 0.22	− 0.15± 0.26	− 0.42 ± 0.10
P for trend	< 0.001	= 0.137	= 0.007	= 0.05	< 0.001	= 0.017	= 0.577	= 0.001

† = completed age, e.g., 4 years = 4.00-4.99 years; * = p-value < 0.05 for differences between boys and girls from the same BMI category.

while rates of normal weight were increasing, in both genders (all p-values for trend < 0.05). In adolescence, normal weight boys and girls were increasing with age, while overweight and obese rates were decreasing, in both genders (all p-values for trend <0.05).

Prevalence of central and total obesity (WtHR ≥ 0.5), by gender and age, are presented in Figures 1, 2. The prevalence of central obesity across age groups follows parallel rates with total obesity (defined according to BMI), with the exception of children aged 4- and 5-y-old. In the whole study population, central obesity was diagnosed in 95.3% and 93.5% of the simple obese boys and girls, respectively (p < 0.001). Additionally, in almost two to three of overweight children (68.6% of boys and 64.3% of girls) coincides with central obesity; while a proportion of 12% of normal weight children were classified as centrally obese. Prevalence of central obesity in the different BMI categories (IOTF definitions) by age and gender is shown in Table III, as well as the secular trends of its development across childhood and adolescence. Moreover, exploring the distribution of total and central obesity by prefecture, we found some extensive areas of high rates, located primarily in the Aegean Sea, Crete and Ionian Sea for both genders.

Health behaviors of children/adolescents on total/central obesity

Further data analysis was conducted to assess the potential effect of demographic and proximal health behaviors (e.g. age, anthropometric and physical fitness measurements, adherence to the Mediterranean diet, physical and sedentary activity levels, and sleeping hours) on total and central obesity incidence in the whole sample, and in children and adolescents, separately. The initial logistic regression analysis in the whole sample (Table IV) revealed that for every one-year increase in the age the risk of total and central obesity was significantly decreased, while better performances in physical fitness measurements were associated with lower odds of being totally or centrally obese, in both genders. Moreover, compliance with current recommendations in lifestyle factors such as Mediterranean diet, physical activity levels, sleeping hours and sedentary activities seems to decrease odds of being totally or centrally obese, in both genders, with the exception of sleeping hours on centrally obese girls (p = 0.818).

Further analysis (Tab. V) by age groups (e.g. children, adolescents) showed some discrimination in the influence of lifestyle factors on total and central obesity, compared with the whole sample. Specifically, it appears that adequate levels of physical activity did not

Fig. 1. Central obesity (%) by age and gender, in Greek children 4- to 17-y-old.

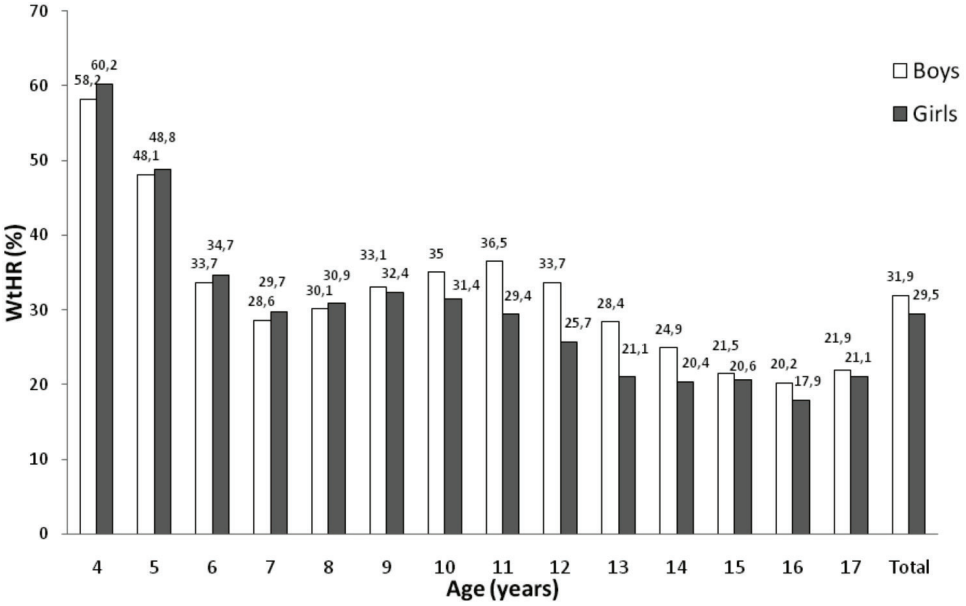
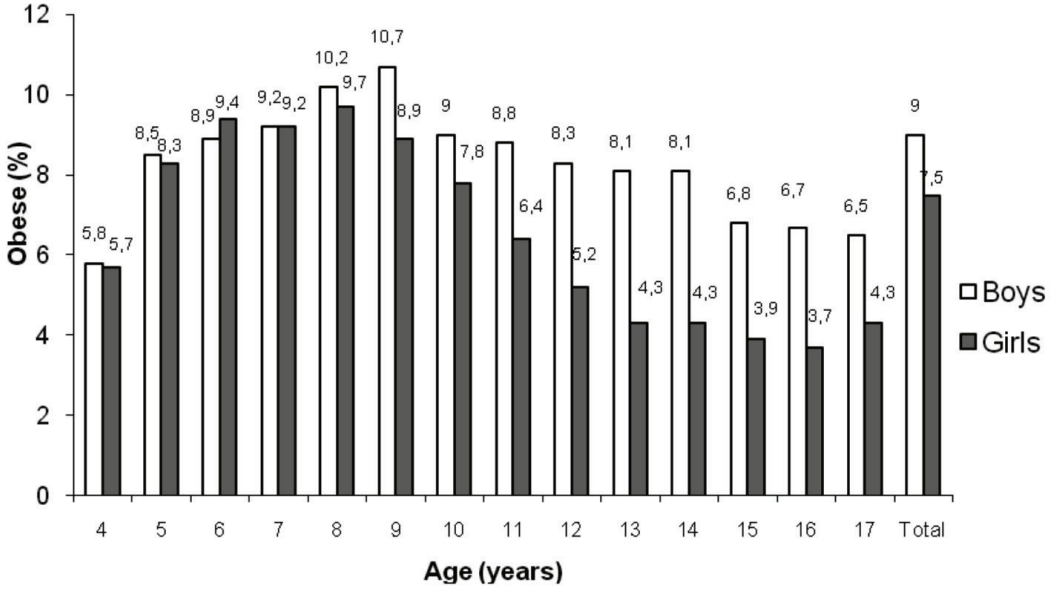


Fig. 2. Total obesity (%) by age and gender, in Greek children 4- to 17-y-old.



have favorable influence on the incidence of total obesity, in children from both sexes. Also, in adolescents of both sexes, increased screen time did not associate with total obesity, while physical activity status and adherence to the Mediterranean diet had not significant effect in total obesity in adolescent boys and girls, respectively. Sleeping hours and physical activity levels of girls aged 4- to 11-y-old did not influence central obesity status. Moreover, in adolescence, physical activity levels in both sexes, screen time in boys and sleeping hours in girls, did not associated with central obesity.

Discussion

The major findings of our study, based on a epidemiologic, population-based survey indicate that: (a) boys and girls transitioned from childhood to adolescence at more favorable levels of adiposity, (b) central obesity exists in a significant proportion of overweight and normal weight (according to IOTF criteria) children and adolescents and (c) compliance with recommendations in Mediterranean diet, physical activity levels, sleeping hours and sedentary activities decrease the risk of total and central obesity, in both genders.

Tab. III. Central obesity (%) by BMI categories (IOTF definitions), gender and age, in 4 to 17-y-old Greek children.

	Boys *				Girls			
Age †	Total	Normal weight	Overweight	Obesity	Total	Normal weight	Overweight	Obesity
Children								
4	58.2	53.1	90.6	100	60.2	52.9	93.8	97.8
5	48.1	38.1	83.5	97.0	48.8	37.5	84.2	98.3
6	33.7	19.8	68.6	92.7	34.7	19.1	68.0	93.9
7	28.6	12.0	62.7	94.2	29.7	13.0	61.0	92.5
8	30.3	10.1	66.2	94.9	30.9	11.2	65.2	92.1
9	33.1	10.0	69.7	95.8	32.4	11.6	66.3	94.7
10	35.0	11.1	74.1	96.2	31.4	11.0	67.2	94.8
11	36.5	12.4	77.5	96.5	29.4	11.1	65.3	93.6
B ± SE per year change	– 2.6 ± 1.3	– 5.4 ± 1.5	– 1.6 ± 1.4	– 0.22 ± 0.35	– 3.7 ± 1.1	– 5.4 ± 1.5	– 3.4 ± 1.3	– 0.54 ± 0.30
P for trend	= 0.095	= 0.013	= 0.312	= 0.551	= 0.017	= 0.011	= 0.039	= 0.134
Adolescents								
12	33.7	11.3	73.7	95.7	25.7	9.7	62.0	92.8
13	28.4	8.0	63.0	94.5	21.1	8.0	55.4	92.8
14	24.9	5.9	55.9	95.3	20.4	8.5	55.0	90.6
15	21.5	5.3	51.2	93.8	20.6	9.6	56.6	93.3
16	20.2	4.7	48.9	94.6	17.9	8.2	53.2	92.9
17	21.9	5.7	53.3	96.6	21.1	9.9	58.6	90.0
B ± SE per year change	– 2.5 ± 0.6	– 1.1 ± 0.4	– 4.3 ± 1.2	0.09 ± 0.26	– 0.93 ± 0.5	0.08 ± 0.22	– 0.63 ± 0.77	– 0.31 ± 0.34
P for trend	= 0.013	= 0.039	= 0.027	= 0.360	= 0.135	= 0.746	= 0.46	= 0.40

† = completed age, e.g., 4 years = 4.00–4.99 years; * = p-value < 0.05 for differences between boys and girls from the same BMI category; the prevalence of abdominal obesity differs significantly between categories of BMI categories in both genders.

To update country data on childhood obesity and evaluate public health interventions, the EYZHN (National Action for Children's Health) program analyzed anthropometric, physical fitness measurements and several lifestyle factors for public and private schoolchildren in pre-elementary to middle school (4- to 17-y-old), using data from about 360,000 boys and girls.

Our study has several strengths. It was conducted in a wide age-range group and examined several anthropometric and lifestyle factors. Moreover, childhood is doubtless an advantageous period to apply effective obesity prevention strategies. At adolescence, physiological (e.g. pubertal stage) and behavioral (e.g. eating disorders, established dietary habits) factors may hold back prevention efforts. A second advantage of the presented data is that it is derived using the same standardized procedure of measurements and are based on almost all the child and adolescent population of Greece. Furthermore, the use of the IOTF cut-off points for BMI classification for thinness, normal-weight [13], overweight, and the obese [14], are the most proper for large epidemiological studies [15] and allow for direct comparisons of our results with those from other countries. Finally, primary and secondary education is compulsory in Greece and, therefore, we were able to study a great proportion of 4- to 17-y-old children and adolescents. The later overcomes the methodological flaws of previous studies performed in Greece that were heterogeneous in terms of design, target- population, quality, theoretical underpinning and outcome measures [9-12, 24], making impractical to compare study findings with the data from other similar projects.

Data from our representative sample of 4- to 17-y-old Greek children and adolescents indicate that the overall prevalence of obesity was higher in boys (9.0%) than in girls (7.5%), ($p < 0.001$). The overall rate of overweight was 22.2% in boys and 21.6% in girls. The prevalence of overweight children was higher in girls younger than 9-y-old, compared to boys, while the proportion of obese girls was elevated in comparison with boys only in 6-y-old and decreasing thereafter. Our findings regarding prevalence of overweight and obesity are consistent with previous data from different regions of Greece [4, 10-12, 24-25]. According to the WHO European Region, prevalence of childhood overweight (including obesity) varied significantly among European countries, ranged from 11% to 33%, with highest rates in Southern European countries (i.e. Greece 33%). In adolescence, prevalence of overweight (including obesity) ranged from 10% (i.e. the Russian Federation) to 23% (Greece) [26]. This study confirms the above notion and suggests that additional actions should be adopted for the treatment of obesity in our country.

Boys and girls transitioned from childhood to adolescence had more favorable levels of overweight and obesity (total and central). Nevertheless, the reductions are less pronounced with girls compared with all children. Probably, dieting with advanced age, especially in girls,

Tab. IV. Associations between demographic and health behaviours and unadjusted odds of total and central obesity, by gender.

Predictors	Total obesity OR (95% CI)	P-value	Central obesity OR (95% CI)	P-value
Boys				
Age (per 1 year)	0.972 (0.966-0.978)	< 0.001	0.958 (0.955-0.962)	< 0.001
Waist circumference (per 1 cm)	1.136 (1.134-1.038)	< 0.001	-	-
Weight (per 1 kg)	-	-	1.036 (1.036-1.037)	< 0.001
Sit and reach test (per 1cm)	0.918 (0.915-0.920)	< 0.001	0.976 (0.974-0.977)	< 0.001
20 meters shuttle run test (per 1 stage)	0.931 (0.929-0.933)	< 0.001	0.955 (0.955-0.956)	< 0.001
10 x 5 meters shuttle run test (per 1 sec)	1.099 (1.094-1.103)	< 0.001	1.097 (1.094-1.101)	< 0.001
Sit-ups in 30 seconds (per 1 sit-up)	0.918 (0.915-0.920)	< 0.001	0.941 (0.940-0.943)	< 0.001
Standing long jump (per 1 cm)	0.995 (0.995-0.995)	< 0.001	0.996 (0.996-0.996)	< 0.001
Adherence to the Mediterranean diet (moderate/high vs low)	1.152 (1.087-1.212)	< 0.001	1.050 (1.002-1.092)	= 0.044
Sleeping hours (sufficient vs insufficient)	1.225 (1.168-1.286)	< 0.001	1.143 (1.109-1.177)	< 0.001
Screen time (acceptable vs increased time)	1.184 (1.123-1.247)	< 0.001	1.054 (1.020-1.099)	= 0.002
Physical activity levels (adequate vs inadequate)	1.037 (1.004-1.066)	= 0.034	1.035 (1.005-1.067)	= 0.023
Girls				
Age (per 1 year)	0.908 (0.901-0.914)	< 0.001	0.920 (0.917-0.924)	<0.001
Waist circumference (per 1 cm)	1.136 (1.133-1.038)	< 0.001	-	-
Weight (per 1 kg)	-	-	1.035 (1.034-1.036)	< 0.001
Sit and reach test (per 1 cm)	0.907 (0.904-0.910)	< 0.001	0.975 (0.973-0.977)	< 0.001
20 meters shuttle run test (per 1 stage)	0.902 (0.899-0.905)	< 0.001	0.945 (0.943-0.946)	< 0.001
10 x 5 meters shuttle run test (per 1 sec)	1.098 (1.093-1.103)	< 0.001	1.096 (1.092-1.100)	< 0.001
Sit-ups in 30 seconds (per 1 sit up)	0.907 (0.904-0.920)	< 0.001	0.939 (0.937-0.940)	< 0.001
Standing long jump (per 1 cm)	0.995 (0.995-0.996)	< 0.001	0.996 (0.996-0.996)	< 0.001
Adherence to the Mediterranean diet (moderate/high vs low)	1.120 (1.039-1.199)	= 0.006	1.069 (1.020-1.116)	= 0.008
Sleeping hours (sufficient vs insufficient)	1.102 (1.064-1.167)	= 0.001	0.996 (0.964-1.029)	0.818
Screen time (acceptable vs increased time)	1.155 (1.081-1.233)	< 0.001	1.086 (1.046-1.128)	< 0.001
Physical activity levels (adequate vs inadequate)	1.104 (1.046-1.166)	< 0.001	1.066 (1.034-1.098)	< 0.001

could be an explanation for the reduction in overweight and obesity after 11 years of age. In fact, study from Yiannakoulia et al., (2004) found that the proportion of Greek girls reporting to be on a diet to lose weight was rising with age (from 11.5- to 15.5-y-old) [27]. The finding that overweight and obesity in boys and girls are declining across age has been reported in Greece and elsewhere [4, 5]. The fact that overweight and obesity were higher in the younger ages reveals that further targeted actions should be focused in these age groups.

Regarding thinness, there is little information in European children and no data from Greece. The prevalence of thinness in the present study is quite similar to that reported from other European countries. Specifical-

Table V. Associations between demographic and health behaviours and unadjusted odds of total and central obesity in children and adolescents, by gender.

	Total obesity OR (95% CI)				Central obesity OR (95% CI)			
	Children		Adolescents		Children		Adolescents	
Boys								
Age (per 1 year)	0.977 (0.968-0.987)	< 0.001	0.936 (0.905-0.968)	< 0.001	1.044 (1.038-1.051)	< 0.001	0.898 (0.879-0.917)	< 0.001
Waist circumference (per 1 cm)	1.177 (1.175-1.180)	< 0.001	1.248 (1.239-1.258)	< 0.001	-	-	-	-
Weight (per 1 kg)	-	-	-	-	1.103 (1.102-1.105)	< 0.001	1.248 (1.239-1.258)	< 0.001
Sit and reach test (per 1cm)	0.980 (0.977-0.993)	< 0.001	0.982 (0.977-0.985)	< 0.001	0.976 (0.975-0.978)	< 0.001	0.978 (0.974-0.981)	< 0.001
20 meters shuttle run test (per 1 stage)	0.924 (0.922-0.926)	< 0.001	0.941 (0.937-0.945)	< 0.001	0.954 (0.953-0.955)	< 0.001	0.956 (0.954-0.958)	< 0.001
10 x 5 meters shuttle run test (per 1 sec)	1.097 (1.092-1.102)	< 0.001	1.109 (1.097-1.120)	< 0.001	1.087 (1.084-1.091)	< 0.001	1.123 (1.113-1.133)	< 0.001
Sit-ups in 30 seconds (per 1 sit-up)	0.919 (0.916-0.922)	< 0.001	0.877 (0.870-0.884)	< 0.001	0.950 (0.948-0.952)	< 0.001	0.895 (0.890-0.900)	< 0.001
Standing long jump (per 1 cm)	0.995 (0.994-0.995)	< 0.001	0.996 (0.996-0.997)	< 0.001	0.996 (0.996-0.996)	< 0.001	0.997 (0.996-0.997)	< 0.001
Adherence to Mediterranean diet (moderate/high vs low)	1.166 (1.089-1.237)	< 0.001	1.186 (1.067-1.290)	= 0.003	1.089 (1.036-1.139)	= 0.001	1.100 (1.018-1.176)	= 0.018
Sleeping hours (sufficient vs insufficient)	1.191 (1.128-1.258)	< 0.001	1.315 (1.186-1.457)	< 0.001	1.136 (1.098-1.175)	< 0.001	1.090 (1.061-1.124)	= 0.010
Screen time (acceptable vs increased time)	1.263 (1.188-1.343)	< 0.001	1.103 (0.994-1.224)	= 0.064	1.151 (1.107-1.196)	< 0.001	1.040 (0.975-1.110)	= 0.229
Physical activity levels (adequate vs inadequate)	1.049 (0.991-1.109)	= 0.097	0.982 (0.886-1.088)	= 0.728	1.043 (1.008-1.080)	= 0.016	0.970 (0.911-1.034)	= 0.354
Girls								
Age (per 1 year)	0.903 (0.893-0.913)	< 0.001	0.994 (0.949-1.040)	= 0.783	0.961 (0.955-0.968)	< 0.001	0.988 (0.966-1.011)	= 0.315
Waist circumference (per 1 cm)	1.163 (1.160-1.166)	< 0.001	1.230 (1.219-1.241)	< 0.001	-	-	-	-
Weight (per 1 kg)	-	-	-	-	1.030 (1.023-1.037)	< 0.001	1.073 (1.072-1.074)	< 0.001
Sit and reach test (per 1 cm)	0.983 (0.980-0.985)	< 0.001	0.980 (0.973-0.987)	< 0.001	0.977 (0.975-0.979)	< 0.001	0.978 (0.974-0.981)	< 0.001
20 meters shuttle run test (per 1 stage)	0.903 (0.900-0.907)	< 0.001	0.905 (0.894-0.915)	< 0.001	0.943 (0.942-0.945)	< 0.001	0.961 (0.957-0.964)	< 0.001
10 x 5 meters shuttle run test (per 1 sec)	1.092 (1.087-1.098)	< 0.001	1.099 (1.086-1.113)	< 0.001	1.088 (1.084-1.092)	< 0.001	1.099 (1.089-1.110)	< 0.001
Sit-ups in 30 seconds (per 1 sit up)	0.913 (0.910-0.917)	< 0.001	0.884 (0.873-0.895)	< 0.001	0.945 (0.943-0.947)	< 0.001	0.922 (0.916-0.928)	< 0.001
Standing long jump (per 1 cm)	0.994 (0.994-0.995)	< 0.001	0.996 (0.996-0.997)	< 0.001	0.995 (0.995-0.996)	< 0.001	0.997 (0.997-0.998)	< 0.001
Adherence to Mediterranean diet (low vs moderate/high)	1.229 (1.143-1.306)	< 0.001	1.095 (0.923-1.247)	= 0.284	1.159 (1.103-1.211)	< 0.001	1.124 (1.039-1.202)	= 0.005
Sleeping hours (sufficient vs insufficient)	1.114 (1.045-1.187)	< 0.001	1.241 (1.082-1.223)	= 0.002	1.022 (0.985-1.060)	= 0.250	1.030 (0.960-1.105)	= 0.405
Screen time (acceptable vs increased time)	1.319 (1.223-1.422)	< 0.001	1.125 (0.975-1.428)	= 0.108	1.224 (1.169-1.280)	< 0.001	1.134 (1.055-1.129)	= 0.001
Physical activity levels (adequate vs inadequate)	1.038 (0.988-1.102)	= 0.220	1.157 (1.009-1.326)	= 0.036	1.012 (0.977-1.047)	= 0.487	1.059 (0.988-1.134)	= 0.106

ly, Boddy et al., 2008 [28], Martinez-Vizcaino et al., 2015 [29], and Lazzeri et al., 2008 [30] have reported overall prevalence of thinness ranging from 6.2% to 9.5% and found significantly higher prevalence in girls, a finding similar to ours. Furthermore, results in children and adolescents from 10 European countries and the USA are in accordance with our findings and proposed that girls and younger children presented higher prevalence of thinness compared to boys and older ones, respectively [31].

Waist to Height ratio has emerged as a central adiposity parameter and significant predictor of risk factors for cardiovascular disease in children and adolescents [9]. We assessed the prevalence of central obesity throughout childhood and adolescence and we specifically focused on its presence in normal weight and overweight children. Our findings proposed that boys were more likely to be centrally obese than girls (31.9% vs 29.5%, $p < 0.001$), and in line with current evidences from nationwide Greek survey of children and adolescents, based on WHtR measurements [19, 32]. On the contrary, a review of 29 studies by de Moraes et al., (2011) incorporate that is not clear what gender has a higher proportion [33]. In the total study population, 30% of children were centrally obese while almost two to three of overweight children and 12% of normal weight were classified as centrally obese. Central obesity rates in Greek children and adolescents are higher than those have reported in Swedish, English and Spanish children and adolescents [34-36], but quite similar to that incorporated from the USA [37] and Greece [11, 19]. In our study, prevalence of central obesity in children 4- to 5-y-old was remarkably high (48 to 60%). Although, a value of 0.5 in WtHR indicates high sensitivity and specificity to detect obesity in individuals aged 6-18 years, results from a study of 5,725 Norwegian children and adolescents recommended that in younger children, this cutoff was not appropriate due to low specificity [38]. Moreover, according to Taylor et al., (2011), due to the residual correlation between WtHR and stature in children, the division of WC by height may be insufficient to properly adjust the height during growth [39]. Evidences from the Bogolusa Heart Study highlighted that normal weight and overweight children with central obesity had increased cardio-metabolic risk in comparison with overweight children without excessive abdominal fat [40]. Those findings, especially in Greece, are of particular concern because the simultaneous presentation of total and central obesity may demand specific intervention programs against abdominal fat accumulation.

To our knowledge this study is the first to examine the association of obesity with several modifiable lifestyle factors simultaneously, in a representative sample of 6-to 17-y-old boys and girls. Adherence to the Mediterranean diet, physical and sedentary activity levels, and physical fitness measurements associated with the risk of being obese (total or/and central) among Greek children and adolescents. The present study reveals that excess body weight was negatively associated with physical fitness in children and adolescents, a finding that is in line with other studies supporting that obese children are not physically fit [41, 42]. The most obvious explanation is that excess weight is a disadvantageous parameter for performance, particularly for weight-bearing activities like running and jumping.

Nationally representative European studies collecting data with the use of a common data protocol on physical activity and nutrition habits, are limited. Specifically, the HBSC study targeting 11-, 13- and 15-y-old [43], the ENERGY study targeting 10/12-y-old [44] and the WHO European COSI study in 6 to 9-y-old children [45]. Our findings in the whole study group support the notion that adherence to Mediterranean diet, adequate physical activity levels, sufficient sleeping hours and acceptable screen time decrease the risk of total and central obesity, in both genders. In line with us, the HBSC study supported a strong and consistent negative association of overweight with dietary habits and physical activity, while the ENERGY study concluded that appropriate dietary behaviors, lack of physical activity and sedentary behaviors are regarded as potential risk factors for becoming overweight/obese [43, 44]. Furthermore, the COSI study in 6- to 9-y-old children from five countries speculated that unhealthy eating behaviors and spending screen time ≥ 2 h/d were positively associated with obesity [45]. In our study, no consistent relations (in children and adolescents) between adequate physical activity levels and total and/or central obesity were noted. Nevertheless, we did not find related studies to compare with for the associations of adequate physical activity with total and central obesity, in children and adolescents, separately.

Limitations of the present study include some methodological issues, and the fact that potential confounding factors, such as sexual maturation, genetic predisposition to obesity etc., have not been evaluated. Dietary habits, physical activity and sedentary time status are based on self-reported data that could be subject to socially desirable reporting bias. However, children responses were anonymous; therefore, participants had no reason to dissemble or misreport their answers. Moreover, although a standard protocol was used to measure anthropometric data, the large number of professionals that have participated as evaluators, may have introduced inter-observer measurement error. Finally, because of the large sample size, statistical significance can easily be achieved.

Conclusions

Despite the aforementioned limitations, this is the first study that reports on the most recent total and central obesity prevalence trends in Greek children and adolescents 4- to 17-y-old. Results revealed that total obesity and overweight rates of both sexes are alarmingly elevated while central obesity coexists in the most overweight and obese children and adolescents. An encouraging finding of our data was that obesity presented decreasing trends in the transition from childhood to adolescence. Finally, it seems that low physical fitness, low adherence

to Mediterranean diet, insufficient sleeping hours, inadequate physical activity levels and increased screen time constitute risk factors of obesity since all are associated with higher odds of total and central obesity. Serious and urgent actions need to be taken from public health policy makers affecting both social and market environment in order not only to prevent a further increase in overweight and obesity rates but, more importantly, to treat obesity and/or the obesity associated co-morbidities.

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The authors declare that there is 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.

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ORIGINAL ARTICLE

A screening focusing on aftereffects of alcohol consumption in a student population. A National cross-sectional survey

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Keywords

Alcohol drinking • College students • Prevention • Alcohol misuse • Screening tool

Summary

Introduction. *Students overestimate alcohol consumption of those around them and underestimate their own, so that quantitative approach may not be the most relevant to assess students' drinking. The main objective was to provide an appropriate tool for screening for students with potential drinking problems.*

Methods. *A multicentre cross-sectional survey was conducted by internet between February and June, 2013 in France. Thirteen questions explored alcohol consumption, including 8 concerning after-effects of drinking episodes (4 items of the AUDIT) and alcohol behaviour (CAGE test). A multiple correspondence analysis (MCA) was conducted to identify profiles of student's alcohol consumption. Partitioning methods were used to group students by mode of alcohol use. The most relevant items included in the MCA were identified. Three questions were identified as most pertinent among the students with potential drinking problems and ranked by a decision tree with the Chi-square Automatic Interaction Detector method. Finally, we assessed the generalisation of the model.*

Results. *A total of 36,427 students participated in the survey: 25,679 were women (70.5% of respondents), sex ratio 0.42 and mean aged 21.2 (sd 3.7 years). Among those who had experimented with alcohol (N = 33,113), three consumption profiles were identified: "simple/non-use" (66.9%), "intermediate consumption" (25.9%) and "problem drinking" (7.2%). For the latter group, the three most relevant items were (Q20) "not able to stop drinking after starting", (Q21) "failed to do what was normally expected", and (Q23) "unable to remember what happened the night before".*

Conclusions. *These results provide healthcare professionals with a 3-item screening tool for students "problem drinking".*

Introduction

Addictive behaviour in students, especially alcohol misuse, is a subject of major concern that has been widely investigated in the literature [1-3]. The negative physical, psychological and sexual consequences associated with alcohol misuse are well known [1-3]. In their study of US college students, Hingson et al. estimated that in a student population of 8,530,318, 10.5% (599,000) were injured in 2001 due to drinking, 12.0% (696,000) were assaulted or hit, and 2.0% (97,000) experienced sexual assault or date rape by another drinking college student [1]. This team later reported that in 1998, 1440 students and in 2005 1825 students from different student populations died from alcohol-related unintentional injuries, for a 3% increase in the rate per 100,000 students per year [2]. Saewyc et al. showed that 16% of both female and male university students had been victims of emotional or physical violence closely related to alcohol misuse [3].

In France, the university student health services (USHS) are responsible for health promotion, surveillance and prevention for students, including screening for addiction problems.

It is difficult for individuals to quantify alcohol consumption, above a certain quantity that varies from person to person [4, 5]. The general population and most particularly young men misperceive their alcohol intake [5-7], as do students, who tend to overestimate that of those around them and underestimate their own [8-10]. In this context, it may be more relevant to identify misuse by measuring the negative consequences of drinking trough specific questionnaire items.

The Alcohol Use Disorders Identification Test (AUDIT) is a self-administered questionnaire of 10 items used to screen for problem drinkers, that is, those whose alco-

hol use during the past year has been harmful or placed them at risk; it has shown good metrological properties in student populations [11, 12]. Its shorter versions, AUDIT-C, AUDIT*2, and AUDIT*3, have also been evaluated among students; like it, they include a quantitative assessment of alcohol consumption and seek to identify which version is most appropriate for screening for excessive drinking in this population [12-15]. They have shown sensitivity ranging from 80% to more than 90% and specificity from 82% to 95% or more and perform at least as effectively as AUDIT [12-14]. To our knowledge, the AUDIT questions that measure excessive consumption of alcohol by its negative effects on memory and behaviour (items 4, 5, 7 and 8) have not been compared with the complete AUDIT questionnaire to measure their screening performance.

Consequently, in order to improve screening of students with drinking problems, the association of directors of French university health centres (Association des Directeurs des Services de Santé Universitaire-ADSSU), the Interministerial mission for combatting drugs and addictive behaviour (Mission Interministérielle de Lutte contre les Drogues et les Conduites Addictives-MILDECA) and the conference of university presidents (Conférence des Présidents d'Université-CPU) set up a project supported by the French Ministry of Higher Education and Scientific Research.

The principal objective of this study was to provide an appropriate tool for screening for potential student drinking problem.

Methods

A multicentre cross-sectional survey was conducted by internet between February 2 and June 30, 2013 in France. An email was sent by participating USHS to students' digital workspaces.

Questionnaire

The questionnaire was devised by a working group from the USHS of Bordeaux, Clermont-Ferrand, and Grenoble, associated with the ADSSU. Relevant items were selected from a review of the literature, with special reference to four French and European surveys (http://www.ovenational.education.fr/; http://www.ofdt.fr/) [16-18]. The questionnaire comprised 76 items that explored sociodemographic and educational characteristics, substance use, physical, psychological, and sexual violence, physical and mental health, social deprivation, and unmet health care needs (Supplemental Table A).

Thirteen questions explored alcohol consumption (Q15-Q23 and Q28-Q31). Four assessed alcohol consumption (as a regular habit), the quantity of alcohol drunk over the past year, in the past 30 days, on a single occasion, and one question measured the number of times the individual had been drunk, according to the OFDT definitions (Q15-Q19) [19]. Eight questions analysed excess alcohol consumption. Four questions came from AUDIT, two that asked about symptoms of dependence (Q20-Q21) and two about harmful use of alcohol (Q22-Q23). These questions were (Q20) "How often during the past year have you found that you were not able to stop drinking once you had started?", (Q21) "How often during the past year have you failed to do what was normally expected from you because of drinking?", (Q22) "How often during the last year have you had a feeling of guilt or remorse after drinking?", and (Q23) "How often during the last year have you been unable to remember what happened the night before because you had been drinking?" The following responses were available: never; less than once a month; once a month; more than once a month; once a week or more. Four questions came from the Cut-Annoyed-Guilty-Eye-opener (CAGE) test (Q28-Q31), which measures problems associated with alcohol consumption at any point in life. The responses to these questions was yes or no: (Q28) "Have you ever felt you needed to cut down on your drinking?", (Q29) "Have people annoyed you by criticising your drinking?", (Q30) "Have you ever felt guilty about drinking?", and (Q31) "Have you ever felt you needed a drink first thing in the morning (eye-opener) to steady your nerves or to get rid of a hangover?" [20].

The online version of the study questionnaire was developed by the Bordeaux School of Public Health (ISPED). Only complete questionnaires were considered. The database was anonymous, created by ISPED, and approved by the French data protection authority (CNIL).

Population

All French USHS (N = 54) were invited to participate in the study. In all, 33, at universities located across France and enrolling 537,092 French students, distributed the questionnaire. Paris-area universities are underrepresented in this group. Among the USHS that did not participate, 10 conducted a different survey (I-share), and the others did not have access to student lists. All students in participating universities were asked to participate through their university e-mail address. Response was fully voluntary.

Statistical analysis

To make the sample representative of the study population, the data were weighted for gender and university discipline with the raking ratio technique. The adjustment was based on data furnished by the French ministry of higher education and research (http://www.enseignement-sup-research.gouv.fr/cid77397/les-effectifs-d-etudiants-dans-le-superieur-en-2012-2013.html). Except for the two variables used for the weighting, all results presented have been weighted.

Descriptive analysis was performed to assess overall and quantitative variables, by calculating percentages and their 95% confidence intervals (95% CI) and means with their standard deviations (sd), respectively. Bivariate analysis was performed with the Chi-square test for qualitative variables and Student's t-test for quantitative variables.

Among the 13 questions exploring alcohol use, the 10 most informative alcohol-related items were kept: the

number of glasses consumed on a single occasion and the number of episodes of drunkenness during the past year, as well as Q20-Q23 and Q28-Q31. A multiple correspondence analysis (MCA) was conducted to identify profiles of consumption by detecting its underlying patterns. We next identified the three items most relevant to characterising each homogeneous pattern cluster by calculating the Cramer's V. Then, we ranked these three questions identified as most pertinent among the students with potential drinking problems by a decision tree with the Chi-square Automatic Interaction Detection (CHAID) method (a decision tree technique, based on adjusted significance testing) [21-23].

The last step of the statistical analysis assessed the generalizability of our model. The database (33,113 students who had experimented with alcohol) was divided in two parts, a learning group (75% of the sample, 24,689 students) and a validation group (25%, 8424 students) through the measurement of a global error rate, an underestimated error rate and an overestimated error rate.

Significance was defined by a threshold of 5% for all statistical tests. Statistical analysis was performed with SAS software (V9.2. SAS Institute Inc., Cary, NC, 2002-2003) and R-3.1.1.

Results

In all, 36,427 students responded to the survey: 25,679 young women (70.5%) and 10,748 young men (29.5%), for a sex ratio of 0.42. Their mean age was 21.2 (sd 3.7) years. The academic divisions enrolling the most respondents were science and technology, agronomics, industry, and teaching (29.0%), arts, letters, languages, and human and social science (24.6%), and health (19.2%) (Tab. I).

Overall, 91.3% of students had experimented with alcohol and 79.0% had used alcohol in the last 30 days; 59.0% reported they had been drunk at least once in the past year (Tab. II). Of students who had experimented with alcohol, 6.9% reported being unable to stop drinking once they had started, 4.9% that they had failed to do what was normally expected from them, 6.0% that they felt guilt or remorse after drinking, and 6.0% that they could not remember what happened the night before at least once in the past month. All harmful alcohol use symptoms were reported more frequently by men (Tab. III).

Among those who had experimented with alcohol (N = 33,113), three consumption profiles were identified: "simple/non-use" (22,164 students, 66.9%), "intermediate consumption" (8,553 students, 25.9%) and "problem drinking" (2,396 students, 7.2%) (Fig. 1). For the "problem drinking" group, the three most relevant items were (Q20) "not able to stop drinking after starting", (Q21) "failed to do what was normally expected", and (Q23) "unable to remember what happened the night before" (Supplemental Table B). In the decision tree, those three relevant items were ranked as follows: (Q23) (Q20), and (Q21).

Tab. I. Socio-demographic and higher education characteristics of overall students included in the survey.

	N[a]	%[b] (n[c])	m[d] (sd[e])
Gender (women)*	36427	70.5 (2,5679)	
Age	36378		21.2 (3.7)
Academic division*	36427		
Science and Technology, Agronomics, Industry, Teaching		29.0 (10,558)	
Art, Letters and Languages, Human and Social sciences		24.6 (8,967)	
Health		19.2 (6,978)	
University institute of Technology		11.1 (4,029)	
Law, Political sciences		8.2 (2,991)	
Commerce, Economic sciences, Management		5.8 (2,097)	
Sport		2.2 (807)	

a = number of individuals with information about the variable; b = percentage; c = number of individuals with that response category; d = means; e = standard deviation; * = non-weighted data.

From the learning portion of the sample (N = 24,689), the three most relevant questions for identifying students with drinking problems were ranked as follows: (Q23) " unable to remember what happened the night before", (Q20) "not able to stop drinking after starting", and (Q21) "failed to do what was normally expected". Among the students who reported that they could not remember who happened the night before (Q23) "at least once a month", 40.3% belonged to the group with drinking problems (Supplemental Material 2), while among those who reported that they were unable to stop drinking (Q20) more than once a month, 15.0% did, as did 6.7% of those who reported that they were unable to do what was normally expected (Q21) more than once a month. Overall, 84.5% of the students in the "problem drinking" group were correctly identified by using a simple decision tree. Inversely, the response "never" to those three questions correctly identified 70.0% of the students belonging to the "simple/non-use" group (Fig. 2).

Thus this model, with three simple questions (unable to remember after drinking, not able to stop drinking, failed to do what was expected due to alcohol intake), has good sensibility and enough specificity for identifying students at risk. This indicates that it might be a good tool for screening.

Discussion

The students with drinking problems can be identified during rapid screening in daily practice, by asking three probing questions, all from AUDIT.

Our results are consistent with those of the 2010 French Health Barometer concerning the annual prevalence of at least one episode of drunkenness (59.0% vs 50.9%,

Tab. II. Descriptive and bivariate analyses of alcohol use among overall students included in the survey.

	Total* N = 36427 %[a] (n[b])	Males** N = 10748 %[a] (n[b])	Females** N = 25679 %[a] (n[b])	p
Alcohol use				
Experimentation	91.3 (33113)	91.9 (9853)	90.9 (23260)	< 0.0001
≥ 1 use in the last 30 days	79.0 (28197)	82.9 (8858)	76.1 (19339)	< 0.0001
≥ 10 use in the last 30 days	8.8 (2615)	14.4 (1470)	4.6 (1145)	< 0.0001
≥ 1 use per day	1.9 (522)	3.3 (332)	0.8 (190)	< 0.0001
≥ 1 drunkenness in the last year	59.0 (20498)	66.6 (7095)	53.2 (13403)	< 0.0001
≥ 3 drunkenness in the last year	33.9 (11076)	43.8 (4595)	26.3 (6481)	< 0.0001
Drinks on a single occasion < 0.0001				
≤ 4 drinks on a single occasion	70.0 (24154)	60.2 (5965)	77.6 (18189)	
5 or 6 drinks on a single occasion	16.5 (5194)	19.0 (1875)	14.6 (3319)	
7 or 9 drinks on a single occasion	7.7 (2252)	10.5 (1006)	5.5 (1246)	
≥ 10 drinks on a single occasion	5.8 (1513)	10.4 (1007)	2.3 (506)	

a = percentage; b = number of individuals with this response category; c = with at least two positive answers; * = data weighted on gender and academic division; * = data weighted on academic division.

Tab. III. Another descriptive and bivariate analyses of alcohol use characteristics among male and female students who had experimented with alcohol.

	Total* N = 33113 %[a] (n[b])	Men** N = 9853 %[a] (n[b])	Women** N = 23260 %[a] (n[b])	p
Not able to stop drinking once you had started (Yes)				< 0.0001
< 1 time per month	9.3 (2,882)	11.0 (1,073)	8.0 (1,809)	
≥ 1 time per month	6.9 (1,851)	10.2 (931)	4.3 (920)	
Failed to do what was normally expected from you because of drinking (Yes)				< 0.0001
< 1 time per month	14.0 (4,348)	16.4 (1,580)	12.1 (2,768)	
≥ 1 time per month	4.9 (1,326)	7.3 (649)	3.2 (677)	
Had a feeling of guilt or remorse after drinking (Yes)				< 0.0001
< 1 time per month	19.9 (6,402)	21.3 (2,055)	18.9 (4,347)	
≥ 1 time per month	6.0 (1,688)	7.8 (699)	4.6 (989)	
Unable to remember what happened the night before because you had been drinking (Yes)				< 0.0001
< 1 time per month	18.5 (5,752)	22.6 (2,228)	15.3 (3,524)	
≥ 1 time per month	6.0 (1,671)	8.8 (856)	3.8 (815)	
Have you ever felt you should cut down on your drinking? (Yes)	14.7 (4,303)	18.6 (1,721)	11.6 (2,582)	< 0.0001
Have people annoyed you by criticising your drinking? (Yes)	10.7 (3,033)	15.8 (1,505)	6.8 (1,528)	< 0.0001
Have you ever felt you are drinking too much? (Yes)	17.3 (5,139)	22.7 (2,169)	13.2 (2,970)	< 0.0001
Have you ever had a drink first thing in the morning to steady your nerves or to get rid of a hangover (eye opener)? (Yes)	1.2 (309)	1.9 (164)	0.7 (145)	< 0.0001
CAGE-Alcohol positive[c]	12.8 (3,716)	17.3 (1,619)	9.4 (2,097)	< 0.0001

a = percentage; b = number of individuals with that response category; c = with at least two positive answers; * = data weighted on gender and academic division; ** = data weighted on academic division.

respectively) and those of the Eurobarometer concerning binge drinking (responding yes to "I usually consume five servings of alcohol or more on the days I drink" (27.4% vs 22%) [24]. Similar results were also observed concerning the amount drunk at one time despite a different threshold and time period (30.7% vs 27.4%) [25].

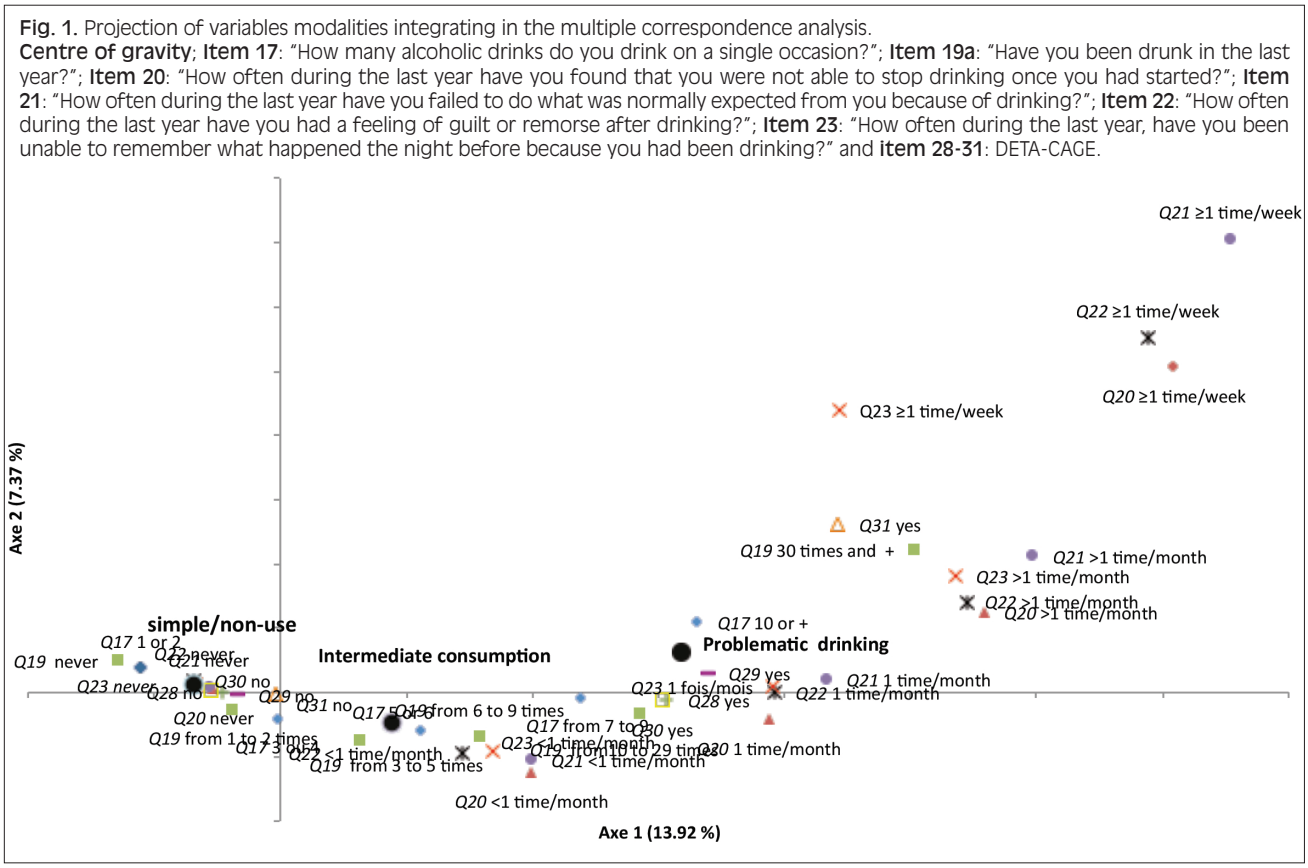


Fig. 1. Projection of variables modalities integrating in the multiple correspondence analysis.
Centre of gravity; **Item 17**: "How many alcoholic drinks do you drink on a single occasion?"; **Item 19a**: "Have you been drunk in the last year?"; **Item 20**: "How often during the last year have you found that you were not able to stop drinking once you had started?"; **Item 21**: "How often during the last year have you failed to do what was normally expected from you because of drinking?"; **Item 22**: "How often during the last year have you had a feeling of guilt or remorse after drinking?"; **Item 23**: "How often during the last year, have you been unable to remember what happened the night before because you had been drinking?" and **item 28-31**: DETA-CAGE.

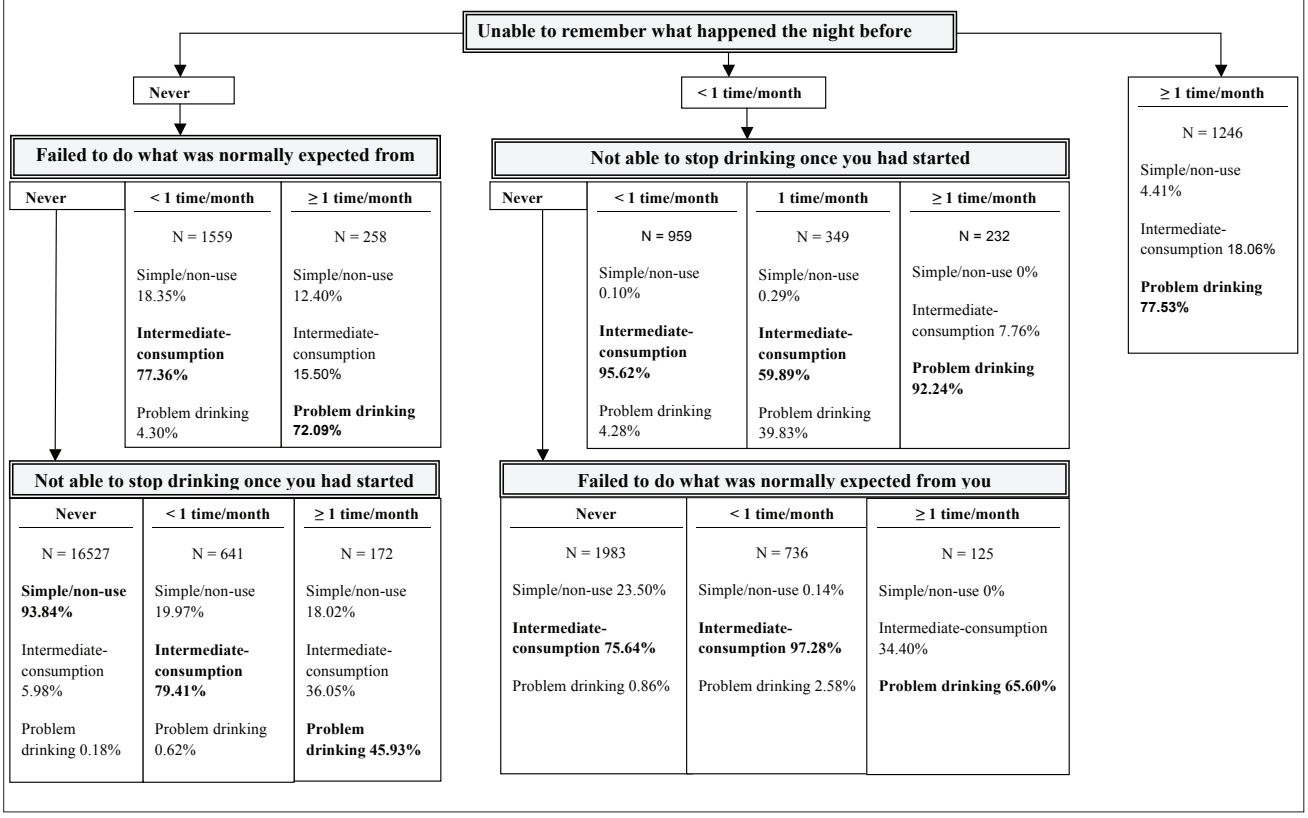
The 30-day prevalence of alcohol consumption among the US university students whose alcohol use was assessed by the ACHA-NCHAII (66.8%) and the Monitoring the Future (MTF) surveys (63.1%) was lower than in our study (79.0%) [26, 27]. Nonetheless, results for the percentage of students who had been drunk at least once were quite similar: The MTF survey reported that 57.9% of American university students had been drunk at least one time in the last 12 months, compared with 59.0% in our survey. Moreover, 32.3% of the American students in the ACHA-NCHAII survey admitted to having forgotten where they were or what they had done under the influence of alcohol, a percentage moderately higher than among French students (24.5%).

One of the issues underlying this study was students' misperceptions of their own alcohol consumption. Our study appears to show a recall bias associated with excessive alcohol consumption. The approaches based on the quantity of alcohol drunk appeared to be much more less discriminant for identifying groups of students at high risk than the questions about the consequences of their drinking. For students with drinking problems, the questions on negative effects on memory and behaviour are informative and contribute to identifying students at high risk. Students thus appear to underestimate the real quantity of alcohol they drank and overlook guilt feelings, but have better perception and report more accurately the consequences of drinking in terms of amnesia, loss of control, and inability to do what was expected of them [4, 5].

To our knowledge, this is the first nationwide study in France intended to analyse students' practices related to addiction. The Health Barometer analyses the health of the French but not specifically that of students and the OVE conducts studies of students without specifically focusing on their health status. This multicentre cross-sectional study was conducted among a very large sample of students. It is nonetheless impossible to estimate the students' participation rates, because the Ministry of Education could not provide student enrolment data by university for the year the study took place. Participation varied according to university. Some students could not be asked to participate because we did not obtain the agreement of their university's communication department. The results are nonetheless homogeneous between the participating universities.

Healthcare professionals but also public authorities now have available three questionnaire items that can be used to assess alcohol consumption and are relevant both for early, routine identification and for initiating prevention campaigns among students with problem drinking. These questions meet the needs of healthcare professionals who have wanted to be able to conduct rapid screening in their everyday practice [28, 29]. Once this screening and identification has been performed, several methods of management are possible: intervention by a healthcare professional, peers, or even self-management on the internet [30-32]. A strategy of correction of misperceptions has also proved effective [33].

Fig. 2. Results of the decision tree (N = 24 689), for students belongs to the simple/non-use group (66.87%), the intermediate group (25.81%) and the problem drinking group (7.32%).



The continuation of this work also leads us to approach this research field from another angle. Do the three questions we identified only allow us to identify students who are problem drinkers through the negative consequences that excessive drinking can have? Or do they further allow us to identify difficulties in controlling alcohol consumption that thus reveal vulnerability to future dependence on it? If the latter is the case, these three questions must be considered factors of vulnerability, similar to those previously identified: age at the beginning of drinking, alcohol consumption and drunkenness in middle school and high school, and the frequency of negative effects of alcohol consumption [34].

Conclusions

Questions about the inability to remember what happened the night before, inability to stop drinking, and inability to do what one is normally expected to do provide information that can be used to screen students with drinking problems, using a threshold frequency of at least once a month. These three key points may also be factors of vulnerability to alcohol. The development of management strategies incorporating them is essential.

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Authors' contributions

JH, AP, VM, SM and LG developed the protocol of the study. JH and SL performed the statistical analysis. All authors interpreted the results. MB, ADL, GB and LG wrote the article, the other authors read it and made revisions for significant content. All authors had full control of the content of the article.

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Supplemental Table A: survey questionnaire

SURVEY: Students' health

We would like you to fill in a questionnaire for a survey on health concerns among students living in France.

The present questionnaire is anonymous and takes around 20 minutes to complete.

Please reply to ALL items

1. Age _____ years old

2. Sex

□ Male□ Female

3. Nationality

□ French □ Other (state which) _____

4. Residence

□ Family or relatives □ Residence hall □ Apartment rental with roommates
□ Apartment rental alone □ Other

5. a) Town of higher education _____

b) Location of University ***(answered "other" if you are in school)*** _____

6. a) Type of higher education establishment:

□ University □ Grandes écoles □ Preparatory courses for Grandes Ecoles
□ Advanced Technician Certificate □ University Institute of Technology
□ Specialised school □Engineering school □Other _____

b) Year of study _____

c) Academic discipline

□ Agronomics □ Art □ Commerce, Economic sciences □ Law □ Teaching
□ Industry □ Letters and Languages □ Management □ Health □ Human and social sciences
□ Science and Technology □ Political Sciences □ Sport □ Other

7. Year of the 1st registration in higher education _____

8. a) Year of high school diploma_____

b) Where high school diploma was awarded_____

9. a) How much do you weigh? □□□kg **b) How tall are you?** □,□□ meters

10. How many average hours do you sleep on average a night? _____ hours

11. Do you have difficulty falling asleep, staying asleep or achieving restorative sleep?

□ Never □ Rarely □ Sometimes □ Often □ Most of the time □ All the time

IF YOU ANSWERED NEVER OR RARELY, GO TO ITEM 13

12. a) When did those difficulties begin?

□ Before the start of the academic year □ After the start of the academic year

b) Did these difficulties and tiredness prevent you from performing your daily activities (school work, job, leisure)?

□ Never □ Rarely □ Sometimes □ Often □ Most of the time □ All the time

The following items are about your feelings during <u>the past 4 weeks</u>. For each question, give the one answer that comes closest to the way you have been feeling.

13. **During the past 4 weeks, how much of the time**

(For each item you will be asked to fill in a bubble in each line)

	All the time	**Most of the time**	**A good bit of the time**	**Some of the time**	**A little of the time**	**None of the time**
a) Have you been very nervous?	1	2	3	4	5	6

b) Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
c) Have you felt calm and peaceful?	1	2	3	4	5	6
d) Have you felt downhearted and depressed	1	2	3	4	5	6
e) Have you been happy	1	2	3	4	5	6

The following items are about alcohol consumption. Choose the most appropriate answer.

14. Have you ever drunk alcohol (wine, beer, cider, liquor, cocktails)?

□ Yes □ No

IF NO, GO TO ITEM 32

IF YES:

15. How often have you drunk alcohol in the last year?

□ Never □ ≤ 1 time a month □ 2 to 3 times a month
□ 1 to 2 times a week □ ≥ 3 times a week □ Every days

16. How often have you drunk alcohol in the last 30 days?

□ Never □ 1 to 2 times □ 3 to 5 times (about 1 time a week)
□ 6 to 9 times (1 to 2 times a week) □ 10 to 19 times (3 to 5 time a week)
□ 20 to 29 times (almost every day) □ ≥ 30 times (≥ 1 time per day)

17. How many alcoholic drinks do you drink on a single occasion?

□ 1 or 2 □ 3 or 4 □ 5 or 6 □ 7 to 9 □ ≥ 10


7cl of aperitif At 18°


10cl of champagne at 12°


2.5cl of whiskey at 45°


2.5cl of pastis at 45°


25 cl of beer at 5°


10 cl of wine at 12°

Be careful, the quantity of alcohol depends on volume drunk: 1 glass of whisky of 10cl is equivalent to 4 units of alcohol, 1 glass of 15cl of liqueur is equivalent to 2 units of alcohol. 1 glass of cocktail with fruit liqueur or spirits and fruit juice is equivalent to 2 units of alcohol.

18. Overall, you drink:

□< 10 drinks of alcohol a year □ 1 to 4 drinks a month
□ 5 to 10 drinks a month □> 10 drinks a year

19. a) Have you been drunk in the last year?

□Never □ 1 to 2 times □ 3 to 5 times □ 6 to 9 times □ 10 to 29 times □ ≥ 30 times

b) On a 1 to 10 points scale, at what point were you drunk the last time?

1: Felt happy *10: So drunk that I couldn't walk*

□1 □2 □3 □4 □5 □6 □7 □8 □9 □10

20. How often during the last year have you found that you were not able to stop drinking once you had started?

□ Never □< 1 time a month □ 1 time a month □>1 time a month □≥ 1 time a week

21. How often during the last year have you failed to do what was normally expected from you because of drinking?
□ Never □< 1 time a month □ 1 time a month □>1 time a month □≥ 1 time a week
22. How often during the last year have you had a feeling of guilt or remorse after drinking?
□ Never □< 1 time a month □ 1 time a month □>1 time a month □≥ 1 time a week
23. How often during the last year have you been unable to remember what happened the night before because you had been drinking?
□ Never □< 1 time a month □ 1 time a month □>1 time a month □≥ 1 time a week
24. Have you been injured as a result of your drinking?
□ Never □ Rarely □ Sometimes □ Often □ Very often
25. Has someone else been injured as a result of your drinking?
□ Never □ Rarely □ Sometimes □ Often □ Very often
26. Have you been raped or experienced sexual assault as a result of your drinking?
□ Never □ Rarely □ Sometimes □ Often □ Very often
27. Have you witnessed any violent incidents (fight, sexual assaults…) as a result of your drinking?
□ Never □ Rarely □ Sometimes □ Often □ Very often

Concerning your alcohol consumption:

28. Have you ever felt you should cut down on your drinking?
□ Yes □ No
29. Have people annoyed you by criticising your drinking?
□ Yes □ No
30. Have you ever felt bad or guilty about your drinking?
□ Yes □ No
31. Have you ever had a drink first thing in the morning to steady your nerves or to get rid of a hangover (eye opener)?
□ Yes □ No

The following items are about marijuana consumption. Choose the most appropriate answer.

32. Have you ever used marijuana?
□ Yes □ No

IF NO, GO TO ITEM 42

IF YES:

33. How old were you the first time? _____ years old
34. Have you used marijuana in the last year?
□ No □ ≤ 1 time a month □ 2 to 4 times a month □ 2 to 3 times a week
□ ≥ 4 times a week
35. How often have you used marijuana during the last 30 days?
□ 0 □ 1 to 2 times □ 3 to 9 times □ ≥10 times □ Every day

Concerning your marijuana consumption:

36. Have you ever felt you should cut down on your use of marijuana?
□ Yes □ No
37. Have people annoyed you by criticising your use of marijuana?
□ Yes □ No
38. Have you ever felt bad or guilty about your use of marijuana?
□ Yes □ No
39. Have you ever used marijuana first thing in the morning to steady your nerves or to get rid of a hangover (eye opener)?
□ Yes □ No

40. Have you smoked cannabis when you were alone?
□ Never □ Rarely □ Sometimes □ Often □ Very often □ Permanently
41. Have you smoked cannabis before midday?
□ Never □ Rarely □ Sometimes □ Often □ Very often □ Permanently
The following items are about tobacco use. Choose the most appropriate answer.
42. Do you smoke currently?
□ I don't smoke and I have never tried
□ I have tried but did not become a smoker
□ I was a smoker but I stopped
□ I smoke occasionally (< 1 cigarette a day)
□ I smoke everyday (at least 1 cigarette a day)

IF YOU DON'T SMOKE CURRENTLY, GO TO ITEM 52

If you smoke, occasionally or daily:
43. How old were you the first time? _____ years old
44. How many cigarettes have you smoked during the last 30 days?
□ <1 □ 1 to 5 □ 6 to 10 □ 11 to 20 □ 21 to 30 □ ≥ 31
45. How many times did you stop smoking for at least 7 days? □□ times
46. How soon after waking do you smoke your first cigarette?
□ Within 5 minutes □ 6 - 30 minutes □ 31 - 60 minutes □ > 60 minutes
□ I don't smoke in the morning
47. Do you find it difficult to refrain from smoking in places where it is forbidden?
□ Yes □ No
48. Which cigarette would you hate to give up?
□ The first in the morning □ Those after lunch □ The last of the day □ An other
49. Do you smoke more frequently in the morning?
□ Yes □ No
50. Do you smoke even when you are sick in bed?
□ Yes □ No
51. Do you use a water pipe?
□ No, never
□ Yes, < 1 time a week
□ Yes, 1 to 3 times a week
□ Yes, > 3 times a week
Have you used the followings substances at least one time in your life and in the last 30 days?

	a) I have used it at least 1 time in my life	**b) I have used it in the last 30 days**
52. Medication for nerves or sleep disturbance	□ Yes □ No	□ Yes □ No
53. Amphetamines	□ Yes □ No	□ Yes □ No
54. Cocaine	□ Yes □ No	□ Yes □ No
55. Ecstasy / MDMA	□ Yes □ No	□ Yes □ No
56. Poppers/glue/solvents	□ Yes □ No	□ Yes □ No

57. Mushrooms / LSD	□ Yes □ No	□ Yes □ No

58. Are you currently in paid employment?
□ No □ Paid job in summer only □ Occasional paid job
□ Paid job < 15 hours a week□ Paid job ≥ 15 hours a week

IF YOU HAVE NO JOB, GO TO ITEM 61

IF YOU HAVE A JOB:

59. Does this job have a relation with your studies? □ Yes □ No

60. Did you ask for your study schedule to be altered so as to continue with your job?
□ Yes □ No

61. Do you do sports?
□ No sport □ Not regularly or < 1 hour a week □ Regularly from 1 to 2 hours a week
□ Regularly from 3 to 4 hours a week □ Regularly ≥ 5 hours a week

62. What medical insurance do you have?

a) Main insurance
□ Student insurance □ Parents' insurance □ Insurance as a salaried worker
□ Other □ Don't know

b) Private health insurance
□ Student private health insurance □ Parents' private health insurance
□ Complementary universal health insurance □ Other □ None □ Don't know

Have you seen a doctor in the last 12 months?

63. Attending physician	**a)** □ Yes □ No	**b) If yes**: □ Campus health centre □ Office □ Other
64. Gynecologist	**a)** □ Yes □ No	**b) If yes**: □ Campus health centre □ Office □ Other **c) Do you use regular means of contraception?** □ Yes □ No **d) Have you ever taken emergency oral contraception?** □ Yes □ No
65. Psychologist / Psychiatrist	**a)** □ Yes □ No	**b) If yes**: □ Campus health centre □ Office □ Other
66. Dentist	**a)** □ Yes □ No	**b) If yes**: □ Campus health centre □ Office □ Other
67. Other medical specialist	**a)** □ Yes □ No	**b) If yes**: □ Campus health centre □ Office □ Other

68. Did you choose an attending physician to be your family physician?
□ Yes □ No □ Don't know

Did you forgo seeing healthcare professionals in the last 12 months? *(Several answers possible)*

69. Attending physician	**a)** □ Yes □ No	**b) If yes**: □ Cost problem □ I didn't have any time □ I couldn't get an appointment when I needed one □ I couldn't get an appointment where I lived □ Other
70. Dentist	**a)** □ Yes □ No	**b) If yes**: □ Cost problem □ I didn't have any time □ I couldn't get an appointment when I needed one □ I couldn't get an appointment where I lived □ Other
71. Ophthalmologist	**a)** □ Yes □ No	**b) If yes**: □ Cost problem □ I didn't have any time □ I couldn't get an appointment when I needed one □ I couldn't get an appointment where I lived □ Other
72. Optician	**a)** □ Yes □ No	**b) If yes**: □ Cost problem □ I didn't have any time □ I couldn't get an appointment where I lived □ Other
73. To buy medication	**a)** □ Yes □ No	**b) If yes**: □ Cost problem □ I didn't have any time □ Other

74. Have you ever been a victim of violence?

a) Psychological?

□ Yes □ No

Add comments below.

_____

_____

_____

b) Physical?

□ Yes □ No
Add comments below.

_____
_____
_____

c) Sexual?
□ Yes □ No
Add comments below.

_____
_____
_____

75. Have you ever behaved violently?

a) Against someone?
□ Never □ Rarely □ Sometimes □ Often □ Very often

b) Against yourself?
□ Never □ Rarely □ Sometimes □ Often □ Very often

c) Committing acts of vandalism?
□ Never □ Rarely □ Sometimes □ Often □ Very often

Add comments below.

_____
_____
_____

76. Answer all the following items:

a) Do you sometimes meet with a social worker (welfare worker, educator)?
□ Yes □ No

b) Do you have complementary health insurance (mutual insurance)?
□ Yes □ No

c) Do you live with a partner?
□ Yes □ No

d) Are you a homeowner or will you be one in the near future?
□ Yes □ No

e) Are there periods in the month when you have real financial difficulties in facing you needs (food, rent, electricity)?
□ Yes □ No

f) Have you participated in any sports activities in the last 12 months?
□ Yes □ No

g) Have you gone to any shows (cinema, theatre) in the last 12 months?
□ Yes □ No

h) Have you gone on vacation during the past 12 months?
□ Yes □ No

i) Have you seen any family members in the past six months (other than your parents or children)?
□ Yes □ No

j) If you had financial, family or health difficulties, is there anyone you could stay with for a few days?
□ Yes □ No

k) If you had financial, family or health difficulties, is there anyone you could give you material aid such as lending you money?
□ Yes □ No

Thank you for taking the time to answer this questionnaire.

Write any comments you may have in the space below.

Consult the results of the statistical analysis on our website: http://www.addictprev.fr/

Comments:

Supplemental Table B: results of the three most discriminant items for the three groups, simple use/non-use, intermediate consumption and problem drinking, identified by the multiple correspondence analysis

Items	Group 1 Simple use/non-use	Group 2 Intermediate consumption	Group 3 Problem drinking
Number of drink on a single occasion	0.542	0.401	0.413
Annual frequency of drunkenness	**0.689**	**0.548**	0.556
Not able to stop drinking after starting	0.541	0.430	**0.679**
Failed to do what was normally expected	0.572	0.465	**0.627**
Guilt feelings	**0.613**	**0.526**	0.553
Unable to remember what happened the night before	**0.652**	**0.551**	**0.645**
Have you ever felt you should cut down on your drinking?	0.400	0.239	0.321
Have people annoyed you by criticising your drinking?	0.332	0.156	0.338
Have you ever felt bad or guilty about your drinking?	0.465	0.291	0.354
Have you ever had a drink first thing in the morning to steady your nerves or to get rid of a hangover (eye opener)?	0.087	0.008	0.144

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ORIGINAL ARTICLE

Energy drink and ginseng consumption by Italian university students: a cross-sectional study

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Keywords

Energy Drink • Ginseng • Behavior • University Students • Italys

Summary

Introduction. *The consumption of energy drinks (ED) and ginseng by young people to enhance their mental and physical performance has become widespread. Reported side-effects of ED have raised doubts regarding their safety. This cross-sectional study investigates the phenomenon.*
Methods. *An anonymous questionnaire was administered to a representative sample of Verona university students. The resulting data were analyzed with Excel 2013, STATA 13 software.*
Results. *ED and ginseng consumption was reported by 38.6% and 37.4% of the students, respectively. More than 70% of ED and ginseng users were 18 to 22 years old. Excluding non-responders, ED consumers were mostly males (51.8% vs 33.0%), contrary to ginseng consumers (females 40.4% vs 30.9%). Being a working student was significantly positively associated both to EDs (OR 1.5) and ginseng use (OR 1.4). The most frequently reported academic and other reasons for ED use were: "to study longer" (47.5%), and "to socialize" (29.1%). The most often used combinations were ED containing alcohol (65.6%) and ginseng-coffee beverages (71.8%).*
Conclusions. *The diffusion of ED and ginseng consumption warrants prevention and monitoring measures, and deserves further analysis.*

Introduction

Energy drinks (ED) are beverages that contain caffeine (in the range of 50 to 550 mg per can or bottle), taurine, l-carnitine, carbohydrates, glucuronolactone, vitamins, and herbal supplements such as ginseng and guarana [1]. They are generally known to stimulate cognitive functions and alertness [2]. With the exception of the caffeine, glucose and guarana extracts, there is an overwhelming lack of evidence to support claims that any of the other ingredients in ED contribute to improving cognitive functioning (an effect attributed especially to taurine) or physical performance [3]. The introduction of a now famous ED in Austria in 1987 was followed by an aggressive marketing campaign, which laid the foundations for the more recent upward trend in ED consumption [4]. Sales of ED were estimated to be worth over 12.5 billion USD in 2012, after a 60% growth recorded from 2008 to 2012 [5].
Ginseng is a generic term commonly used to describe a number of different botanical compounds that belong to the genus Panax [6]. In Korea, China, the Himalayan region, Vietnam, Japan and Northern America (regions where it has grown in the wild for thousands of years), ginseng has always been a popular herbal remedy believed to have beneficial effects on global health. Ginseng proponents suggest that it can enhance mental and physical vigor, ease childbirth, and treat inflammatory diseases [7]. The Istituto Superiore di Sanità (Italian National Health Institute) Working Group on smoking, alcohol consumption and drug use, ranks ginseng among the so-called smart drugs, considered as all the natural or artificial compounds not prohibited by existing laws on illicit drugs that may contain active substances with suspected or known psychoactive properties [8].
A study conducted in 2011 [9] found that 48.3% of 439 students attending a Turkish university had used ED at least once in their life, while the prevalence rate of ongoing users was 33%. At another Turkish university, the prevalence rate of ginseng use in 2005 [10] had only been about 6%. The prevalence rates of ED use among university students range from 36.4% to 70.1% in Northern America [11, 12], and are around 38% in Central-South America [13, 14]. As for ginseng consumption, an American study [15] found that 3.3% of the surveyed population had used the herb in the previous week. There are still very few data on the prevalence rates of ginseng use available in literature.
In 2011, the European Food Safety Authority (EFSA) commissioned a study to gather ED consumption data in 16 countries of the European Union. The survey showed that 68% of adolescents (10-18 years old), 30% of adults (18-65 years old), and 18% of children (3-10 years old) had consumed ED at least once in the previous year. The average consumption was 2 liters a month in adults, 2.1 in adolescents, and 0.49 in children [5].
Italy has far from negligible prevalence rates of ED consumption too. In 2012, 41% of 15- to 19-year-old Italian students had used ED, especially among males (54%) [16]. A multicenter survey conducted on Ital-

ian adolescents and young adults (14 to 35 years old) found that 20.1% of respondents had used ED at least once in their life [17]. According to another Italian survey, 56.9% of students attending the Faculty of Medicine in Messina used ED [18]. ED consumption is often justified as a way to stay awake, increase energy levels, boost performance during physical exercise, or remain concentrated while studying, or it is drunk together with alcohol while partying [14, 16, 19, 20]. The tendency to mix ED with alcohol, especially at parties [14, 16, 18-20], can also lead to other risk-taking behavior, such as smoking tobacco and cannabis [21].

The adverse effects of ED consumption generally relate to sympathomimetic effects due to an excessive intake of caffeine [22], i.e. irritability, anxiety, restlessness, insomnia, gastrointestinal upsets, tremors, tachycardia, psychomotor agitation and, in rare cases, death [4]. Other side effects described as being related to ED use are "jolt and crash episodes", headaches, heart palpitations [20], hypertonia (due to vasoconstriction), and bronchial dilation [23, 24]. One publication summarized the demonstrated psychological effects of ED on cognitive functions, mood, sleep, decision-making, and their overall impact on well-being and quality of life [12]. ED consumption is also thought to have a role in the epidemic of obesity and type 2 diabetes, due to the combination of sucrose and caffeine possibly altering the metabolic pathways, and to insulin resistance possibly being induced by caffeine acting as an adenosine receptor antagonist [21, 25-27].

In addition, the misguided conviction of being less subject to the effects of alcohol when it is mixed with an ED prompts individuals to drive without being fully aware of the real risk of road accidents [5]. This can also be interpreted as an adverse effect of people's inadequate knowledge about ED.

In fact, the proportion of individuals aware of the health-related risks of ED was far lower (57.4%) than the same individuals' perception of risks related to alcohol assumption (92.9%) [17].

The adverse effects of ginseng are due to a complex mix of numerous potentially bioactive constituents (ginsenosides) [7] and include hypertension, irritability, insomnia, and skin rash [28]. There is little evidence to support its effect in raising blood pressure [29]. Preliminary non-randomized controlled studies have suggested a possible lipid profile improving effect [29].

The aim of the present study was to investigate ED and ginseng consumption in a representative sample of students attending university in north-east Italy.

Materials and methods

This cross-sectional study was conducted during the 2014-2015 academic year and involved students on Bachelor's (three-year) and Master's (six-year) degree courses in health care at a university of Verona (Northern Italy). The Bachelor's (BSc) degree students were attending courses on: Biomedical Laboratory Techniques, Cardiovascular Perfusion Techniques, Dental Hygiene, Imaging and Radiotherapy Techniques, Midwifery, Nursing, Physiotherapy, Psychiatric Rehabilitation Techniques, Speech and Language Therapy; the Master's degree (MSc) students sampled were studying Medicine and Dentistry.

Data were collected by means of an anonymous multiple-choice questionnaire administered to BSc and MSc students attending the 1st and 3rd years, and to MSc students attending the 5th year. No 3rd-year students of Physiotherapy, Dental Hygiene and Psychiatric Rehabilitation Techniques were sampled because these degree courses were not taking place at the time of our study.

A representative number of students was selected according to the number of students enrolled in the investigated degree courses.

The validated questionnaire obtained information on students' socio-demographic characteristics, such as age, sex, degree course, occupation (working students vs. non-working/unemployed student), nationality, Italian area of residence (Northern *vs* Central-Southern Italy). The survey also sought details on the students' parents, e.g. age, educational level (primary school, secondary school, high school, university), and occupation (self-employed, employee, unemployed, retired, other). The highest educational level achieved by at least one parent was considered as a measure of the family's level of education, and defined as "low" for primary or secondary school, or "high" for high school or university.

Students were then questioned about their university career and use of ED and ginseng. Considering the frequency of their use in the 6 months prior to the survey, possible answers were: never (0 times), rarely (1-10), sometimes (11-30), often (31-90), or very often (≥ 91 times) in order to evaluate a recent behavioral anamnesis.

Some of the reasons for using ED were related to the academic sphere (e.g. to improve alertness and study longer, concentration while studying, exam performance and attention in classroom). A positive response for at least one of the above reasons was taken as an indication that ED were used for the purpose of cognitive enhancement (CE).

Other possible reasons investigated were: to improve social skills, or performance when driving, practicing sports, or at work.

Some final questions concerned whether ED and/or ginseng were consumed alone or in combination with alcohol, tea, illicit drugs, or medicines.

The data collected from this study were processed in compliance with Italian privacy law (N. 196/2003). All students were over 18 years old (legal age in Italy) and they were assured of the confidentiality of their responses. They were adequately informed about the purposes of the survey and their participation was entirely voluntary.

To ensure a high response rate, the survey was brief, easy to complete (taking less than 15 minutes) and administered at the beginning of a lecture. All questionnaires were collected immediately and the information was entered in a database.

The prevalence rates of the socio-demographic and academic characteristics of the different groups were calculated differently: on the total sample (not recalculated); on the use of ED or ginseng calculated on the total sample; and on the use of ED or ginseng by students' working status. The combined rates therefore do not always reach 100%.

Statistical and data analysis

The data processing and the calculation of the confidence intervals (95%) for ED and ginseng users were done with Excel 2013 and STATA 13.

The statistical analyses were conducted using Fisher's exact test or the chi-square test with Yates's correction, assuming significance for $p < 0.05$.

Multiple logistic regressions models (MLRM) were also performed to evaluate the following variables as possible predictors for EDs or ginseng use by students (all sample, working or not working students): male gender, being a Bachelor's degree student, age (years), coming from the North of Italy and parents' high educational level. As outcomes we tested the use of the following substances: EDs alone, EDs mixed with alcohol, ginseng alone, ginseng coffee.

Results

Analysis of the whole sample of 899 students investigated

A total of 1107 questionnaires were administered and the response rate was 89.4%. Of the 990 questionnaires completed, 899 had been compiled properly and formed the object of our analysis.

The survey showed that 38.6% (95% CI 35.4-41.9%, n = 347) and 37.4% (95% CI 34.2-40.6%, n = 336) of the total sample of 899 students had used ED and ginseng, respectively.

The age of the sample ranged between 18 and 39 years (mean 21 years). Males accounted for 30.3% (n = 272) of the sample, females for 68.8% (n = 619), and 0.9% did not specify their gender (Tabs. I and II).

The students were Italian in 90.3% of cases, while 2.7% were foreigners, and 7.0% did not state their nationality. Most of the students (84.5%) came from Northern Italy (Tabs. I and II).

As shown in Tables I and II, most of MSc students were attending Medicine (23.4%), while most of BSc students were on Nursing courses (47.3%).

Figure 1 shows the socio-demographic features of the students' 1,798 parents. Not all students completing the questionnaire provided all the required information on both parents, in which case they were referred to as "non-responders". Most parents (50.2%) were 51-60 years old, many had a high educational level (46.9%), and most were in employment (74.3%).

No statistically significant association emerged between a low family educational level and the students' use of either ED (Fisher's exact test) or ginseng (chi-square with Yates' correction).

Analysis and comparison of sampled students by working status

Tables I and II shows the distribution of the sample grouped as working students (21.9%) and non-working students (75.4%) in relation to their ED and ginseng use, gender, age bracket, place of residence, and type of university course. Only 2.7% of the students did not report their employment status.

The two groups were comparable in terms of gender composition (p = ns, Fisher's exact test). The most prevalent age range in both groups was between 18 and 22 years (78.0% of the non-working students, and 66.0% of the working students). Considering the data calculated on the total sample, the non-working students were mostly from Central-Southern Italy (83.0%), while the working students were largely from Northern Italy (22.9%) and this difference was statistically significant ($p < 0.05$, Fisher's exact test).

The courses attended by the highest percentage of the non-working students in our sample were: Medicine (83.8%) among the MSc students, and Cardiovascular Perfusion Techniques (100.0%) among the BSc students. The highest proportions of the working students were studying Medicine for MSc (15.7%), and Psychiatric Rehabilitation Techniques for BSc (60.0%).

Analysis of ED and ginseng users

The main features of ED and ginseng users are as follows (Tabs. I and II):

- ED consumers differed by gender (33.0% of the female sample *vs* 51.8% of the male sample; $p < 0.05$, Fisher's exact test), and so did ginseng users (40.4% of the female sample *vs* 30.9% of the male sample; $p < 0.05$, Fisher's exact test).
- More than 70% of the ED and ginseng users were between 18 and 22 years old. In this age group the percentage of ED users was significantly higher than among the older students (40.6% *vs* 34.2%, $p < 0.05$, Fisher's exact test). There was no statistically significant difference in the percentage of ginseng consumers between the students aged 18-22 years (37.6%) and the older students (36.1%) (p = ns, Fisher's exact test).
- Most of the ED users were from Central-Southern Italy (43.6%), while most of the ginseng users were from Northern Italy (38.2%); for both ED and ginseng use there was no significant difference relating to their area of residence (p = ns, Fisher's exact test).
- In our sample, 25.1% of ED users and 24.7% of ginseng users were working students. Among all the working students in the sample, the prevalence rates of ED and ginseng consumption were 44.2% and 42.1%, respectively. Instead, among all non-working students, the prevalence rates of ED and ginseng consumption were 37.3% and 36.3%, respectively. According to multiple logistic regression model (MLRM), being a working student was significantly

Tab. I. Distribution of total sample by working status and ED use in relation to socio-demographic characteristics and university course (prevalence rate).

	Total sample		Non-working students			Working students		
	Pop. (899)	ED users (347)	Pop. (678)	ED users (253)		Pop. (197)	ED users (87)	
Variables	A (%)	B (%)	B (%)	B (%)	C (%)	B (%)	B (%)	C (%)
Gender								
Female	68.8	33.0°	75.4	*23.6^*	71.6	22.1	8.7	26.5
Male	30.3	51.8°	76.5	*38.6^*	74.5	22.1	12.1	23.4
Non-responders	0.9	25	37.5	25	100	0	0	0
Age (years)*								
18-22	74.9	78.7	78	81.8	81.8	66	70.1	70.1
23-27	17.7	19	16.1	16.2	16.2	23.9	26.4	26.4
≥ 28	4.8	0.9	3.9	0.4	0.4	8.1	2.3	2.3
Non-responders	2.6	1.4	2	1.6	1.6	2	1.1	1.1
Area of residence								
Northern Italy	84.5	39	75.9	*27.8#*	71.3	*22.9§*	*10.5∞*	27
Central-Southern Italy	10.5	43.6	*83.0§*	*38.3#*	87.8	12.8	*3.2∞*	7.3
Non-responders	5	21.7	52.2	13	60	23.9	8.7	40
Degree course								
Medicine	23.4	31.9	83.8	26.2	82.1	15.7	5.2	16.4
Dentistry	5.9	54.7	81.1	41.5	75.9	13.2	11.3	20.7
Nursing	47.3	40.7	70.8	27.5	67.6	24.9	12	29.5
Biomedical Laboratory Techniques	5.2	51.1	85.1	42.6	83.3	14.9	8.5	16.7
Speech and Language Therapy	4.2	23.7	73.7	13.2	55.6	23.7	10.5	44.4
Midwifery	4.2	26.3	78.9	23.7	90	21.1	2.6	10
Imaging-Radiotherapy Techniques	3.8	50	67.6	35.3	70.6	32.4	14.7	29.4
Physiotherapy	3	48.1	63	33.3	69.2	37	14.8	30.8
Cardiovascular Perfusion Techniques	1.3	0	100	0	0	0	0	0
Dental Hygiene	1.1	30	60	30	100	30	0	0
Psychiatric Rehabilitation Techniques	0.6	40	40	20	50	60	20	50

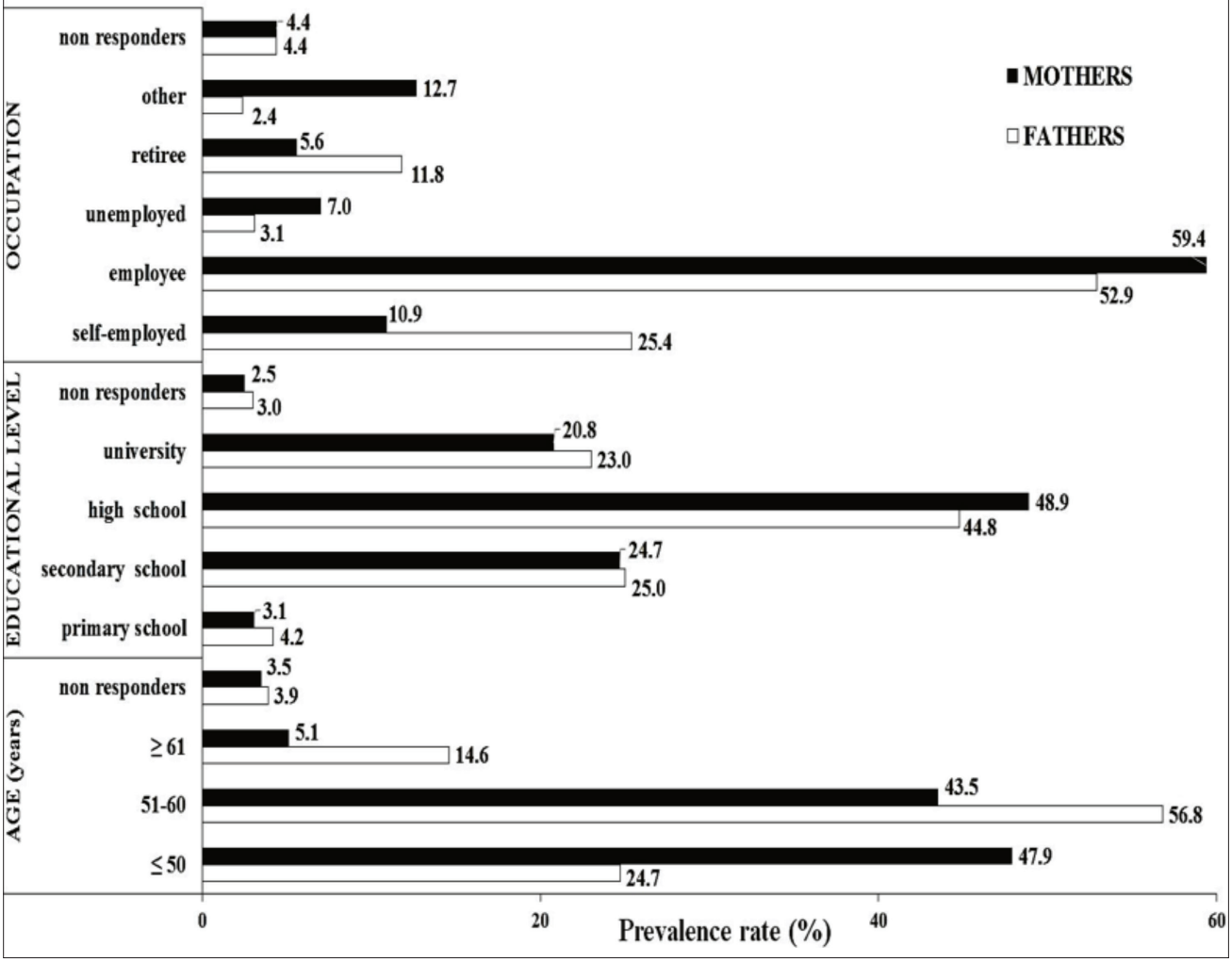
Pop.: population; A: Non-recalculated prevalence rate; B: Prevalence rate calculated on total sample; C: Prevalence rate calculated on ED users; * age reported as non-recalculated prevalence rate; °; ^; §; #; ∞: $p < 0.05$.

Tab. II. Distribution of total sample by working status and ginseng use in relation to socio-demographic characteristics and university course (prevalence rate).

	Total sample		Non-working students			Working students		
	Pop. (899)	Ginseng users (336)	Pop. (678)	Ginseng users (246)		Pop. (197)	Ginseng users (83)	
Variables	A (%)	B (%)	B (%)	B (%)	C (%)	B (%)	B (%)	C (%)
Gender								
Female	68.8	*40.4**	75.4	28.9	71.6	22.1	*29.6 '*	73.5
Male	30.3	*30.9**	76.5	24.6	79.8	22.1	*20.6 '*	67.5
Non-responders	0.9	25.0	37.5	0.0	0.0	0.0	25.0	100.0
Age (years)**								
18-22	74.9	75.3	78.0	75.0	75.0	66.0	78.0	78.0
23-27	17.7	19.0	16.1	18.7	18.7	23.9	20.5	20.5
≥ 28	4.8	2.7	3.9	2.8	2.8	8.1	2.4	2.4
Non-responders	2.6	3.0	2.0	3.3	3.3	2.0	0.0	0.0
Area of residence								
Northern Italy	84.5	38.2	75.9	27.9	72.4	*22.9§*	*10.0&*	26.2
Central-Southern Italy	10.5	30.9	*83.0§*	26.6	89.7	12.8	*3.2&*	10.3
Non-responders	5.0	37.0	52.2	19.6	52.9	23.9	8.7	23.5
Degree course								
Medicine	23.4	39.5	83.8	31.0	78.3	15.7	8.1	20.5
Dentistry	5.9	35.8	81.1	30.2	84.2	13.2	5.7	15.8
Nursing	47.3	34.6	70.8	23.5	68.0	24.9	9.6	27.9
Biomedical Laboratory Techniques	5.2	51.1	85.1	40.4	79.2	14.9	10.6	20.8
Speech and Language Therapy	4.2	52.6	73.7	36.8	70.0	23.7	15.8	30.0
Midwifery	4.2	47.4	78.9	34.2	72.2	21.1	13.2	27.8
Imaging-Radiotherapy Techniques	3.8	32.4	67.6	26.5	81.8	32.4	5.9	18.2
Physiotherapy	3.0	33.3	63.0	25.9	77.8	37.0	7.4	22.2
Cardiovascular Perfusion Techniques	1.3	0.0	100.0	0.0	0.0	0.0	0.0	0.0
Dental Hygiene	1.1	20.0	60.0	10.0	50.0	30.0	10.0	50.0
Psychiatric Rehabilitation Techniques	0.6	60.0	40.0	40.0	66.7	60.0	20.0	33.3

Pop.: population; A: Non-recalculated prevalence rate; B: Prevalence rate calculated on total sample; C: Prevalence rate calculated on ginseng users; ** age reported as non-recalculated prevalence rate; *, &,', §: $p < 0.05$.

Fig. 1. Socio demographic features of students' parents. (Prevalence rate).



($p < 0.05$) positively associated to EDs use (OR 1.5) and to ginseng use (OR 1.4), as shown in Table III.

- The courses attended by the highest percentage of the ED consumers were: Dentistry (54.7%) among the MSc; and Biomedical Laboratory Techniques (51.1%) among the BSc. The highest proportions of ginseng users were studying Medicine for MSc (39.5%), or Psychiatric Rehabilitation Techniques for BSc (60.0%).

No statistically significant differences emerged relating to the type of degree course attended by or ginseng users (MSc 38.8% *vs* BSc 36.8%; p = ns, Fisher's exact test). Instead, according to MLRM, being a Bachelor's degree student was significantly ($p < 0.05$) positively associated to EDs use (OR 1.4) (Tab. III).

Figure 2 shows the prevalence rates of ED and ginseng users in the various years of the different degree courses. Among the students of Medicine, ED use decreased from the first year (33.7%) to the fifth (28.3%), while ginseng consumption rose from the first year (38.5%) to the third (47.8%), then decreased by the fifth year (35.0%).

As for the students of Dentistry, ED use dropped significantly from the first year (72.0%) to the third (31.3%), ($p < 0.05$, Fisher's exact test), then increased again by the fifth (50.0%). The same trend was seen for ginseng consumers, with 48.0% in the 1st year, 6.3% in the 3rd, and 50.0% in the fifth.

In the first year of BSc, ED use ranged from 52.4% (in Biomedical Laboratory Techniques) to 22.2% (in Speech and Language Therapy), while ginseng use ranged from 66.7% (in Speech and Language Therapy) to 20.0% (in Dental hygiene).

In the 3rd year of BSc, ED use ranged from 58.3% (in Imaging-Radiotherapy Techniques) to 25.0% (in Speech and Language Therapy), while ginseng consumption ranged from 57.7% (in Biomedical Laboratory Techniques) to 33.3% (in Imaging-Radiotherapy Techniques).

Overall, the differences in the prevalence rates of ED and ginseng consumption by year of each degree course were never statistically significant (p = ns, Fisher's exact test), except between the 1st and 3rd years of Dentistry for both ED and ginseng, and between the 3rd and 5th years of Dentistry for ginseng alone ($p < 0.05$, Fisher's exact test). ED and ginseng consumption was also compared among students in the same years of different degree courses.

Tab. III. Multiple Logistic Regression Models for EDs or ginseng s use predictors.

Predictors Outcomes of the models	Male gender	Age (years)	Coming from North of Italy	Being a Bachelor's degree student	Being a working student	Parents' high educational level
EDs use by all students (n = 899) * (Pseudo-R2 = 0.0447)	**OR 2.3* (1.7-3.2)**	**OR 0.9* (0.8-0.9)**	OR 1.0 (0.6-1.6)	**OR 1.4* (1.0-1.9)**	**OR 1.5* (1.0-2.1)**	OR 0.9 (0.6-1.3)
Ginseng use by all students (n = 899) * (Pseudo-R2 = 0.0121)	**OR 0.7* (0.5-0.9)**	OR 1.0 (0.9-1.0)	OR 1.1 (0.7-1.8)	OR 0.8 (0.6-1.2)	**OR 1.4* (1.0-2.0)**	OR 1.0 (0.7-1.4)
EDs use by working students (n = 197) * (Pseudo-R2 = 0.0440)	**OR 1.9* (1.0-3.7)**	**OR 0.9* (0.8-1.0)**	OR 3.1 (0.8-12.5)	OR 1.4 (0.7-3.1)	-^	OR 1.3 (0.6-2.6)
Ginseng use by working students (n = 197) * (Pseudo-R2 = 0.0838)	**OR 0.3* (0.2-0.7)**	**OR 0.9* (0.8-1.0)**	OR 2.0 (0.5-8.1)	OR 0.6 (0.3-1.4)	-^	OR 1.2 (0.5-2.5)
EDs use by not working students (n = 678) * (Pseudo-R2 = 0.0504)	**OR 2.5* (1.7-3.6)**	**OR 0.8* (0.8-0.9)**	OR 0.8 (0.5-1.4)	OR 1.4 (1.0-2.0)	-^	OR 0.8 (0.5-1.2)
Ginseng use by not working students (n = 678) (Pseudo-R2 = 0.0025)	OR 0.8 (0.5-1.2)	OR 1.0 (1.0-1.1)	OR 1.1 (0.6-1.9)	OR 0.9 (0.6-1.3)	-^	OR 0.9 (0.6-1.5)
EDs use to improve alertness and study longer by all students (n = 899) * (Pseudo-R2 = 0.0321)	**OR 2.2* (1.5-3.2)**	**OR 0.9* (0.8-1.0)**	OR 1.2 (0.6-2.2)	**OR 1.7* (1.1-2.6)**	OR 1.3 (0.8-2.0)	OR 0.9 (0.8-2.0)
EDs plus alcohol use by all students (n = 899) * (Pseudo-R2 = 0.0574)	**OR 2.9* (2.0-4.1)**	**OR 0.9* (0.8-0.9)**	OR 0.8 (0.5-1.3)	OR 1.3 (0.9-1.9)	OR 1.4 (1.0-2.1)	OR 0.8 (0.5-1.2)
Ginseng-coffee use by all students (n = 899) * (Pseudo-R2 = 0.0163)	**OR 0.6* (0.4-0.9)**	OR 1.0 (0.9-1.0)	OR 1.2 (0.7-2.2)	OR 0.7 (0.5-1.1)	**OR 1.5* (1.0-2.1)**	OR 1.1 (0.7-1.7)

* $p < 0.05$; -^ Not evaluated.

Analysis of working students engaging in ED or ginseng use

The population of working students using ED revealed no statistically significant differences by distribution between BSc (44.6%) and MSc (42.5%), (p = ns, Fisher's exact test). On the other hand, there was a statistically significant difference between working students using ED aged 18-22 years (46.9%) and those aged 23 or more (39.7%), ($p < 0.05$, Fisher's exact test). Male gender was significantly positively (OR 1.9, $p < 0.05$, MLRM) associated with EDs use by working students (Tab. III).

The population of working students using ginseng was not homogeneous by gender (29.6% females *vs* 20.6% males; $p < 0.05$, Fisher's exact test). Male gender (OR 0.3, $p < 0.05$, MLRM) and age (OR 0.9. $p < 0.05$, MLRM) were significantly negatively associated to ginseng use by this type of students (Tab. III).

Analysis of non-working students engaging in ED or ginseng use

The population of non-working students using ED differed statistically by gender (23.6% females *vs* 38.6% males; $p < 0.05$, Fisher's exact test), but not by type of degree courses (35.2% MSc *vs* 38.3% BSc; p = ns, Fisher's exact test).

Age (OR 0.8. $p < 0.05$, MLRM) was significantly negatively associated to ginseng use by this type of students (Tab. III).

The population of non-working students using ginseng was homogeneous in terms of gender (28.9% females *vs* 24.6% males), area of residence (27.9% from Northern and 26.6% from Central-Southern Italy), and MSc (37.0%) *vs* BSc (36.0%), (p = ns, Fisher's exact test). The same applied to the age factor, with no statistically significant difference between ginseng users aged 18-22 years (35.0%) and those aged 23 and over (39.3%), (p = ns, Fisher's exact test).

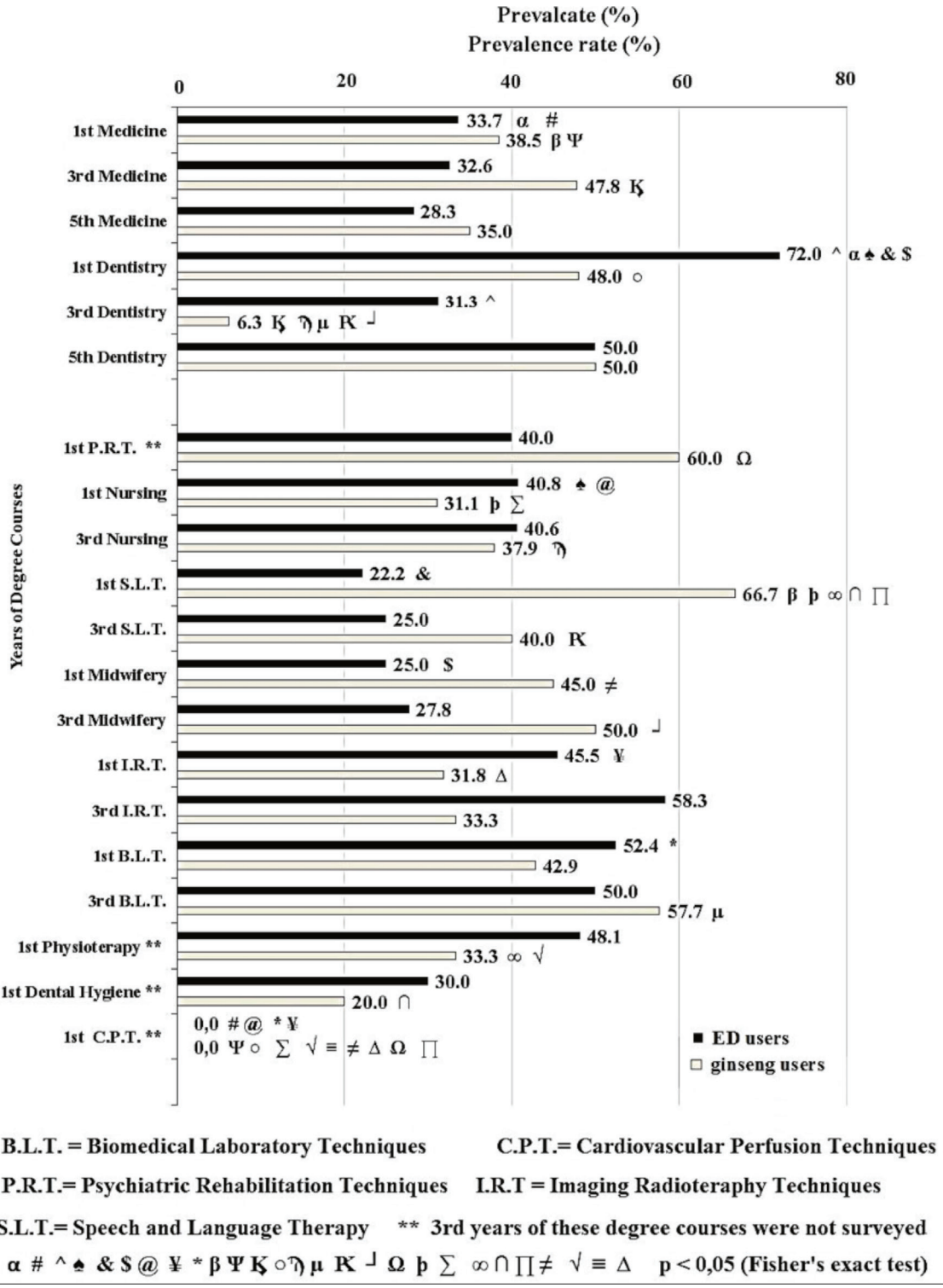
Reasons for substance use and their associations

Table IV shows the prevalence rates of the reasons for using ED and whether they were used alone or combined with other substances.

For the sample as a whole (n = 899), the prevalence rate of ED use for at least one 'academic' reason was 22.7%, while it was 21.2% for at least one 'other' reason; this difference was statistically significant ($p < 0.01$, Fisher's exact test).

The most frequently mentioned academic reason for using ED was "to improve alertness and study longer" (47.5%), while the most common other reason was "to socialize, at parties or the disco for instance" (29.1%).

Fig. 2. Distribution of ED and ginseng users by degree and year of course. (Prevalence rate).



Whatever the motives for using ED, the most often reported frequency of their usage was 1-10 times in the previous six months (93.1%).

ED and alcohol emerged as a very common combination (65.6%), comparable with the prevalence rate of ED used alone (65.7%). Only a minority of respondents combined ED with medicines (4.1%), or illicit drugs (5.5%).

As for the prevalence rates of ginseng use, alone or combined with other substances. The most common associations were ginseng with coffee (71.8%), while it was rarely combined with drugs (2.4%). The reported frequency of ginseng usage (alone or combined with other substances) was most often "1-10 times in the previous six months" (80.7%) (Fig. 3).

Tab. IV. ED use: reasons for use and associations with other substances (prevalence rate).

Use in previous 6 months		1-10 times	11-30 times	31-90 times	≥ 91 times	Total
Associations with EDs	No	53.3%	9.5%	1.7%	1.2%	**65.7%**
	Alcohol	45.2%	12.4%	4.9%	3.1%	**65.6%**
	Coffee	9.5%	2.6%	0.6%	0.0%	**12.7%**
	Tea	5.2%	1.2%	0.9%	0.0%	**7.3%**
	Drugs	2.6%	2.0%	0.6%	0.3%	**5.5%**
	Medicines	2.9%	0.9%	0.3%	0.0%	**4.1%**
Reasons for ED use: to improve	Alertness and study longer	38.0%	6.9%	2.6%	0.0%	**47.5%**
	Concentration while studying	24.2%	4.9%	1.7%	0.0%	**30.8%**
	Social skills (*)	21.3%	4.9%	1.7%	1.2%	**29.1%**
	Sports performance	19.6%	2.3%	1.4%	0.3%	**23.6%**
	Exam performance	13.5%	1.7%	2.0%	0.3%	**17.5%**
	Driving performance	8.6%	2.9%	0.9%	0.0%	**12.4%**
	Attention in classroom	10.1%	1.2%	0.9%	0.0%	**12.2%**
	Working performance	7.8%	2.6%	1.4%	0.0%	**11.8%**

(*) To socialize (e.g. at parties, disco, bars).

Fig. 3. Distribution of ginseng use associated or not with other substances. (Prevalence rate).

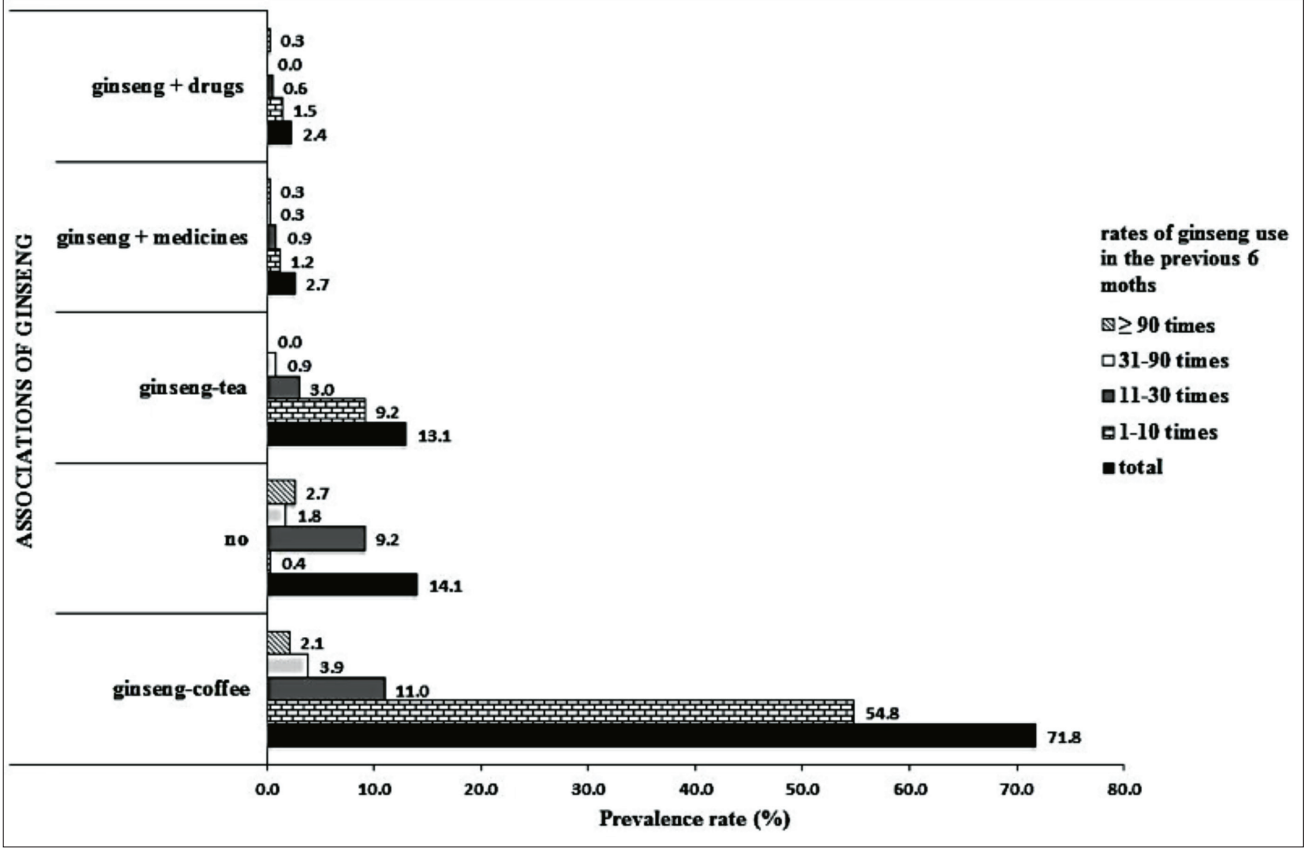


Table V shows the socio-demographic characteristics and degree courses of students using ED in order to study longer, and the features of students mixing ED with alcohol or ginseng coffee.

The population of students who used ED "to improve alertness and study longer" differed by gender (15.2% of the female sample and 26.1% of the male sample; $p < 0.05$, Fisher's exact test), and the majority (80.0%) were 18-22 years old. There was also a statistically significant difference in the proportion of these ED consumers between the younger students 18-22 years old (19.6%) and those aged 23 and over (15.8), ($p < 0.05$, Fisher's exact test). Being a Bachelor's degree student (OR 1.7, $p < 0.05$, MLRM) was significantly positively associated to EDs use for this reason (Tab. III). On the other hand, they were homogeneous in terms of area of residence (18.7% from Northern, 19.1% from Central-Southern Italy; p = ns, Fisher's exact test). Finally, this type of ED user was found in similar proportions among the working students (21.8%) and non-working students (17.7%) (p = ns, Fisher's exact test).

Tab. V. Socio-demographic features of ED and ginseng users by personal characteristics and university course, reason for usage (to improve alertness and study longer), and association with alcohol or coffee (prevalence rate).

Variables	ED users (to study longer) (N = 165)		ED+alcohol users (N = 228)		Ginseng+coffee users (N = 241)	
	A (%)	B (%)	A (%)	B (%)	A (%)	C (%)
Gender						
Female	*15.2**	46.1	*19.7°*	59.8	*29.6^*	73.5
Male	*26.1**	50.4	*38.6°*	74.5	*20.6^*	67.5
Non-responders	0.0	0.0	12.5	50.0	25.0	100.0
Age (years)**						
18-22	80.0	80.0	80.7	80.7	74.7	74.7
23-27	18.8	18.8	18.4	18.4	18.3	18.3
≥ 28	0.6	0.6	0.0	0.0	2.9	2.9
Non-responders	0.6	0.6	0.9	0.9	4.1	4.1
Area of residence						
Northern Italy	18.7	48.0	25.4	65.2	27.8	73.5
Central-Southern Italy	19.1	43.9	31.9	73.2	19.1	94.7
Non-responders	10.9	50.0	10.9	50.0	26.1	70.6
Degree course						
Medicine	15.2	47.8	19.0	59.7	30.5	77.1
Dentistry	18.9	34.4	47.2	86.2	26.4	73.7
Nursing	19.3	47.4	25.4	62.4	24.7	71.4
Biomedical Laboratory Techniques	25.5	50.0	34.0	66.7	27.7	59.1
Speech and Language Therapy	10.5	44.4	15.8	66.7	39.5	75.0
Midwifery	18.4	70.0	18.4	70.0	39.5	83.3
Imaging and Radiotherapy Techniques	20.6	41.2	41.2	82.4	14.7	45.5
Physiotherapy	25.9	53.8	29.6	61.5	25.9	77.8
Cardiovascular Perfusion Techniques	0.0	0.0	0.0	0.0	0.0	0.0
Dental Hygiene	20.0	66.7	20.0	66.7	10.0	50.0
Psychiatric Rehabilitation Techniques	40.0	100.0	40.0	100.0	40.0	66.7

A: Prevalence rate calculated on total sample; B = Prevalence rate calculated on ED users; C: Prevalence rate calculated on ginseng users; ** age reported as non-recalculated prevalence rate; *; °; ^ = $p < 0.05$.

Students mixing ED with alcohol differed significantly by gender (19.7% females *vs* 38.6% males; $p<0.05$, Fisher's exact test), but not by area of residence (25.4% from Northern, 31.9% from Central-Southern Italy; p = ns, Fisher's exact test). Here again, there was a statistically significant age-related difference, with 80.7% of these ED plus alcohol consumers among the 18- to 22-year-olds, as opposed to 20.8% among the older students ($p < 0.05$, Fisher's exact test), but no difference between MSc (24.7%) and BSc students (25.6%), (p = ns, Fisher's exact test), or between working students (29.2%) and non-working students (25.4%), (p = ns, Fisher's exact test).

In the population of ginseng-coffee users there were between students aged 18- to 22-year-olds (26.8%) and older students (25.3%), between areas of residence in Northern (27.8%) *vs* Central-Southern Italy (19.1%), or between students attending MSc (29.7%) *vs* BSc (25.6%). Ginseng-coffee users did show a statistically significant gender-related difference (29.6% females *vs* 20.6% males, $p < 0.05$, Fisher's exact test). Finally, being a working student (OR 1.5, $p < 0.05$, MLRM) was significantly positively associated to ginseng-coffee (Tab. III).

Discussion

To our knowledge, this is one of very few studies to have attempted to establish the prevalence of ED and ginseng use by Italian university students. In our sample of 899 students, 38.6% had used ED, and 37.4% had used ginseng in the 6 months prior to answering our questionnaire.

Considering that the age range of our sample of students was 18-28 years (not considering the only two subjects aged 30 and 39 years), the prevalence rate of their ED use is more than twice as high as the figure the EFSA found in Europe for the same age group in 2011 (38.6% vs.15.9%) [5].

Consistently with the findings of a Belgian study [30] conducted on university students aged 19-23, we found no association between families' educational level and ED or ginseng usage. This would mean that their use is not associated with any specific social patterns, but a broad social phenomenon, particularly among young people [9, 11, 12, 14].

The statistically significant age-related difference emerging in ED use in our sample (with 18- to 22-year-olds using ED far more than older students) is probably

because these drinks are readily accessible to children, adolescents, and young adults [31]. The growth in their popularity may be partly attributed to their availability in bars and clubs, where ED have been used as mixers. But nowadays consumers can find pre-mixed alcoholic energy drinks at any local supermarket or grocery store [31]. It would be desirable for the distribution and sale of these products to be regulated.

Our population of ED users confirmed a gender-related trend seen in other studies, with a larger consumption of these drinks by males [14, 16, 30, 32-34]. One possible explanation for this gender disparity comes from Miller's research [32]: a greater ED consumption among college males would be linked to "jock identity", conformity to masculine norms, and risk-taking behavior.

Although no statistically significant difference in ED use by area of residence emerged from our analysis, our population was skewed towards Central-Southern Italy (out-of-town students). As seen in another study, this might reflect a tendency to behave differently when far from home and parental control, especially as regards any predisposition towards substance use [32].

On the total sample (n = 899), we found a statistically significant difference between EDs or ginseng or ginseng-coffee use and being a working *vs* a non-working student, probably due to the higher load of stress experienced by this type of students.

ED consumption was associated with socializing by 29.1% of the users in our sample – a result in line with the findings of another Italian survey, which found a statistically significant association between ED use and going to the disco or other recreational places [17].

Our study confirms that the use of ED mixed with alcohol is becoming increasingly common among youth and young adults [35, 36] (65.7% of the ED users in our sample had mixed ED with alcohol at least once in the previous 6 months). Combining ED with alcoholic beverages would increase the sensation of pleasure, and might reduce the depressant effects and/or increase the excitatory effects of alcohol [37, 18, 38].

The potential risks of associating ED with alcohol include: underestimation of alcoholic drunkenness, excessive diuresis, dehydration, changes in heart rhythm (palpitations, arrhythmias), high blood pressure, neural symptoms (irritability, disturbed sensation, tremors, muscle twitching), gastrointestinal disorders, and worsening depression [39, 40].

Educational campaigns should be conducted on various levels to contrast the massive marketing campaigns of ED manufacturers, which insinuate that ED can improve mental performance without any side effects. It is worth noting that the most often advertised reason for using these drinks "to prolong wakefulness" is reflected in people's justification for their use, as seen in the literature [11, 41] and in the present study. In actual fact, in stark contrast with this belief, the consequences of using ED include excessive daytime drowsiness and slower reaction times, which nullify the perceived short-term effects [1]. ED generally offer a quick fix for temporary difficulties, but their prolonged use can affect an individual's quality of life. Their psychological effects may be dose-dependent [1], so heavy consumers should be warned and advised to reduce their frequency of consumption. As the adverse effects of excessive ED use are generally prompted by the high caffeine intake, there should be an evidence-based upper limit for the quantity of caffeine allowable in a single serving of any drink [42]. While the caffeine in ED can have severe implications, adverse effects have also been reported in association with other substances they contain, such as taurine, guarana, and ginseng [39, 40, 43, 44].

An important strength of the present study lies in the large number of participants (899), surveyed by entering university classrooms (an approach that enabled us to provide students with all the information they needed to answer the questionnaire).

The present study also has some aspects that need to be mentioned: there were more women than men, due to the greater female presence at Italian medical schools; most of the respondents were from Northern Italy because of the university's location, so the results may not be generalizable to all Italian university students; and the data were collected only at a single point in time, whereas a longitudinal study would be more appropriate to support the study's findings.

Further research is needed, also on the possible adverse effects of ED and ginseng consumption. A multicenter study on a larger sample could also consider even younger people (of school age) and compare them with older groups.

Conclusions

Our study has shown a wide diffusion of EDs and ginseng among the sampled students, in particular among the younger ones and working students. There was a male predominance in the use of ED, and a female one for ginseng. These data point out possible risks both for dependence and acute and chronic health risks related in particular to EDs use/abuse. Therefore, health education programs are suitable: informing peopl – starting from primary school age, also involving children's parents and general practioners, through the local health services – could raise awareness of EDs health risks.

Aknowledgements

The authors declare that there is no conflicts of interest with manufacturing factories of energy drink and ginseng.

Authors' contributions

SM: design, organization and supervision of the study. Drafting of the text.

DG: data analysis, participation in the drafting of the article, statistical analysis.

SP: data analysis, participation in the drafting of the article, statistical analysis.
JP: data analysis, participation in the drafting of the article.
AS: preparation and validation of the questionnaire, distribution of the questionnaire, data collection and data entry in the database.
SF: preparation and validation of the questionnaire, distribution of the questionnaire, data collection and data entry in the database.
EC: preparation and validation of the questionnaire.

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ORIGINAL ARTICLE

Energy drink consumption: a survey in high school students and associated psychological effects

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Keywords

Energy drinks • Abuse • Caffeine • Alcohol • Young

Summary

Introduction. *Energy drinks represent an emerging health problem among young people. Energy drinks generally refer to a class of beverages containing sugars and various combinations of bioactive ingredients such as caffeine, taurine etc. Also the mix of energy drinks with alcohol is fairly frequent among young people and could be associated with dangerous effects.*
Methods. *In 2016-2017, a cross-sectional study was conducted in 1581 students attending eight high school in the Marche Region. Data were collected via an anonymous self-administered questionnaire.*
Results. *The 27.7% of students use energy drinks and the majority, corresponding with the 93.0%, are aware of the main ingredients contained in energy drinks. The main activities for which young people use these drinks are: sport, leisure, pleasure, study. Young people who admit to using alcohol mixed with energy drinks more than 4 times a month are an alarming fact.*
Conclusions. *This research confirms that energy drinks are used more by young males and especially by those who practice sports. Furthermore, the use these beverages to increase the concentration in the study and to be more brilliant in free time, is confirmed.*

Introduction

The first energy drink was introduced in Austria in 1987 and was launched in the United States (US) in 1997 [1]. Since then, the consumption of energy drinks has increased dramatically although they have become twice as expensive as traditional soft drinks. In 2011, the European Food Safety Authority (EFSA) commissioned a study to gather consumption data for energy drinks in 16 countries of the European Union. They found that the average consumption was 2 L/month in adolescents and 0.49 L/week in children [2, 3].
In the last decade the consumption of energy drinks has increased among athletes and the general public, above all in young individuals.
"Energy drinks" (EDs) generally refer to a class of beverages containing sugars and various combinations of bioactive ingredients such as caffeine, taurine, glucuronolactone, B-group vitamins, inositol, niacin, panthenol, extracts of guarana, green tea and ginger, purported to "energize" the body and the mind. Energy drinks have been found to improve attention and/or reaction times and indices of alertness in some studies; the combination of caffeine and glucose can ameliorate deficits in cognitive performance and subjective fatigue during extended periods of cognitive demand [4]. However, several ingredients may have unwanted health consequences in youngsters and should be used carefully. The health risks associated with energy drink consumption are primarily related to their caffeine content: a caffeine overdose can cause palpitations, hypertension, dieresis, central nervous system stimulation, nausea, vomiting, marked hypocalcemia, metabolic acidosis, convulsions and in rare cases, also death. Therefore, the European Union regulates the labeling of EDs by requiring that beverages characterized by "a high caffeine content", in a proportion in excess of 150 mg/l, show the following message clearly: "High caffeine content" [5]. In particular, in Italy the maximum concentration of caffeine is 32 mg/100 ml and must be specified on warning labels, such as "high caffeine content" and "not recommended for children, pregnant women or people sensitive to caffeine" [6]. Caffeine can also have a neuro-pharmacological effect, in fact it can increase the tendency to alcohol addiction. International studies have also indicated that mixing energy drinks with alcohol is fairly frequent among college students: this may be dangerous given the stimulant nature of energy drinks and depressant characteristics of the alcohol (the stimulant effect can mask how a person is intoxicated by alcohol); moreover, this mix is very dehydrating and will hinder the body's ability to metabolize alcohol, thus increasing intoxication [7, 8]. Another side effect of energy drink consumption is the increased risk of obesity due to their high sugar content [9]. The potential adverse health effects of the other ingredients of energy drinks (guarana, taurine, glucuronolactone, B vitamins) are not well known, so further studies are required [10]. Past observations have suggested that young adults can easily access energy drinks, but there is scarce evidence on the reason why they consume this kind of beverage and whether they are aware of their potential health hazards [9].

In accordance, this study examined the frequency of consumption of energy drinks in groups of high school students in the Marche Region (Italy) and investigated the factors for preferring these drinks and their eventual use with alcohol and during physical activity. It also analyzed and investigated the sensation of anxiety and depression in young people.

Methods

In 2016-2017, a cross-sectional study was conducted in students attending eight high schools in the Marche Region, and in particular in the district of Macerata, Ancona and Ascoli Piceno. This survey included 1581 students, after approval was obtained from the administrators of each faculty, and verbal consent was received from each participant. Data were collected via an anonymous self-administered questionnaire; it consisted of 30 questions on students sociodemographic characteristics, personal habits, total caffeinated fluid intake, knowledge of energy drinks and habits (possible consumption during physical exercise and in association with alcohol). Before arriving at the final form of questionnaire we tested it on 120 people to check if the questions were interpreted as intended and to assess the extent of response, we only assigned validity to our questionnaire after the validation process was completed. Another important step in the construction of the questionnaire was our focus on reliability: results have to be reproducible; in our case, reliability was assessed by comparison with similar data obtained from other researches in PubMed.

To evaluate the consumption of caffeine we were inspired by the CCQ (Caffeine Consumption Questionnaire) [11], slightly modified according to our requirements. The data about the association of energy drinks and alcohol were collected using BAES (Biphasic Alcohol Effects Scale) [12], a self-report, unipolar adjective rating scale that is designed to measure both stimulant and sedative effects of mixing alcohol with energy drinks. It consists of fourteen items that comprise two sub-scales (stimulant and sedative). Individuals were instructed to rate the extent to which drinking alcohol produced these feelings, from 0 (not at all) to 10 (extremely).

Results

Table I shows the characteristics of the sample, uncovering some unhealthy lifestyle choices in this group, such as the habit of smoking, the use/abuse of substances and the intake of hunger sedatives. Analyzing the data showed that the majority of these students (1130 subjects), corresponding with 93.0%, are aware of the main ingredients contained in energy drinks; the most commonly reported ingredients are caffeine, taurine and sugars. The part (7.0%) who is not aware often confuses energy drinks with sport or soft drinks. On the other hand, the majority of students (64.3%) are not aware of the possible side effects caused by abusing energy drinks. However, among the side effects associated with their consumption, they admit to having experienced insomnia, anxiety, tachycardia and gastrointestinal disturbances above all (Fig. 1).

Students who use energy drinks are 436 (27.7%) and we have seen that men were more likely than women to make use of these beverages; their assumption happens especially during the hours when sports are being practiced, followed by consumption during hours of study and leisure. Only 215 students from the participants said they do not use energy drinks because they "think it is unhealthy", while 770, the majority, answered they do not feel as if they need to use them, and don't question themselves about their risks.

The main activities during which young people use these drinks are: sport (97), free time (82), pleasure (72), study (60). During sport activities which see the largest percentage of energy drinks consumption among young people, the most popular brand was Red Bull (321) as it is most strongly associated with sport competitions.

If we ask the student sample to indicate on which occasion their choosing an energy drink is a first choice, we can say that they are strongly motivated by the need for energy (193), staying awake (145) and because they like the taste (139), finally to increase athletic performance (71).

Though to a much lesser extent, the data on energy drinks consumption as first choice (32) during study (to increase concentration) and at the wheel to drive for longer periods (32) should be noted. Analyzing the consumption of energy drinks as second and third choice shows that, no less than 131 participants, turn to energy drinks to increase athletic performance as a second choice (259 as third choice), 143 to increase concentration during study (240 as third choice), 61 to aid in hangover recovery (352 as third choice) and finally 64 to stimulate their metabolism (350 as third choice).

Even if mixing energy drinks with alcohol is not frequent, and the prevailing answers are "sometimes" and "rarely", the percentage of students who consume energy drinks with a frequency of 1 to 4 is relevant. The most worrying data shows that 44 students admit to drinking cocktails of alcohol and energy drinks more than 4 times per month (Fig. 2).

Consumers of these cocktails were then asked to estimate (using numbers) sensations they experienced as shown in the BAES table, and the results showed that the stimulating effects such as " elated", " energized", "excited", "stimulated", "talkative", "up" and "vigorous" prevailed, though with very little difference between them (Fig. 3).

Discussion

As in previous researches, this study has allowed to photograph the situation of the population of young people regarding the adoption of incorrect lifestyles that expose young people to risks to their health (smoking, alcohol, drugs…) [13-15]. Most of the students in our sample who responded to the questionnaire know the main ingredients of energy drinks, but are not aware of the possible side effects that their abuse may cause. This is very seri-

Fig. 1. Side effects reported by students.

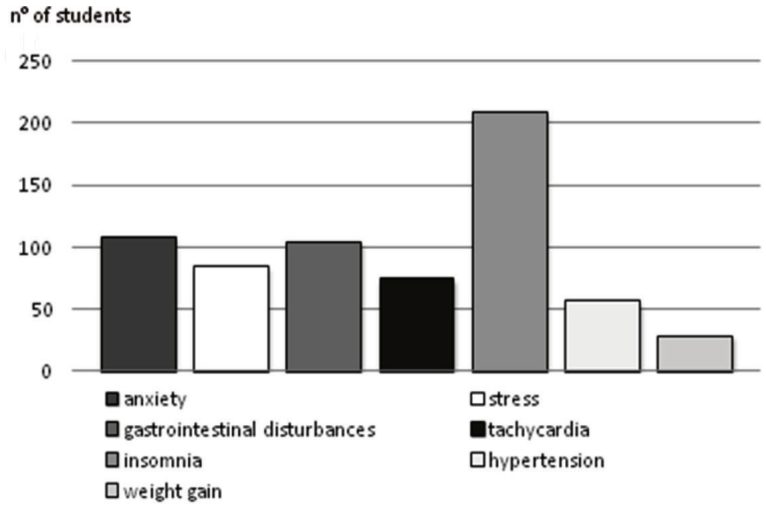
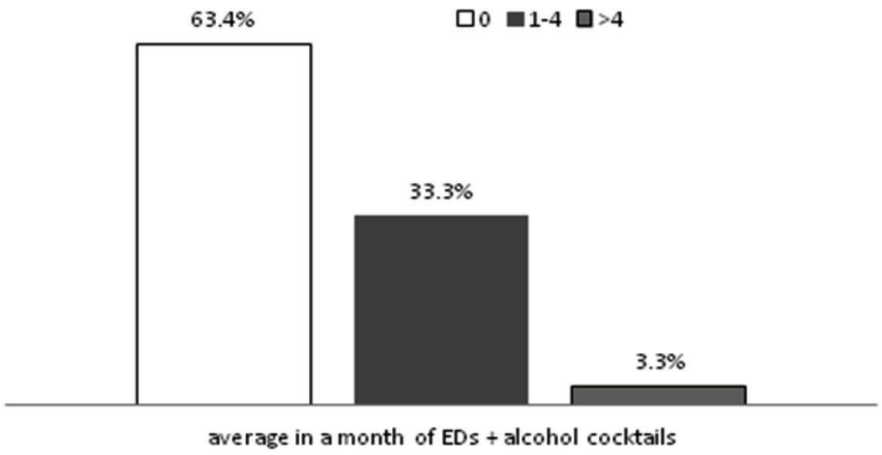


Fig. 2. The use (%) of energy drinks mixed with alcohol in a month.

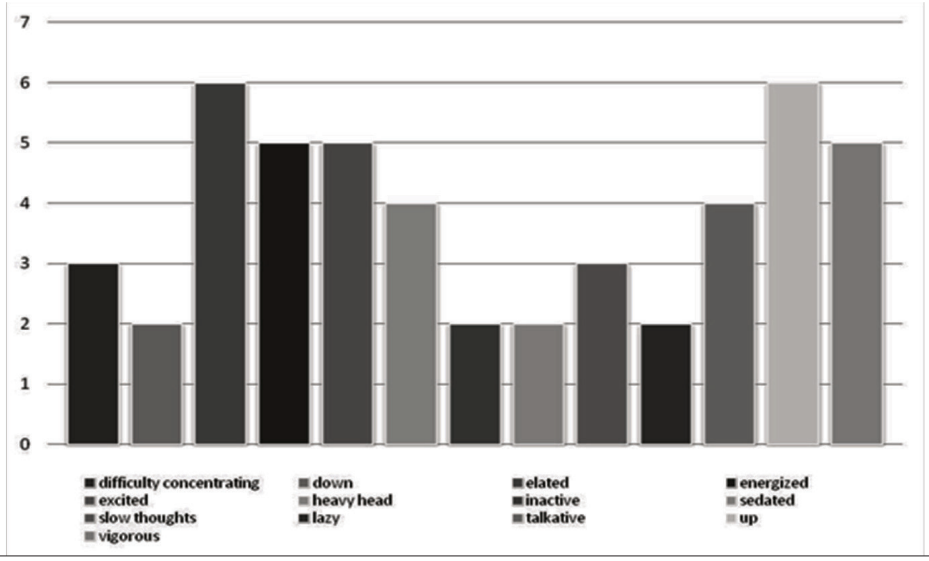


ous because it means that today's youth are not aware of what they can face, acting without awareness of possible consequences. Previous studies also showed that abusing energy drinks may lead young people to take other illegal substances, and this is even more severe [16-18]. Therefore, education is very important, first of all by the family and then by the school, in order to warn children and adolescents of the possible risks they may incur by simply drinking a can. Education on the health hazards of various nutritional elements and/or supplements can be included in school curricula.

In our study, 436 out of 1.581 students use energy drinks. It is worrying that the students who consume energy drinks, though aware of the side-effects associated with their use such as insomnia, anxiety, tachycardia and gastrointestinal disturbances, continue to use these drinks in association with sport activities, free time, fun and study. Even more worrying is the data showing energy drink as the first choice of drink during study to increase concentration and at the wheel in order to increase concentration and feel less tired [19]. Finally, an analysis of the consumption of energy drinks as second and third choice drink show an increase in students who turn to EDs to increase athletic performance, their concentration in study, combat hangover symptoms and lastly to stimulate their metabolism [9, 20, 21].

Our study shows that young people see energy drinks as containing stimulating, helpful substances which aid them in activities such as driving, study and sports. This notion represents an unhealthy lifestyle that is dangerous to oneself and others, caused in part by the superficiality of some young people, but mostly by the image that producers of energy drinks publicize through mass media by associating successful competitive performances with their consumption.

Fig. 3. Feelings associated (BAES: 0-10) with the use of energy drinks mixed with alcohol.



Among our participants, the consumption of energy drinks mixed with alcohol was not high, in fact the answers which were chosen most frequently were "sometimes" and "rarely" with an average of 1 or 2 cocktails being consumed in a month.
However, it is worrying and dangerous to see that in a sample of young people of high school age, 36.3% admitted to consuming energy drinks mixed with alcohol with a frequency going from once a month to more than 4 times a month.
This data increases in relevance when confronted with the results of a study on 500 students of the University of Messina which found that 56.9% of students were using energy drinks and of that percentage, 48.6% is used to drinking EDs mixed with alcohol [22]. In our study, consumers of these cocktails were then asked to estimate (using numbers) their sensations as shown in the BAES table, and results showed that stimulating effects such as "elated", " energized", "excited", "stimulated", "talkative", "up" and "vigorous" prevailed, though with very little difference between them.
The problem deriving from perceiving these sensations is the habit of consuming these mixtures, as it can lead to a risk of alcohol dependency [23]. The energy drink market has grown exponentially over the past decade. Energy drinks marketing strategies include sporting events and athlete sponsorships, alcohol-alternative promotion, and product placement in the media (including Facebook and video games) oriented to children, adolescents, and young adults. Newer alcoholic energy drinks, the cans of which resemble their nonalcoholic counterparts, target risk-taking youth. Contrasting with brand design is the voluntary fine-print warning label on some products, which state that they may not be safe for children, those who are sensitive to caffeine, or for pregnant or nursing women [6, 24]. The use of EDs by young people does not only create problems associated with caffeine, but also sugars. The literature shows that in EDs, sugars are present in amounts able to cause insomnia, nervousness, headache, tachycardia and seizures [25-27].
The absence of precise rules in many countries has resulted in the aggressive marketing of energy drinks over the world, targeted primarily at adolescents and young adults (men in particular) [28].
Producers have taken advantage of shortcomings in international and community legislation which, allow drinks in Europe to contain quantities of caffeine over 150 mg/100 ml by simply adding a warning label which is often not read, and in the US, producers are not obliged to label the amount of caffeine on the product.

Conclusions

The amount of data available allows us to illustrate a picture of the use of EDs in the younger population.
Our study confirms energy drinks are used mostly by young males and people taking part in sports. The tendency to use these drinks to help increase concentration during study and be on the ball in their free time was also confirmed.
The promotion of these drinks feeds the market of illusions, consolidating the opinion that any problem can be overcome with a drink, alcohol or a pill.
This illusion could determine a serious problem of public health also between the italian young people [29, 30].

Acknowledgments

The authors express their gratitude to all participant schools and declare that they have no conflict of interest.

Authors' contributions

SS: conceived and coordinate the study, evaluated the results and wrote the manuscript. FP: coordinated and

contributed to the manuscript writing. MT: contributed to critically revised the manuscript. LK: contributed to critically revised the manuscript. FC: contributed to the recruitment of the participants, the acquisition of epidemiological data. IG: contributed to the supervision of the study and evaluated the results.

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ORIGINAL ARTICLE

Mental and physical effects of energy drinks consumption in an Italian young people group: a pilot study

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Keywords

Energy Drinks • Young • Physical Stress • Mental Stress

Summary

Introduction. *The primary consumers of energy drinks were athletes, to combat fatigue, but in reality, thanks to their fast expansion and economic growth, young adults and teenagers represent the new target market. Consumption of energy drinks by both recreational and competitive athletes has increased dramatically in recent years, though they are often unaware of what is being ingested, believing to improve their physical and psychological performance. The literature shows contradictions about the capacity of energy drinks to enhance psychophysical results. In relation to probable adverse effects induced by the irregular consumption of energy drinks, which in several cases are not so clear, we decided to investigate the possible relationship between the intake of energy drinks and the presence of mental and physical stress in young people and athletes.*
Methods. *Two experimental sessions, separated at least by 1 week, according to a randomized cross-over design, following this protocol were conducted: in the first session a mental and physical stress was conducted without the consumption of energy drinks, the second after energy drinks consumption. BAI (Beck Anxiety Inventory) and BDI (Beck Depression Inventory II) test have been used to test the mental stress, and a "cycle ergometer test" to test the physical stress.*
Results. *BAI and BDI tests results showed that before the consumption of energy drinks, subjects are considered in the range of "minimal level of anxiety", (10 and 60 percentiles) and do not report a level of depression. After the energy drinks consumption, a "mild level of anxiety" has been recorded, and the BDI showed a case with a pathological profile. The physical test recorded a small increase in the maximum heart rate was verified with the intake of an energetic beverage.*
Conclusions. *The stimulating effect of Energy Drinks EDs on nervous system and cardiovascular system, must be checked and studied in deeper detail, because it may represent a risk for the health of young athletes.*

Introduction

The World Health Organization (WHO) defines energy drinks (EDs) as beverages able to offer users an energy boost related to their ingredients (caffeine, amino acids, herbal extracts, carbohydrates and vitamins) all of which are marketed as energizing and stimulating [1].
Furthermore the same category of energy drinks also includes sport drinks, soft drinks, nutraceutical drinks, and supplement foods which have lower percentages of consumption. But it is very important not to confuse them as they are used for different purposes, and regulate them with specific law [2]. Sports drinks are hydro-saline beverages with a low-carbohydrate content, without caffeine, for post-workout rehydration purposes to compensate the loss of water and electrolytes. Soft drinks are characterized by the addition of flavorings and sweeteners; nutraceuticals are expressly ingested to enhance the health state and usually contain bioactive compounds such as concentrated extracts from teas, fruits, vegetables or herbs and antioxidants [3].
Numerous debates have been raised in relation to the reduction caused by energy drinks in the proper nutritional intake within food standards code. Several countries have drafted various limitations in order to regulate and control the labeling, distribution and sale of energy drinks for their argued caffeine content [4].
The primary consumers of energy drinks were athletes, to combat fatigue, but in reality, thanks to their fast expansion and economic growth, young adults and teenagers represent the new target market. Consumption of energy drinks by both recreational and competitive athletes has increased dramatically in recent years, though they are often unaware of what is being ingested, believing to improve their physical and psychological performance [5]. Furthermore, among athletes, the popularity of caffeine and caffeine-containing products has also increased because this substance has been removed from the World Anti-Doping Agency list of banned substances [6].The literature shows contradictions about the capacity of energy drinks to enhance physical results, and there are several possible mechanisms that could explain improvements, suggesting the high energy compound effect on the central nervous system is most likely responsible [7]. It is well known that the combination of more sugars (glucose with fructose or maltodextrin and

fructose) seems to increase the quantity of energy provided during exercise [8]. Energy drinks, in fact, contain high quantity of carbohydrates (25-30 grams in 240 ml) and this amount does not conformity to the recommended content of 6-8% (6-8 grams in 100 ml) suggested by the International Society of Sport Nutrition (ISSN), the American College of Sports Medicine [9, 10], and the Panel on Dietetic Products, Nutrition and Allergies of European Food Safety Authority (EFSA) [11].
These doubts about health problems arising from the use of energy drinks represent a health problem still to be explored, not only from a physical but also psychological point of view.
Since the initial diffusion of caffeinated drinks, their regulatory aspect has always shown some deficiencies due to the lack of knowledge about the physiological effects produced by the substances used in manufacturing, compromising the level of security in their composition. In fact, exacerbation and increase of anxiety, acutemania, seizures, and mood and behavior problems have been correlated to the use of energy drinks [12]. Furthermore, in young people, high levels of soft drink consumption are related to psychological problems, with emotional symptoms including mental distress, hyperactivity, behavioural problems, and suicidal behaviors [13, 14].
In relation to probable adverse effects induced by the irregular consumption of energy drinks, which in several cases are not so clear, we decided to investigate the possible relationship between the intake of energy drinks and the presence of mental and physical stress in young people and athletes.

Methods

Study population

Ten healthy young adults, five females and five males, aged 18 to 27 years, were recruited for a mental and physical stress test. The study was carried out in healthy, non-obese, non medicated individuals who were taking any medication affecting cardiovascular or autonomic regulation, non-medicated individuals who were free from any somatic or mental condition. The study criteria were met by 20 individuals, 10 females and 10 males, and the experimental protocol has been described.
Only 10 individuals accepted to participate.The sample selected was invited not to take drinks with caffeine (as energy drinks) and substances of abuse in the week before the test, and in the hours before the physical and mental test.
The mean height of the participants was 167.7 ± 1.0 cm, their body weight was 59.8 ± 2.4 kg, and the medium BMI (Body Mass Index) was 21.5. Some participants were trained (subjects with regular physical activity); in particular inside the female group only the participant 1 and 2 were trained. Inside the male group only the number 6 was untrained. Three of participants, inside the untrained group, reported to smoke regularly.

Study design

The adherence to the protocol including the mental and physical tests was made by each subject who completed and signed the informed consent. This form was valid for all tests performed. In particular, the informed consent was important for the physical test. In fact, during the exam to raise the heart rate, it is possible, to perceive some complications like chest pain or on the electrocardiogram, and this was generally followed by the spontaneous interruption of the exercise. In some cases, it is possible to verify cardiac arrhythmias with early stoppage of the test.

Psychological and physical tests

All experiments took place in a quiet, temperature-controlled (22°C) laboratory and started between 08.00 a.m. and 09.00 a.m. Every subject attended 2 separate experimental sessions (each session separated at least by 1 week) according to a randomized cross-over design. The first week, on arrival at the laboratory subjects were asked to sit in a comfortable armchair; subjects were called one at a time to the cardiologist's surgery, and were given their ECG under physical stress. A baseline recording was made for 20 minutes. Then, a mental arithmetic stress test was performed for 5 minutes: subjects subtracted the number 6 or 7 (chosen at random) continuously from a random 3 or 2 digit number and were instructed to give a written response. Each mental stress task was presented to the subjects on a monitor and comprised 60 unique calculations, with a 5-second interval between each calculation. Immediately after the mental task, subjects were asked to rate their perceived stress using BAI (Beck Anxiety Inventory) [15] and BDI (Beck Depression Inventory II) [16] tests.
The BAI and BDI-II tests in young adults were validated [17, 18].
The BAI is a self-report instrument that allows to measure the severity of anxiety symptoms in adults; it was designed to include anxiety symptoms only minimally overlapping with those of a depressive nature. The test consists of 21 items (descriptions of somatic symptoms of anxiety, subjective or related to phobias), to be evaluated on a four-point scale (0 to 3). The clinical study can then continue assessment using DSM (Diagnostic and Statistical Manual of Mental Disorders) criteria to arrive at a specific diagnostic category and plan interventions targeting the underlying cause of the respondent's anxious symptomatology and/or diagnosis.
The BDI-II is a self-report instrument that allows you to assess the severity of depression in adults and adolescents (including a psychiatric diagnosis). The test consists of 21 items and gives a total score and scores on two areas: Somatic-Affective and Cognitive.
After the BAI and BDI tests, physical stress was evaluated using a "cycle ergometer test" with the purpose of estimating maximal oxygen consumption (VO2max). It is an instrumental examination which consists in the recording of an electrocardiogram before, during and after the execution of a physical effort, increasing blood pressure and blood flow to the coronary arteries thus

ensuring the influx of blood to the cardiac muscle. Our performance was executed with a cycle ergometer, regularly calibrated, a particular exercise bike with regulators and electronic controllers of the effort that is made by the patient. In this way it was possible to evaluate the cardiovascular reaction to physical exercise, particularly concentrating on heart rate, pressor response and variation of the ECG to achieve the maximum heart rate (MHR) expected for the age of the subject. The calculation of the MHR was performed by using the Karvonen formula that keeps the difference between the theoretical maximum heart rate (220) and the age of the subject in correlation, ensuring the most accurate method for making the measurements in percentages: prediction of HR_{max} (220 bpm - age). The preparation test included a recommendation for the subject to have a light breakfast, avoiding alcohol consumption, in the hour before the test was administered. Before starting the cycling exercise, all procedures were briefly explained to all the patients. Exercise testing protocols were assisted by supervising laboratory staff. It was recommended that selected protocol be based on the limitations of each individual. The electrode pads of the ECG were placed on the thorax of the patient in order to record a basal electrocardiogram. In this manner it was possible to extrapolate an overview of the cardiovascular profile characterized by the load (Watt), the maximum heart rate (bpm), percentage on the theoretical heart rate, the maximum systolic and diastolic pressure. Analysis is divided into three phases: 1) pre-exercise; 2) exercise; 3) post-exercise. For each period time, number of heartbeats, blood pressure and potency were monitored as collection data. In the first phase, physical effort started at 0 Watts by pedaling on the exercise bike and becomes progressive during the exercise period, through gradual increments (steps) given by resistance from the bicycle ergometer pedals, until reaching the point of highest fatigue. This increase of strength was provided by additing 25 Watts every minute (25Wx1) until reaching exhaustion. When the subject was no longer able to stand a further increase, they were told to finish with a final sprint which allowed to measure maximum heart rate. In the final stage, post-exercise, the patient continued to pedal without workload. The collection data included the measurement of blood pressure taken at rest, in both a supine (at the end of the exercise period) and standing position (prior to cycling), necessary to determine the presence of any ECG abnormalities that might interfere with test results. The desired testing endpoint was researched within 8 to 12 minutes of testing. The second week, the same protocol was repeated. On arriving at the laboratory, the test subjects, not wearing blindfolds, ingested either 250 ml of an energy drink containing caffeine (114 mg), taurine (1420 mg), and glucuronolactone (84.2 mg), sucrose, and glucose (39.1 g). Subjects were asked to ingest their drink at a convenient pace over a 4 minute period. After 15/20 minutes, the ECG was carried out again under physical stress, followed by the mental stress task and participants were then asked to rate their perceived stress using BAI and BDI tests. The study was conducted in accordance with the latest version of the Declaration of Helsinki.

Statistical analysis

The data were processed using an Excel Workbook (Microsoft Office), and analyzed using XLSTAT Software [19]. The Student t-test was used to analyze possible relationship between the data recorded before and after the Energy drinks consumption, and statistical significance was established at $p<0.05$. The t-Student test was chosen, among other non-parametric tests such as Wilcoxon-Mann-Whitney (WMW), which are frequently applied in medical statistics to compare the outcomes of a treatment, because the WMW has a very high probability of rejection of the null hypothesis. This happens when the means or medians are equal [20, 21].

Results

Psychological Tests

After a mental task, subjects were asked to rate their perceived stress rate using BAI and BDI tests.

BAI test. Before the consumption of energy drinks, subjects with a raw score between 0 (10 percentiles) and 7 (60 percentiles) were considered in the range of "minimal level of anxiety", they were considered on average and do not represent a particular clinical significance.

Only two cases presented a raw score between 8 (70 percentiles) and 15 (80 percentiles), meaning that they were in the range showing a "mild level of anxiety"; it is significant that these two cases were women, who usually have higher anxiety levels than men.

After drinking the energy drink, scores remained the same or decreased; only one measured 11 (80 percentiles), meaning a "mild level of anxiety" (Fig. 1).

BDI test. Before drinking energy drinks, the total scores in percentiles were less than 85, were considered on average and without a particular clinical significance: the subjects did not report a level of depression with a clinical significance (Fig. 2).

Percentiles related to somatic-affective factors all come under 85, so the subjects did not present significant symptoms of depression.

Cognitive factors were all under 85 percent (no clinical significance) except in four cases: three were 90, meaning the subject presents cognitive manifestations bordering on pathological aspects; the other was 97, indicating a particular difficult situation with cognitive symptoms of depression (that only in some cases can be considered serious).

Furthermore, in the test after drinking EDs, the total scores in percentiles were under 85%, so they not play a clinical significance.

For the somatic-affective factors, we found only one case measuring 90% (so the score was higher than before). This case has to be considered bordering on pathological.

Fig. 1. Score in percentiles of BAI before and after drinking EDs.

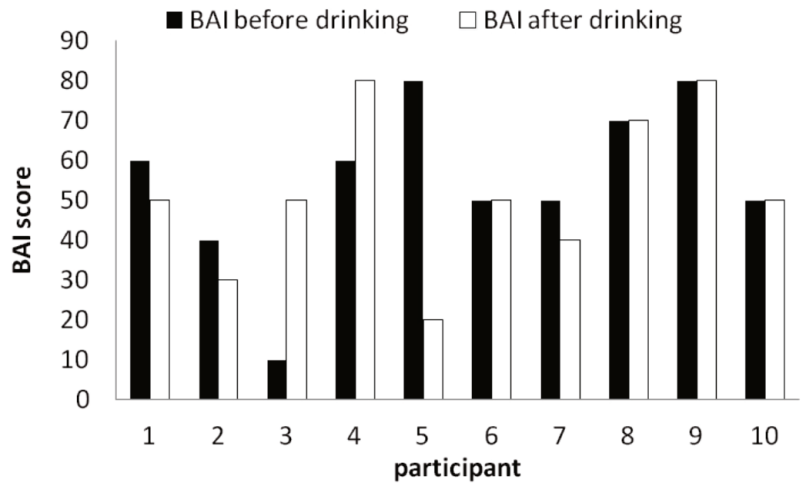
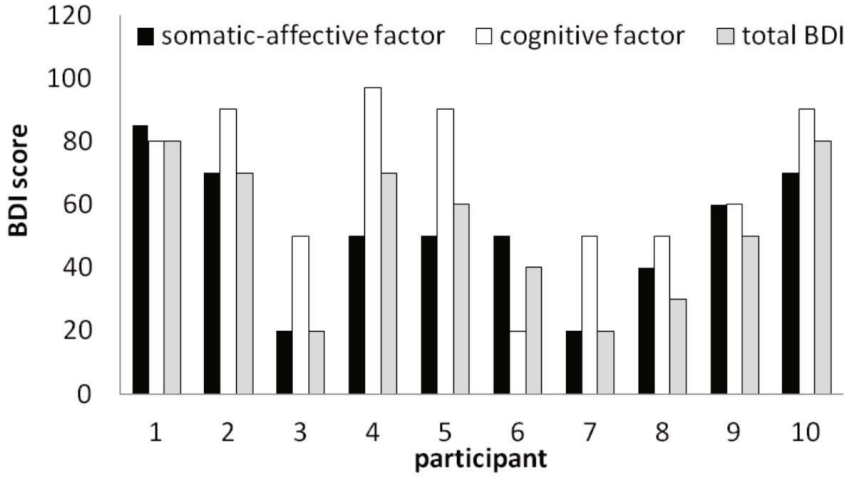


Fig. 2. BDI score before drinking an energy drink: black is the somatic-affective factor, white is the cognitive factor and grey is the total BDI.

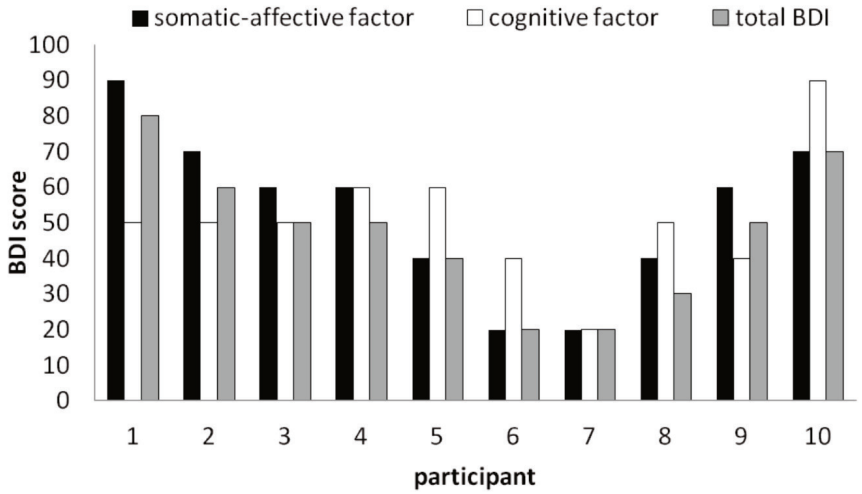


The comparison between the BDI-II of somatic-affective sphere, before and after the EDs intake, showed a t-Student value ($t(18) = 1.77$, $p = 0.09$) that, even if not statistically significant, represents a medical relevance.
Scores for the cognitive factors decreased significantly and only one of the subjects presented values greater than 85 percentiles (the cut-off point) (Fig. 3).
Statistical analysis (t Student), applied to the psychological test, showed a statistically significant by comparing the BDI-II values of the cognitive sphere before and after the intake of EDs ($t(18) = 2.1$, $p = 0.05$).

Physical tests

The physical test was conducted only with 8 participants because two were ill. The effects of energy drinks appear to be largely on cardiac parameters (Tab. I); in fact in the conditions compared, a small increase in the maximum heart rate was verified with the intake of an energetic beverage.
In the post-workout phase, an efficient background is demonstrated by evaluating recovery at the third minute, which is a critical time, of the heart rate. A rapid drop in the heart rate is seen when it drops below 120 bpm, as index of an high vagal tone and therefore associated with good physical performance and health status.
For trained subjects, recovery time is good because is achieved in a short time after exercise, after which the heart rate returns near the baseline; instead, untrained subjects reached maximal heart rate during the exercise period and leading to a slow recovery.
As is possible to appreciate in Table I, our results show the second and fifth female and the last male showed a fast recovery phase, while other participants displayed no great discrepancy between both conditions.
For resting subjects, HRR (Hearth Rate Recovery) was lower, meaning that their performance corresponds to an untrained physical state. For each individual, a parallel observation about cardiovascular changes procured with or without ingesting energy drinks is reported below. In the following results, which have been listed progressively, females are shown first, followed by males.

Fig. 3. BDI score after drinking an energy drinks: black is the somatic-affective factor, white is the cognitive factor and grey is the total BDI.



Females.

- Pre-exercise: different heart rate was noted, slightly higher after energy drink ingestion. Exercise: stable condition in both states (with and without energy drink). Post-exercise: weak recovery period, a little stronger after ingesting the energy drink, highlighted by a slow achievement of baseline blood pressure.
- Pre-exercise: a stable setting for both conditions. Exercise: prominent increase of heart rate after ingesting the energy drink, during the lead-up period. Post-exercise: fast recovery for both, but more marked with the energy drink.
- Pre-exercise: equilibrium between the two conditions. Exercise: no divergence, only slight anomalies observed. Post-exercise: very slow recovery.
- Pre-exercise: no differences were observed for both conditions because an identical heart rate was maintained. Exercise: in the first phase we observed an increase in heart rate after the energy drink was ingested, which then remained unchanged. Post-exercise: recovery is moderate.
- Pre-exercise: flattening of the heart rate for both states. Exercise: energy drink ingestion initially produces an increase of the heart rate, compared to when EDs were not consumed. Post-exercise: faster recovery is noted without the intake of energy drinks, through a strong reduction of heart beat noted near the baseline at the third minute.

Males.

- Pre-exercise: a higher heart rate is observed on ingestion of the energy drink. Exercise: a higher heart rate remains constant for the whole period thanks to the energy drink. Post-exercise: a quick drop with energy drink and modest recovery compared to the other state, in which the heart rate is higher. Basically, the overall recovery phase was weak.
- Pre-exercise: a small increase in heart rate is observed without the ingestion of energy drinks. Exercise: the entire phase is characterized by a flattening of heart activity. Post-exercise: in the energy drink state, the recovery phase was faster than in the other condition, because the heart rate decreased, but being

Tab. I. The maximum heart rate before and after EDs drinking and the Heart rate recovery (25 watts) before and after EDs drinking, at the third minute.

Participants	MHB* before EDs (heart beats)	MHB* after EDs (heart beats)	HRR** before EDs (heart beats)	HRR** after EDs (heart beats)
Females				
1 (trained)	185	187	125	129
2 (trained)	166	164	107	94
3 (no trained)	185	187	129	142
4 (no trained)	178	176	109	112
5 (no trained)	174	174	87	89
Males				
6 (no trained)	176	168	137	129
7 (no trained)	200	194	144	133
8 (trained)	174	185	111	115

* = MHB maximum heart rate; ** = Hearth Rate Recovery.

higher compared to the baseline, the entire recovery was sluggish.
- Pre-exercise: phase of equilibrium in which the maximum heart rate is kept stable during both states. Exercise: there was a brief increase of the heart rate in the first time period. Post-exercise: moderate recovery without any changes in heart rate at the third minute.

Discussion

Physical stress. Judging from outcomes obtained in this study, energy drinks are able to provide an additional burst of energy in a short-time period, more noticeable in trained subjects than untrained. For this reason, to achieve benefits from the intake of an energy drink, short and low-intensity activities are most favored. On the basis of the full results obtained from this investigation we can appreciate how energy drinks are able to stimulate the cardiovascular system for a short time after their intake [22], leading to an initial increase in heart rate, which then drops and remains constant the second time around. This has been observed in particular for physically trained subjects; in fact, the beverage provides a larger energy boost in the first instant of exercise because metabolic processes occur in a brief stretch of time. The sports activities most suited to exploiting the effects energy drinks claim to give are short term and high intensity strength activities. In many cases, before an event or during training, serious adverse effects can arise such as restlessness and irritability, an excessive increase of blood pressure and dehydration problems. Medical illnesses, especially underlying heart problems, have to be checked with physicians before using energy beverages, because their ingestion may exacerbate these conditions.
Psychological stress. Regarding the BAI test, as in a previous study, during our experimental phase we found that the energy drinks gives significant improvements in mental performance including choice reaction time, concentration (number cancellation) and memory (immediate recall), which reflected increased subjective alertness. These consistent and wide-ranging improvements in performance are interpreted as reflecting the effects of the combination of ingredients [23]. However, there are also articles that contradict this thesis [22], so this hypothesis needs to be further investigated with studies and research. Considering BDI test, after taking the energy drinks, anxiety levels decreased or remained unchanged; only in two cases did they increase and this might depend on the tolerance that individuals develop to caffeine. The intake of energy drinks has also caused an increase in somatic-affective factors, decreasing cognitive ones and the perception of depression. Probably this kind of "tolerance" is due to the fact that, nowadays, modern society is accustomed to "fast, excited" states, and a small dose (1 can) of an energy drink no longer affects the psyche of our subjects. The fact that in some cases anxiety and depression decrease after drinking EDs makes us also think that those feelings of mild dysphoria, mood-swings and anxiety were due to not knowing what would happen in the experiment; in fact, in the second phase of the experiment, they returned to normal levels. Energy drinks are known to improve mental performance, for example concentration and memory. Three studies published in two articles by Kennedy and Scholey demonstrate the positive effects of energy drinks on cognitive performance [25, 26]. In our case, anxiety and depression decreased after the ingestion of energy drinks, but they are the most common psychological side effects of energy drink abuse: probably it is an initial effect that will then change; for example, alcohol, cannabis, and other substances people abuse also initially give an illusion of wellness, and when doses are increased, these effects take a turn for the worse (drowsiness, stomach pain, nausea, vomiting etc.) [27, 28]. Precisely this apparently positive effect, may lead the user to increase doses of these substances, with the risk of incurring negative effects and posing a risk to health. Our result seems to agree with what is written in literature [25, 26, 29, 30].

Conclusions

Energy drinks are emerging as a public health threat and, increasingly consumed by youth internationally, are often marketed through youth-oriented media and venues [31-33]. Another crucial issue is the communication of health benefits to consumers in order to provide the knowledge necessary for them to make an informed choice. On this question, a strong policy that contrasts the excessive consumption of energy drinks among young people does not yet exist. Furthermore, alterations in mood, such as stress, anxiety and depression, are factors that have to be seen as if they were an alarm bell. The stimulating effect of EDs on the cardiovascular system in relation with physical activity must be checked and studied in deeper detail, because it may represent a risk for the health of young athletes, at times exacerbating congenital heart diseases. In accordance with other pilot studies, the authors aim to verify the results of this pilot study applying the same experimental protocol to a larger group of young people [34, 35].

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All the authors declare that they have no conflict of interest.

Authors' contributions

FP: conceived and coordinate the study, evaluated the results and wrote the manuscript. IG: coordinated and contributed to the manuscript writing. DE: contributed to the recruitment of the participants, the acquisition of epidemiological data. PP: contributed to the recruitment of the participants. GB: contributed to the recruitment of the participants, supervision during the physical test. PC: contributed to critically revised the manuscript. LK: contributed to critically revised the manuscript. SS: conceived and contributed to the supervision of the study, evaluated the results.

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ORIGINAL ARTICLE

Effects of anticoagulation therapy with vitamin K antagonists on hospitalizations and emergency room accesses in Grosseto (Italy)

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Keywords

Warfarin • Hospitalization • Italy

Summary

Introduction. *A lot of drug groups are associated with preventable drug-related admissions. Coumarin derivatives, prescribed for the treatment and prevention of deep vein thrombosis or pulmonary embolism or prevention of systemic embolism or stroke in patients with prosthetic heart valves or atrial fibrillation, are often associated with bleeding. The aim of our study was to analyze how the anticoagulant therapy with VKAs could affects the hospitalizations and the visits to emergency room in the elderly population (> 65 years old).*
Methods. *In 2013 we conducted a cross sectional study analyzing the database of all pharmaceutical prescriptions, selecting patients living in Grosseto (Italy), which received at least two prescriptions of coumarin derivatives in 2012. We analyzed the admissions to hospital and the accesses to the emergency rooms (ERs) made by each patient, focusing especially on those related to bleeding. For each access to ER we recorded the date, time of stay, diagnosis and outcome. For each hospitalization the information we recorded were the date of admission and discharge diagnosis.*
Results. *3684 patients were included in our study. 261 (7.1%) patients visited the emergency room for bleeding; 37 (1%) for intracranial bleeding. The accesses made by men were higher than those made by women. The average time of stay in ER was 349 minutes. The admissions to hospital were 96 (2.6%); 42 (1.1%) were admitted to hospital with a diagnosis of major vascular event. 53 patients (20.3%), accessed to the ER more than one time. The 11.5% was admitted to the hospital more than one time.*
Conclusions. *Our study showed that VKAs are responsible of an increase of the accesses to ER and of the admissions to hospital. However, it would be interesting to enlarge the sample size including patients living in other provinces or in other regions, with a lower age and treated also with TSOACs, in order to evaluate the real cost-effectiveness of anticoagulant therapy.*

Introduction

The World Health Organization has defined pharmacovigilance as "the science and activities relating to the detection, assessment, understanding, and prevention of adverse effects or any other drug-related problems" [1]. Such "drug-related problems" include adverse drug reactions (ADRs), unintended injuries or complications that arise from iatrogenic drug related causes and which cause or prolong hospital admission and result in disability or death [2-4]. The risk of ADRs is necessarily an inherent risk of all drug therapy and is modulated by several factors, including dose and frequency of administration, genotype, and pharmacokinetic characteristics of special populations, such as pediatric and geriatric patients and those with hepatic or renal impairment. Due to the high frequency and potentially serious consequences, ADRs may have a dramatic impact in clinical practice both from a clinical and economic perspective [5]. As described by Formenti et al. in their study [6] the onset of adverse reactions (ADRs) represents a sentinel event and, in addition to diminishing the quality of life of the patient increases the number of medical visits, hospitalizations and even the deaths, resulting in an overall increase in health care costs. Containing the number of ADRs, therefore, would result in a cost reduction for the ADRs treatment, diagnostic examinations and differential diagnosis analysis [7-9].
Estimates from France suggest that up to 123,000 patients a year present to their general practitioner with an ADRs [10]. ADRs account for 4.2-30% of hospital admissions in the USA and Canada, 5.7-18.8% of admissions in Australia, and 2.5-10.6% of admissions in Europe [8].
A national study from the USA estimated that 11.4-35.5% of emergency department visits in older adults are due to drug-related causes [5, 11].
Howard et al. [8] showed in their systematic review that the drug groups most frequently associated with all types of preventable drug-related admissions were antiplatelets, diuretics, nonsteroidal anti-inflammatory drugs (NSAIDs) and anticoagulants.
Coumarin derivatives, such as warfarin, acenocoumarol and phenprocoumon are prescribed for different indications such as treatment and prevention of deep vein thrombosis or pulmonary embolism or prevention of

systemic embolism or stroke in patients with prosthetic heart valves or atrial fibrillation [12]. Because warfarin and other coumarin derivatives inhibit the vitamin K dependent synthesis of biologically active clotting factors, they are also called vitamin K antagonists (VKAs). The main adverse effect associated with coumarin derivatives is bleeding. Several studies have shown that the incidence of major bleeding in patients on warfarin ranges from 0.4%-7.2% per year [13]. Minor bleeding rates can be as high as 15.4% per year [14]. Fatal bleeding events occur at rates of 1.3 per 100 patient-years, according to a meta-analysis of 33 studies [15].
However in different studies is not always used the same definitions, methods, and consequently, the results vary a lot; moreover the follow-up time is variable and often the studies focus only on a category of patients. The aim of our study was to analyze the impact of the anticoagulant therapy with VKAs in the elderly population – over 65 years old, defined according to the World Health Organization definition of "elderly person" [16, 17] –, through the hospitalizations and the visits to emergency room, using only current data flows. This kind of analysis let us to analyze this phenomenon in a "real setting", in order to quantify the impact of the side effects in a not selected sample, with co-morbidities, with advanced age and with possible problems of compliance with therapy. This is particularly interesting for anticoagulants which are often used in old people with a lot of pathologies, and often living in not adequate domestic environments.

Methods

In 2013 we conducted a cross sectional study in the Demographic and Epidemiological System of the AUSL Toscana Sud Est. We analyzed the database of all pharmaceutical prescriptions: this is a database containing all the information about the loanable pharmaceutical prescriptions, derived from the flows directed to the National Health System.
We selected patients living in Grosseto – Italy, total resident population: 223,652 [18] –, which received at least two prescriptions of VKAs in 2012. This selection was chosen in order to identify patients subjected to long term therapy with anticoagulants. We collected information about age, gender, type of medication, city of residence. Then, through a unique identification code, we identified and analyzed the admissions to hospital (we considered the admissions to all Italian hospitals) and the accesses to the emergency rooms (ER – we considered the ERs of Grosseto, Castel del Piano, Orbetello, Pitigliano, Massa Marittima –) made by each patient. These data derived from the archives containing the flows of the hospital discharge records and accesses to ER, sent to the Region and then to the National Health System. We focused especially on the group of accesses to ER and hospitalizations related to bleeding, occurred within 4 months after the prescription of the anticoagulant. In fact, the daily dose of this kind of therapy varies a lot and could be interrupted for surgery (even for minor surgery). So we estimated, by default, the maximum time between two prescriptions (sometimes 2 packs for each prescription) in 4 months: so considered the time span was from January 2012 to April 2013.
For each hospitalization the information we recorded were the date of admission and discharge diagnosis. In the hospital discharge records all the diagnoses are codified with the code ICD IX, so we considered all the diagnoses containing the words "hemorrhage" and its derivatives and "anemia" and its derivatives.
For each access to ER we recorded the date, time of stay, diagnosis and outcome. Diagnoses related to the accesses to the ER are not codified using ICD IX, so we considered all the diagnoses related to bleedings in progress, previous or chronic.
The results were organized in a database and then exported for statistical analysis. The collected data were processed using the software Stata® SE, version 12.1 (StataCorp, College Station, Texas, USA). The level of significance was set at $p < 0.05$.

Results

From the pharmaceutical prescriptions database we extracted 4368 patients who received at least two prescriptions of VKAs in 2012; 2304 (52.74%) were men.
3684 patients (84.34% of the entire sample) were > 65 years old, 1889 were men (51.3%). The average age was 78.2 years (79.2 for women CI 95% 78.9-79.5; and 77.2 for men CI 95% 76.9-77.5).

Accesses to Emergency Rooms

261 (7.1%) patients visited the emergency room for bleeding; 37 (1%) for intracranial bleeding. Patients who accessed to ER were represented especially by men (7.9%): women were the 6.2%, and this difference was statistically different ($p < 0.05$). The average time of stay in ER was 349 minutes (CI 95% 296.1-402.2); for women 385 minutes (CI 95% 295.0-476.4); for men 325 minutes (CI 95% 260.5-389.8).
Table I shows the principal diagnoses recorded during the visits to ER.

Admissions to hospital

The admissions to hospital were 96 (2.6%); 42 (1.1%) were admitted to hospital with a diagnosis of major vas-

Tab. I. Accesses to ER.

Diagnosis	Percentage (%)
Epistaxis	24
Cerebral hemorrhage	11
Anemia	14
Conjunctival hemorrhage	9
Hematuria	11
Rectorragy	6
Other bleeding	25

cular event. There is not any statistically significant difference in the admission to hospital or in the accesses to ER due to the prescribed medication (warfarin or acenocoumarol).
53 patients (20.3%), accessed to the ER more than one time. The 11.5% was admitted to the hospital more than one time.
Figure 1 shows the principal diagnoses recorded during the admissions to hospital in the considered time-span.

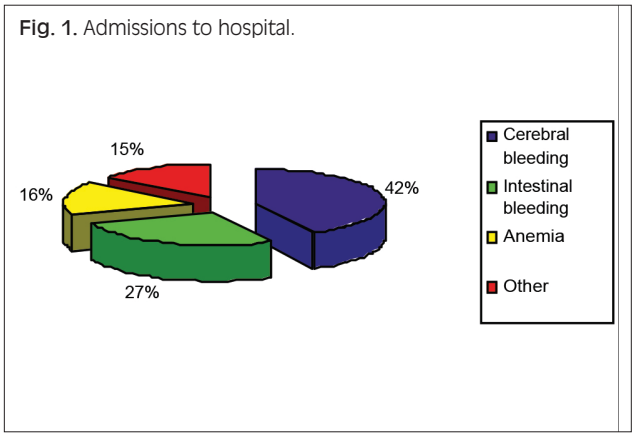

Fig. 1. Admissions to hospital.

Discussion and conclusions

The 84.34% of the sample analyzed in our study was > 65 years old, and was represented especially by men (51.3%) with an average age of 78.2 years. These data are partially in line with those found by Kim et al. in 2010: of the 2346 subjects enrolled in their study to evaluate the hospitalization costs associated with warfarin-related bleeding events, 1804 were females (77%) and about two-thirds of the subjects were over the age of 75 years (< 70 years: 12%, 71-75 years: 23%, 76-80 years: 28%, 81-85 years: 25%, ≥ 86 years: 12%) [19].
In our study the accesses to the emergency room for bleeding (7.1%) were higher than the admissions to hospital (2.6%). In a meta analysis conducted in 2012 [15] it was showed that during the initial 3 months of anticoagulation, the rate of major bleeding was 2.06% (CI 2.04% to 2.08%) and the rate of fatal bleeding was 0.37% (CI 0.36% to 0.38%). The rate of intracranial bleeding was 1.48% (CI 1.40% to 1.56%). Chai-Adisaksopha et al. in 2014 showed in their study that total bleeding occurred in 30.42% patients treated with vitamin K antagonists (VKAs), a lower percentage compared to the 24.86% of patients treated with Target-Specific Oral Anticoagulants (TSOACs); however, major gastrointestinal bleeding occurred in 2.09% of patients treated with TSOACs and 1.70% of patients treated with VKAs [20]. Dale et al. in fact showed that warfarin is associated with greater thrombin suppression in the brain and pathological thrombosis at sites of atherosclerotic plaque disruption [21].
The problem of bleeding after the therapy with VKAs is important for the costs that this phenomenon creates in the National Health System. Sameer R. Ghate et al. [22] showed that during the 12 months after the treatment there was a significantly higher increase of major gastrointestinal or intracranial bleeding event and consequently of hospitalizations, hospital days, and ER visits. In the 12 months after the warfarin start date, the mean adjusted annual costs obtained from the regression model were $42,574, $36,571, $22,824, and $22,507 for subjects with intracranial bleeding, major gastrointestinal bleeding, minor gastrointestinal bleeding, and no bleeding, respectively [22]. The mean length of stay was 7.8 days (SD: 7.1). For the entire cohort, warfarin-related bleeding resulted in an average increased cost of hospitalization of $508.30 per warfarin user [19].
The most important limit of our study is that the study sample is not representative of the Italian population: in fact our sample is composed by patients older than 65 years and treated with VKAs; furthermore, our sample is composed only by patients living in Grosseto Province. Our study is limited to 2013 because the organizational changes in the Local Health Units, led to the fusion of three Local Health Unit (Grosseto, Siena and Arezzo) in a unique Unit, with the following fusion of the databases, which is not totally finished yet. So the previous databases have not been implemented in the same way, therefore it is impossible to repeat the analysis with updated data, although we think that significant differences would not be found.
It could be interesting to complete our study enlarging the sample size, and enrolling patients living in other provinces or in other regions in Italy, with a lower age and treated also with TSOACs. We encourage other colleagues to conduct with us a multicentre study in order to identify significant differences that could influence the costs for the National Health System and evaluate the real cost-effectiveness of anticoagulant therapy. Moreover, it would be interesting to investigate the quality of life [23-25] of patients treated with the two classes of drugs and to evaluate the impact of these therapies on this non-economic aspect of healthcare.

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The authors declare that there is no conflict of interest.

Authors' contributions

FN had the idea for the article, collaborated in performing the study, carried out the data analysis and collaborate in writing the article.
GT collaborated in writing the article and helped to conceptualize ideas.
NN collaborated in writing the article and helped to conceptualize ideas.
SD helped to conceptualize ideas.
PP collaborated in writing the article and helped to conceptualize ideas.

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ORIGINAL ARTICLE

Knowledge, attitude and practice about leptospirosis prevention among town service workers in northeastern Malaysia: a cross sectional study

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Keywords

Town Service Workers • Leptospirosis • Knowledge • Attitude • Practice

Summary

Introduction. *Many efforts have been done to reduce leptospirosis infections in Malaysia especially among high risk groups including town service workers. Town service workers are more likely to be exposed to the leptospiral infection resulting from their occupational activities.*

Methods. *A cross sectional study was conducted in northeastern Malaysia involving 321 town service workers who were subjected to answer an interviewer-guided validated questionnaire which consists of sociodemographic, knowledge, attitude and practice information. Data were entered and analyzed using SPSS Version 20.*

Results. *All of the respondents were Malay with mean (SD) age of 40.6 (10.28) years old. The mean (SD) duration of employment was 12.1 (9.62) years. Fifty four respondents (16.8%) had never heard of leptospirosis. Among the respondents, 215 (67.0%) of them had poor knowledge on leptospirosis. Meanwhile, 167 (52.0%) and only 128 (39.9%) of them had satisfactory attitude and practice respectively. It was found that knowledge on risk factors for leptospirosis was lacking. There were high risk attitudes such as drinking habit and protective equipment used during working with the favourable answers ranged from 67.3% to 89.1%. The weakest area identified in their practice was also on the use of protective equipment.*

Conclusions. *The workers' level of knowledge and practice were relatively poor despite an overall good practice on leptospirosis. This finding might expose them to an increased risk of contracting leptospirosis. Identified weak areas in their knowledge, attitude and practice will assist the policy makers to develop a focused and well-directed intervention program on leptospirosis infection.*

Introduction

Leptospirosis is known to be the most widespread re-emerging zoonotic disease of global distribution affecting humans in tropical, subtropical and temperate zones. It was reported that Seychelles has the highest incidence of leptospirosis in the world with annual incidence of 432.1 per million population while the United States of America reported the lowest annual incidence of 0.1 per million population [1]. However, due to lack of surveillance, the exact number of human cases worldwide is not known [2].

Additionally, most countries in South East Asia region are endemic to the disease. Despite this, leptospirosis is still under-reported due to wide range of clinical presentations associated with acute leptospiral infection [3, 4]. In Malaysia, the data from the Ministry of Health showed that the prevalence of leptospirosis increased dramatically from 2004 to 2009 with the case fatality rates over the period varied from 1.8% to 7.6% with an average of 4.44% [5, 6].

Town service workers are more likely to be exposed to the leptospiral infection resulting from their occupational activities in solid waste management in every step of the process, from the point where residents handle wastes in the home for collection or recycling, to the point of ultimate disposal [7-11]. The seroprevalence of leptospirosis among town service workers in Malaysia was reported as ranging from 17.9% to 24.7% [11, 12]. A recent study by Azfar et al. (2017) reported a slight higher seroprevalence of leptospirosis among town service workers in Kelantan, Malaysia which was 25.5% [13].

In humans, leptospirosis can cause a wide range of symptoms ranging from mild (influenza like illness symptoms) to severe (Weils' syndrome) clinical manifestations. If it is not treated, progression of the disease will lead to complications such as renal failure, meningitis, liver damage, respiratory distress and widespread haemorrhage [14].

Humans may be exposed to leptospirosis through occupational, recreational, or environmental factors [4]. Occupations such as town service workers, paddy planters, army and health care workers impose higher risk of leptospiral infection due to presence of occupational and environmental determinants for human leptospirosis [12, 15, 16]. Town service workers are more likely to be exposed to the leptospiral infection resulting from their occupational activities in solid waste management in every step of the waste management process. Hence, this study was conducted to determine the knowledge,

attitude and practice levels towards leptospirosis among town service workers in northeastern Malaysia.

Methods

A cross sectional study involving 321 town service workers was carried out in northeastern Malaysia. Town service workers from four job categories namely garbage collector, town cleaner, landscaper and lorry driver or mechanic were selected for this study. Garbage collector collects garbage from residential, commercial and industrial area and dumps garbage from containers onto the designated truck for disposal at landfills. Town cleaner duties concerned with sweeping, collection and removal of litter, detritus and leaves from public spaces including roads, pavements, drains, wet market and public precincts. Meanwhile, landscaper performs a range of duties such as transporting and planting new vegetation, mulching, fertilizing, watering, as well as cut and trim grass using either manual hand tools or power-operated equipment. Lorry driver who drives lorry for garbage collection to the landfill may also assist garbage collectors to perform their job. On the other hand, lorry mechanics repair and do maintenance jobs including washing the lorries used for garbage collection.

Sample size was calculated based on 35% of satisfactory attitude on leptospirosis among town service workers [12] at 95% CI and considering the non-response rate of 10%, the estimated sample size required was 385. The sampling frame was the list of town service workers who work as any of the four job categories for more than six months to ensure that they were really engaged with the working activities. Those who did not work during the study period were excluded from the study. A simple random sampling was applied to select the respondents.

The respondents were subjected to answer an interviewer guided questionnaire which consisted of sociodemographic, knowledge, attitude and practice information. The validated questionnaire revealed a Cronbach alpha for knowledge, attitude and practice domain as 0.96, 0.71 and 0.74 respectively. It was designed to be completed within 15 minutes for an average respondent. The language used was Bahasa Malaysia which is the mother tongue of the workers. Only one researcher conducted the interview to prevent the problem of inter-interviewer variations. The interviewer would explain any technical terms that were unclear to respondents.

Knowledge section started with the question of whether the respondents had ever heard about leptospirosis and the source that they heard the information from. Only those who had heard of the disease would proceed to answer the rest of the knowledge questions. They were designed to be answered as "correct", "incorrect" and "do not know". For scoring, "2" marks were given for correct response, "1" mark for "do not know" and "0" mark for "incorrect" response. There were 24 knowledge questions which covered causes, signs, symptoms, complications, treatment, prevention and risk factors of leptospirosis.

For attitude section, there were 12 questions which covered safe work practices, personal protective equipment (PPE) and general practices. Questions on attitude are designed to be answered using a Likert scale of "strongly agree", "agree", "not sure", "not agree" and "strongly not agree". For positive attitude items, scores of "4", "3", "2", "1", and "0" for "strongly agree", "agree", "not sure", "not agree", and "strongly not agree" were given respectively. Meanwhile, the above scoring system was reversed for negative attitude items.

The questions on practice domain were also designed to be answered using a Likert scale of "never", "seldom", "sometimes", "often" and "always". For good practice items, scores of "4", "3", "2", "1", and "0" for "always", "often", "sometimes", "seldom", and "never", were given respectively. For bad practice items, the above scoring system was reversed. A total of 14 questions on practices were asked containing questions on safe work practice, the use of PPE during work and off work general practices.

Ethical clearance was obtained from Research and Ethic Committee (Human), Universiti Sains Malaysia, Health Campus (Reference No: USMKK / PPSP / JEPeM [261.3(7)]). All respondents were explained about the study and consent were obtained before conducting the study. Confidentiality of the data were kept throughout the study.

Data were entered and analyzed using SPSS Version 20.0 [17]. All continuous variables were described using mean (SD) whereas for categorical variables, frequencies and percentages were presented. The mean (SD) for each item of the KAP was also analyzed. The proportion of respondents who gave the correct answer for each item in the knowledge domain was expressed as a correct percentage. The proportions for positive attitude and good practice for each item of the KAP were also displayed. Those who answered "strongly agree" or "agree" for the attitude that they should have and "disagree" or "strongly disagree" for the attitude that they should not have are considered as having positive attitude. The proportions for good practice include those who answered "always" or "often" for the practice that they should adopt and "never" or "seldom" for the practice that they should avoid.

The scores for knowledge, attitude and practice were transformed into percentage scores by dividing the scores obtained by the respondents with the possible maximum scores and multiplied by 100. The categories of knowledge, attitude and practice scores were decided by consensus among the researchers. For knowledge category, the respondents who had never heard of leptospirosis were considered to have "poor knowledge". Those who scored less than 72% were considered to have "moderate knowledge" and those who scored 72% or more were considered to have "good knowledge". The difference between "moderate" and "good" knowledge depended on the mean percentage of total knowledge score among those who had "ever heard of the disease".

Considering the maximum possible score of four points for each item in the attitude and practice domains, the total maximum scores for attitude and practice domain

were 48 and 56 respectively. Allowing the minimum average of three points for each item, a total score of less than 36 (3 points x 12 items) out of 48 indicates unsatisfactory attitude while a total score of less than 42 (3 points x 14 items) out of 56 indicates unsatisfactory practice. Hence, those with a score of less than 75% were considered unsatisfactory whereas a score of 75% or more were considered as satisfactory for both attitude and practice domains.

Results

A total of 321 out of 385 workers who were eligible for this study were recruited giving a response rate of 83.4%. All respondents were Malay with mean (SD) age of 40.6 (10.28) years old and it ranged from 20 to 68 years old. Majority of the respondents were married (83.5%) with mean (SD) number of children was 3.7 (2.49). For level of education, a slightly higher proportion (51.4%) of the respondents who had undergone at least upper secondary school level as compared to those who had undergone up to lower secondary school (48.6%). The mean (SD) duration of employment was 12.1 (9.62) years and it ranged from 1-37 years.

Table I displays the descriptive statistics of domains and sub domains of the KAP on leptospirosis. Only data from 267 workers who had ever heard about leptospirosis were analyzed for knowledge domain. Whereas for attitude and practice domain, they were analyzed for all recruited respondents. Total mean (SD) percentage score for knowledge, attitude and practice were 69.3 (9.95), 73.3 (13.61) and 70.4 (14.81) respectively. In knowledge sub domain, the lowest mean percentage score was on "risk factors for leptospirosis" whereas the mean percentage score for attitude domain was relatively similar. For practice domain, the mean (SD) percentage score on "PPE" was low [55.9 (30.11)] as compared to "safe work practice" and "general practice" sub domains.

Table II shows the category of knowledge, attitude and practice on leptospirosis prevention among the respondents. There were 54 workers (16.8%) had never heard about leptospirosis. For those who ever heard about leptospirosis, 116 (43.4%) of them had heard about leptospirosis through television or radio, 85 (31.8%) heard through newspaper or magazine, 50 (18.7%) heard through health education, doctor's consultation or poster/pamphlet about leptospirosis and 16 (6.0%) of them heard from other source such as neighbour's experience. Tables III, IV and V show the mean score for each item and percentage of workers who were able to "answer correctly" for knowledge questions, had "positive attitude" and practiced "good practice" on leptospirosis prevention.

Discussion

Knowledge, attitude and practice (KAP) questionnaires have been used widely in public health studies as a tool to gather information on knowledge and attitude on certain subjects and to assess the practice on the matter among those at risk. Exploration of KAP in population of interest will facilitate health professionals in understanding the workers' barrier to action and enabling factors that helps the target population to adopt recommended preventive actions [18-21].

In the present study, there was a high percentage of respondents who ever heard about leptospirosis (83.2%). This finding was almost similar to a study in Brazil among urban slum residents whereby only less than 10% of the respondents had never heard of leptospirosis [22]. Similarly, in a study among the butchers and their assistant in slaughterhouses, about 78% of them had heard of the disease [23]. In an earlier study among canoeist in North Wales also reported a very high percentage (95%) of respondents who had heard of leptospirosis and it was largely due to British Canoe Union active roles in conducting health promotion program in the high risk group [24]. Conversely, a study among town service workers in 2008 found that only about 13% of the respondents had heard about leptospirosis [20].

Most of the respondents who had heard about leptospirosis, heard it through television or radio while about a third of them heard about it from newspaper or magazine. This findings was similar to other studies on leptospirosis in Malaysia and the probable reason were due to the continuous effort by the Malaysian government

Tab. I. Descriptive statistics of domain and sub domain percentage KAP scores among respondents.

Domain Sub domain	Min % score	Max % score	Mean (SD) % score
Total knowledge score among ever heard (n = 267)	**37.5**	**91.7**	**69.3 (9.95)**
Causes	25.0	100.0	73.8 (15.08)
Signs, symptoms and complication	18.8	100.0	59.9 (13.02)
Treatment and Prevention	33.3	100.0	88.5 (14.94)
Risk factors	0	100.0	52.8 (30.02)
Total attitude (n = 321)	**31.3**	**100.0**	**73.3 (13.61)**
Safe work practice and PPE	25.0	100.0	73.5 (17.88)
General practices (off work)	20.0	100.0	73.0 (15.50)
Total practice (n = 321)	**30.4**	**100.0**	**70.4 (14.81)**
Safe work practice	0	100.0	71.7 (19.64)
Personal protective equipment	0	100.0	55.9 (30.11)
General practices (off work)	35.0	100.0	84.0 (15.39)

Tab. II. Category of knowledge, attitude and practice score among town service workers.

Category	Frequency	%
Knowledge		
Good (score ≥ 72%)	106	33.0
Moderate (score < 72%)	161	50.2
Poor (never heard)	54	16.8
Attitude		
Satisfactory (score ≥ 75%)	167	52.0
Unsatisfactory (score < 75%)	154	48.0
Practice		
Satisfactory (score ≥ 75%)	128	39.9
Unsatisfactory (score < 75%)	193	60.1

through its Ministry of Health as well by the active roles of printed or electronic media in public health promotion and education [20, 21]. In addition, when there was an outbreak of leptospirosis, reports in local news and media will be more extensive. Since leptospirosis was included as notifiable disease in 2011, there was a better surveillance of the disease and specific health promotion program for leptospirosis being carried out [5, 6]. These factors might contribute to a higher percentage of respondents who ever heard of leptospirosis in the present study.

Consistently, those who had heard about leptospirosis got the information mostly from the media and public health inspectors [23]. In addition, a study among 460 Sri Lankan government school students aged from 12 to 16 years old reported that 100% of the them had heard of leptospirosis. It was found that the most common source of information regarding leptospirosis were television followed by school, newspapers, the medical officer of health or public health inspector, banners and posters and lastly from educational programs [25].

In the present study, 161 from 267 respondents who had heard of leptospirosis were categorized as having moderate knowledge (about 50% of the total 321 respondents) and 33% (106 of 321 respondents) had good knowledge on leptospirosis. In contrast, a study in 2008 among similar target group found that only about 13% of 296 respondents had heard of leptospirosis [26]. In a study among 300 villagers in highly endemic area in Thailand also found that majority of respondents had poor knowledge (80%) meanwhile those with moderate and good knowledge were 11% and 9% respectively [27].

In the present study, sub domain for "risk factors" was identified as the weakest area in which few of the respondents scored zero percent. Knowledge on risk factors for leptospirosis is very crucial as it will increase awareness which will encourage themselves to apply

Tab. III. Knowledge items with mean (SD) score and percentage (%) of correct answers (N = 267).

Knowledge items	Mean (SD)	Correct N (%)
Causes		
"Leptospirosis is a disease caused by microorganism"	1.8 (0.48)	208 (77.9)
"It is a zoonotic disease"	1.8 (0.54)	214 (80.1)
"Leptospirosis can enter our body through cuts"	1.4 (0.75)	139 (52.1)
"Leptospirosis can enter our body through contaminated food"	1.7 (0.61)	209 (78.3)
"Leptospirosis can be transmitted through mosquito bites"	1.0 (0.85)	93 (34.8)
"Human can be infected by shaking hands with infected persons"	1.3 (0.74)	127 (47.6)
Signs, symptoms and complications		
"Infected person may have myalgia"	1.5 (0.65)	151 (56.6)
"Infected person may have jaundice"	1.1 (0.76)	93 (34.8)
"Infected person may free from any symptom"	0.8 (0.71)	43 (16.1)
"It can cause death"	1.8 (0.45)	228 (85.4)
"It can cause lung cancer"	0.7 (0.65)	29 (10.9)
"It can cause kidney failure"	1.3 (0.68)	110 (41.2)
"It can cause liver damage"	1.3 (0.68)	107 (40.1)
"It can cause diabetes"	1.1 (0.73)	91 (34.1)
Risk factors		
"Eat while working is a risk to get leptospirosis"	1.1 (0.87)	115 (43.1)
"Drink while working is a risk to get leptospirosis"	1.1 (0.86)	114 (42.7)
"Smoke while working is a risk to get leptospirosis"	0.9 (0.85)	81 (30.3)
"Town service workers is not considered as a risk group"	1.1 (0.85)	117 (43.8)
Treatment and prevention		
"The disease is treatable"	1.7 (0.52)	197 (73.8)
"The disease can be detected by blood investigation"	1.8 (0.44)	222 (83.1)
"The disease can be prevented by taking bath after working"	1.8 (0.56)	225 (84.3)
"The disease can be prevented by maintaining house compound cleanliness"	1.9 (0.31)	252 (94.4)
"The disease can be prevented by avoiding walking through flood"	1.6 (0.66)	194 (72.7)
"Wearing rubber gloves during work can prevent leptospirosis"	1.8 (0.52)	219 (82.0)

Tab. IV. Attitude items with mean score (SD) and percentage (%) for positive attitude (N = 321).

Attitude items	Mean (SD)	Positive attitude N (%) [a]
Safe work practice and PPE		
"Drink while working is not a problem"	2.17 (1.44)	143 (44.6)
"I need a "safe work practice" course in order to prevent from getting the disease"	3.24 (0.76)	291 (90.6)
"Rubber gloves is important equipment during working"	3.23 (0.97)	286 (89.1)
"Wearing gloves during working is troublesome"	2.96 (1.32)	228 (71.0)
"Wearing gloves during working make our work slower"	3.02 (1.323)	229 (71.4)
"Wearing gloves during working make me feel discomfort"	2.84 (1.37)	216 (67.3)
"Wearing boots make our work slower"	3.12 (1.24)	237 (73.9)
General practice (off work)		
"I must know about leptospirosis"	3.14 (1.01)	281 (87.6)
"I don't mind to wear any type of shoe"	1.89 (1.43)	115 (35.8)
"I should make sure that my house is free from rats"	3.31 (0.88)	297 (92.5)
"I don't mind if the dustbin in my house had no cover"	3.37 (1.18)	271 (84.4)
"I don't feel worry walking through flood"	2.90 (1.35)	219 (68.2)

a = proportion positive attitude who answered "strongly agree" or "agree" for attitude that they should have and "strongly disagree" or "disagree" for attitude that they should not have.

Tab. V. Practice items with mean (SD) score and percentage (%) for good practice (n = 321).

Practice items	Mean (SD)	Good practice n (%) [a]
Safe work practice		
"I eat while working"	3.02 (1.13)	219 (68.2)
"I drink while working"	2.75 (1.25)	201 (62.6)
"I smoke while working"	3.17 (1.21)	237 (73.8)
"Reminding my colleague to follow the working procedure"	2.52 (1.43)	170 (52.9)
Personal protective equipment		
"I'm wearing rubber gloves during working"	2.10 (1.69)	139 (43.3)
"I'm wearing boots during working"	2.37 (1.66)	164 (51.1)
"I'm wearing long sleeves shirt during working"	3.05 (1.46)	228 (71.0)
"I'm wearing mask during working"	1.12 (1.54)	63 (19.7)
"I will make sure the glove is in good condition before use it"	2.55 (1.66)	191 (59.5)
General practice (off work)		
"I'll make sure my house is free from rats"	3.05 (1.28)	222 (69.1)
"I walk through flood"	2.91 (1.16)	220 (68.6)
"I cover the food"	3.73 (0.77)	300 (93.4)
"I'm looking after the goat after working hour"	3.57 (1.16)	283 (88.2)
"I'm looking after the cattle after working hour"	3.54 (1.20)	282 (87.8)

[a] = proportion good practice who answered "always" or "often" for practice that they should adopt and "never" or "seldom" for practice that they should avoid.

good work practices that may protect them from leptospiral infection. Knowledge score on "causes" and "treatment and prevention" subdomains were relatively better compared to the "risk factors". This may suggest that they knew leptospirosis is caused by a microorganism in rat's urine and the disease is treatable but had no or little knowledge on the factors that exposes them to the disease.

About 84% of the respondents did not know that infected person may be asymptomatic. It strengthened the fact that most of the people are not aware of having the disease as infected person may be asymptomatic. A national Sri Lankan household survey in 2009 involving 601 participants also reported lack of knowledge on clinical features of leptospirosis [28]. Knowledge on sign, symptoms and complications are important as treatment at early stage of the disease may prevent the disease progression to severe stage that may result in poorer outcome. These findings were almost similar to study done among town service workers and among army personnel in Kelantan [21, 26].

In the present study, it was found that there was a slightly higher proportion of those with satisfactory (about 52%) than unsatisfactory attitude. The important area that were identified as considering higher risk attitude while respondents were not at work were "wearing any type of shoes" and "walking in flood" as only 35.8% and 68.2% gave favourable answers respectively. For at-

titude at workplace, it was found that high risk attitude during working like drinking habit and PPE usage were quite alarming. These findings were consistent with the low score in the "risk factors" subdomain in the knowledge domain.

Regarding practice on leptospirosis prevention, most of the respondents were categorized as having unsatisfactory practice. This could be due to their inability to perceive the benefits of safe work practice and compliance to use PPE during working. The weak areas were on "PPE", "safe work practice" and "general practice (off work)" subdomains. Items in the "PPE" subdomain showed that low percentage of them were wearing boots (51%), rubber gloves (43%) and mask (20%) while working. These findings were almost similar to the study among town service workers [26].

In conclusion, the level of knowledge and practice were relatively poor despite an overall good practice on leptospirosis. This finding might expose them to an increased risk of contracting leptospirosis. Identified weak areas in their knowledge, attitude and practice will assist the policy makers to develop a focused and well-directed intervention program on leptospirosis infection.

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Authors' contributions

ZMA, SMN and BDA conceived, designed, coordinated and supervised the research project including data collection. AMR, OM, WMZ and YNA performed the data quality control, performed the statistical analyses and evaluated the results. All authors involved in the writing and revising the manuscript, gave their contribution to improve the paper and approved the final manuscript.

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ORIGINAL ARTICLE

Overview of Japanese encephalitis disease and its prevention. Focus on IC51 vaccine (IXIARO®)

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Keywords

Japanese encephalitis disease • Japanese encephalitis virus • IC51 vaccine (IXIARO®) • Travel medicine

Summary

Japanese encephalitis (JE) is a vector-borne disease caused by the Japanese encephalitis virus (JEV). JEV is transmitted by mosquitoes to a wide range of vertebrate hosts, including birds and mammals. Domestic animals, especially pigs, are generally implicated as reservoirs of the virus, while humans are not part of the natural transmission cycle and cannot pass the virus to other hosts. Although JEV infection is very common in endemic areas (many countries in Asia), less than 1% of people affected develop clinical disease, and severe disease affects about 1 case per 250 JEV infections. Although rare, severe disease can be devastating; among the 30,000-50,000 global cases per year, approximately 20-30% of patients die and 30-50% of survivors develop significant neurological sequelae. JE is a significant public health problem for residents in endemic areas and may constitute a substantial risk for travelers to these areas. The epidemiology of JE and its risk to travelers have changed, and continue to evolve. The rapid economic growth of Asian countries has led to a surge in both inbound and outbound travel, making Asia the second most-visited region in the world after Europe, with 279 million international travelers in 2015. The top destination is China, followed by Thailand, Hong Kong, Malaysia and Japan, and the number of travelers is forecast to reach 535 million by 2030 (+ 4.9% per year). Because of the lack of treatment and the infeasibility of eliminating the vector, vaccination is recognized as the most efficacious means of preventing JE. The IC51 vaccine (IXIARO®) is a purified, inactivated, whole virus vaccine against JE. It is safe, well tolerated, efficacious and can be administered to children, adults and the elderly. The vaccination schedule involves administering 2 doses four weeks apart. For adults, a rapid schedule (0-7 days) is available, which could greatly enhance the feasibility of its use. Healthcare workers should inform both short- and long-term travelers of the risk of JE in each period of the year and recommend vaccination. Indeed, it has been shown that short-term travelers are also at risk, not only in rural environments, but also in cities and coastal towns, especially in tourist localities where excursions to country areas are organized.

Introduction

Japanese encephalitis (JE) is a vector-borne disease caused by the Japanese encephalitis virus (JEV), which is a single-stranded RNA virus belonging to the genus *Flavivirus* (*Flaviviridae* family) and is closely related to the West Nile encephalitis virus [1].

There are five JEV genotypes in the world, but genotype 1 circulates much more than the others [2, 3]. JEV is transmitted by mosquitoes to a wide range of vertebrate hosts, including birds and mammals. With regard to human infection, domestic animals, especially pigs, are generally implicated as reservoirs of the virus. As humans are not part of the natural transmission cycle, they cannot pass the virus to other hosts [4].

While JEV infection is very common in endemic areas (many countries in Asia), less than 1% of people affected develop clinical disease [2], and severe disease affects about 1 case per 250 JEV infections. Although rare, severe disease can be devastating; among the 30,000-50,000 global cases per year, approximately 20-30% of patients die and 30-50% of survivors develop significant neurological sequelae [5].

JE is a significant public health problem for residents in endemic areas and may constitute a substantial risk for travelers to endemic areas. Because of the lack of treatment and the infeasibility of eliminating the vector, vaccination is recognized as the most efficacious means of preventing JE [4].

This overview focuses on the epidemiology and clinical features of JE and describes the use of the IC51 vaccine (IXIARO®) in travel medicine.

Epidemiology of japanese encephalitis

The vector of the JEV is the *Culex* mosquito, specifically *Culex tritaeniorhyncus*, which lives and breeds in water pools and flooded rice fields. This type of mosquito bites during night, with two peaks in biting time: a few hours after sunset and around midnight [6].

Humans and some kinds of animals, such as cattle or horses, are dead-end hosts of JEV, as they cannot develop a sufficient level of viremia to re-infect other mosquitoes. Transmission among humans is not possible, although transfusion-related JEV transmission was reported in two immunocompromised lung transplant recipients in Hong Kong, resulting in one case of severe encephalitis and one case of asymptomatic infection with seroconversion [7].

Culex tritaeniorhyncus lives throughout South-East Asia and tropical areas. However, its presence also extends

to the Middle East and Africa, and has recently been reported even in Europe. Within endemic areas, transmission varies according to climatic conditions: in temperate areas of Asia, transmission is seasonal, especially during summer and fall; in subtropical and tropical areas, transmission occurs during the monsoon season, but can be prolonged throughout the year [2].

The global incidence of JE is unknown, as the intensity and quality of JE surveillance systems and the availability of diagnostic laboratory testing vary throughout the world [8]. JE surveillance was not well established until 2012 and a 2011 systematic review of JE disease burden [8] estimated that approximately 68,000 cases occurred globally each year, and that only about 10% of these cases were reported to the WHO [9]. In 2011, Campbell et al. [8] reported that, owing to the lack of efficacious surveillance systems, it was difficult obtain a precise estimate of the incidence of JE. Nevertheless, it was estimated that 3 billion people living in 24 countries in the WHO's South-East Asian and Western Pacific regions are at risk of JE [8, 3]. The authors estimated that about 67,900 cases per year occurred in JE-endemic countries, with half of these cases in China alone. They also classified countries in terms of their incidence (high, medium and low) and the availability of vaccination (vaccination implemented, vaccination program expanding, and no vaccination) (Figure 1). Although Japan, the Republic of Korea and Taiwan are "high-incidence" countries, their routine vaccination programs have enabled them to limit JE incidence to 0.003 per 100,000 in the overall population. Vaccination began in Japan in 1954, in Taiwan in 1968, and in Korea in 1970. In Japan, however, following the withdrawal of the recommendation in 2005, vaccination coverage declined; although the recommendation was reinstated in 2009, coverage remains low. By contrast, China, which is also a high-incidence country, does not have a quality vaccination program (vaccination was implemented from 1981 to 2007, but non-uniformly in children; a routine vaccination program was started in 2008). Consequently, the incidence of JE is 3.3 overall, 12.7 in children aged 0-14 years and 1.0 in subjects aged > 15 years. Some countries where JE incidence is high do not implement any, or are only now developing, vaccination programs: Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines and East Timor. Moreover, countries such as India and Nepal are improving their routine vaccination programs.

Medium-incidence countries (such as Indonesia and Papua New Guinea) have a total incidence rate of 1.7 per 100,000, while those with low incidence have a rate of 1.0 per 100,000 (Fig. 1).

Annual incidence varies by age-group, with children being the most affected [8]. Most adults are immune to infection, as natural infection with JEV (or other *Flaviviruses* that share antigens inducing cross-protection) is probably able to confer lifelong immunity. This explains why children are the most susceptible subjects [3, 4]. It is noteworthy that, where childhood vaccination programs are implemented, there is a shift to a greater proportion of cases in other unvaccinated age-groups [3].

In 2016, 22 of 24 (92%) countries at risk of JE ran surveillance systems, compared with 75% in 2012; some of these systems are national, some are sub-national and others use sentinel surveillance. In 12 of the 24 countries (50%), an immunization program was in place in 2016. Some of these immunization programs are national, others are sub-national in all risk areas, and several are sub-national but do not include all risk areas [9].

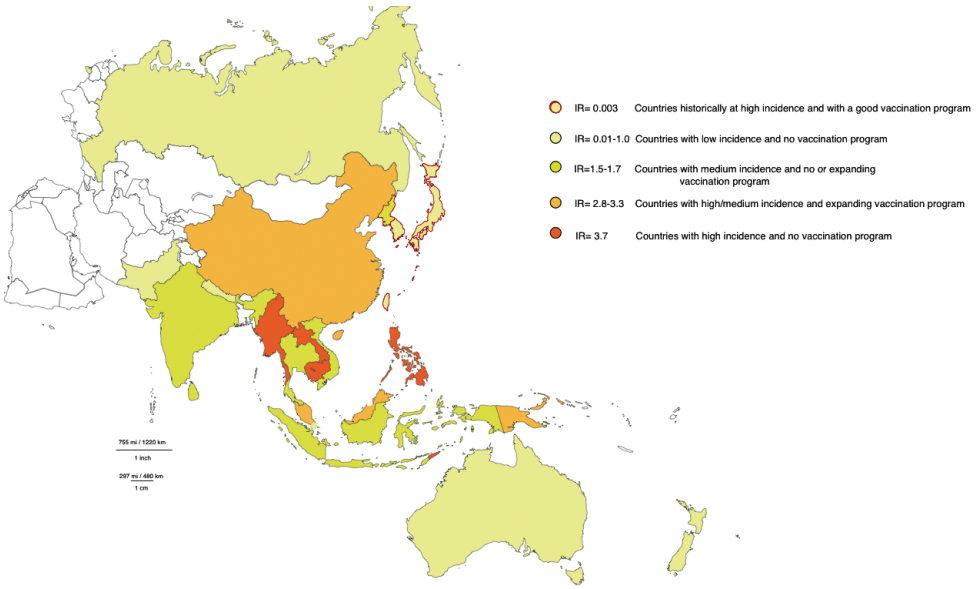
Recently, several factors have changed the geographical distribution of JE. First of all, international travel, especially for reasons of work, has increased. Indeed, the growth of US-based multinational corporations in Asia in the last twenty years has led to a significant rise in the number of business travelers. The business travelers frequently have to spend short or long periods in Asia, where they acquire the same risk of JE as the local population. Furthermore, changes have taken place in agriculture; in many areas of Asia, both rice production and pig farming have been intensified, which could lead to the development of a high risk of JE even in suburban areas, which were not at risk some years ago. The epidemiology of JE is also influenced by ecological and climatic factors. The appearance of JEV in Tibet (a cold, high-altitude region) and the emergence of two new serotypes of JEV in Australia provide confirmation that the spread of JEV is unpredictable. Moreover, global warming may lead to a change in the peak and duration of JEV transmission [5].

Clinical features and treatment

Most infections are asymptomatic or cause non-specific febrile symptoms [4]. Incubation time ranges from 5 to 15 days; the non-specific febrile illness that frequently constitutes the clinical presentation of JE may be the only manifestation in some patients [10]. Other symptoms can occur: coryza, diarrhea and rigor, followed by the onset of altered consciousness and seizures (convulsions occur in 75% of children) [3] or vomiting after several days [11]. When the disease is severe, affecting the central nervous system, the clinical features are mental status changes, focal neurological deficits, weakness and, after a few days, even movement disorders. Moreover, classical symptoms associated with severe JE are Parkinsonian syndrome, with mask-like face, tremor, cogwheel rigidity and choreoathetoid movements. Rarely, the clinical presentation can include acute facial paralyses (similar to poliomyelitis paralyses) [2]. Blood tests reveal neutrophilia, hyponatremia, elevated liver enzymes and cerebrospinal fluid (CSF) pleocytosis, with lymphocytic predominance [12, 2]. JE is most commonly diagnosed by testing for JEV IgM in the CSF, which is reliably positive if the CSF sample is taken at least one week into illness [13]. Other laboratory tests are molecular methods [14].

The treatment of JE usually consists of supportive care, as there are no specific antiviral therapies. The case fatality rate is high, and it is estimated that, in endemic areas, approximately one third of patients admitted to hospital

Fig. 1. Incidence of JE and vaccination policies in Asian countries [8].



die [11]. JE-related deaths are due to raised intracranial pressure, hypoglycemia and seizures [2, 3]. JE disease also causes serious brain damage in about 25-50% of survivors, the highest rate of sequelae being reported in children [3, 15]. Moreover, in pregnant women, the infection can cause harm to the fetus.

Even though JE is rare, it is potentially a severe life-changing illness and requires prevention. Vaccination is the cornerstone of JE control.

International travelers

The epidemiology of JE and its risk to travelers have changed, and continue to evolve. Estimates of JE incidence among Western travelers to tropical and subtropical destinations show that the usual itineraries followed by tourists or businesspeople are considered at low risk of infection (< 1 per 1,000,000 travelers per month) [16]. The risk of travelers contracting JE varies according to their travel itinerary. The overall risk of about 1/400,000 visits [17] may, however, increase for certain high-risk groups, such as those who stay for long periods in rural areas, approaching the risk of 1/50,000 per week seen in the local population [18, 19]. From 1973 to 2008, 55 cases were reported in travelers, most of whom were "high-risk travelers" who had spent a month or more in endemic regions (especially rural areas) and had acquired the same risk of JE as the local population. The case-fatality rate was 18% and 24 survivors developed sequelae. Even though long stays have always been considered a risk factor, the authors found that 13 of the 55 cases occurred in travelers who had stayed for less than 1 month: 10 for 2-4 weeks and 3 for 10-12 days trip [20]. This demonstrates that short-term travelers also have a JE disease risk.

Kollaritsch H. et al. [21] also reported JE cases in travelers, and from 1973 to 2015 the Centers for Disease Control and Prevention reported 79 cases among travelers or expatriates from non-endemic countries [2]. Some of these cases could have been avoided if travelers had been vaccinated in accordance with current recommendations.

More recently, a fatal case of JEV infection following short-term travel to Thailand was reported. In this case, JE viral RNA was detected in urine and whole blood 26 and 28 days, respectively, after the onset of symptoms. Furthermore, live virus was isolated from a urine specimen taken on day 14. This suggests that testing whole blood and urine could offer additional diagnostic information in certain situations [22].

It is well known that most cases of JE are asymptomatic. Consequently, a large number of infections may go unrecognized. Moreover, in the near future, JE could become a major public health issue even in non-endemic countries, as a result of cultural, economic and climatic changes [16].

Travel is changing; the increased speed and availability of transport (especially by air) encourages inter-regional and international tourism and business trips. In terms of population, Asia and the Pacific are the world's largest regions, with 4.1 billion inhabitants in 2015, about 56% of the world's total population [23]. The rapid economic growth of Asian countries has led to a surge in both inbound and outbound travel, making Asia the second most-visited region in the world after Europe, with 279 million international travelers in 2015. The top destination is China, followed by Thailand, Hong Kong, Malaysia and Japan, and the number of travelers is forecast to reach 535 million by 2030 (+4.9% per year). According to the United Nations Word Tourism Organization (UNWTO), this unprecedented growth in travel is the consequence of the Asian economic boom. Further,

a large and growing number of "free and independent travelers", identifiable as a "consumer class", especially young Asian travelers aged 15-34 years ("millennials"), use online travel agencies and mobile technology. Their destinations are often secondary and tertiary cities, which could be potentially at risk of JE [24]. Furthermore, if they travel alone or simply organize their travel on their own, they are unlikely to be informed about JE, its symptoms and prevention. 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.

Prevention of japanese encephalitis

Travelers to JE-endemic areas can reduce their exposure to vectors by adopting personal protective measures: using mosquito-repellent agents, wearing appropriate clothing, avoiding outdoor activities in the evening, using permethrin-impregnated mosquito nets and staying in rooms with air conditioning. In combination with these protective measures, vaccination against JEV infection can provide travelers with safe and effective protection [21, 25].

Different vaccines against JE have been available since the 1950s [14]. First-generation JE vaccines were of various types; mouse brain-derived inactivated vaccines, inactivated vaccines cultivated on primary hamster kidney cells, live attenuated vaccines based on strain SA 14-14-2 and cultivated on primary hamster kidney cells have been widely used for the past few decades [26]. Although mouse brain-derived vaccine was used for several decades in the US and Europe, its production has been discontinued, owing to its unsatisfactory safety profile [24, 25]. A formalin-inactivated vaccine developed in China in 1968 was cultivated in Vero cells and used the P-3 strain; this was widely used and displayed a good safety and effectiveness profile [25, 26]. A live-attenuated SA14-14-2 vaccine authorized in 1988 is currently used in Nepal, India, Sri Lanka, Thailand and South Korea, and has proved to be a valid tool in terms of effectiveness and safety [25].

More interestingly, second-generation JE vaccines are available, which use a lower dosage scheme and exhibit a better safety profile; these constitute a noteworthy option for the protection of residents of endemic areas and travelers. They are the IC51 Japanese encephalitis vaccine (IXIARO®) and the chimeric Japanese encephalitis vaccine.

Overall, vaccination has dramatically reduced the number of JE cases in several Asian countries [8]. However, as the virus is maintained in animal reservoirs, non-immune travelers remain at risk of infection in all endemic areas of South-East Asia.

Development of the IC51 vaccine (IXIARO®)

The IC51 vaccine (IXIARO®) is a purified, inactivated, whole virus vaccine against JE. Developed at the Walter Reed Army Institute of Research, it is based on an SA14-14-2 virus strain cultivated in Vero cells and formulated with 0.1% aluminum hydroxide [27]. Each 0.5 ml dose of IC51 vaccine comes in a ready-to-use liquid format that contains 0.1% aluminum hydroxide as an adjuvant; it does not contain stabilizers such as porcine gelatin or preservatives such as thimerosal.

Several clinical trials have positively evaluated the immunogenicity and safety of the IC51 vaccine. The first studies were carried out to evaluate its immunogenicity and safety in comparison with placebo or mouse-brain JE vaccines. The encouraging results, in terms of immune response and low reactogenicity, of a phase I study [21] that evaluated different doses of vaccine (0.4 micrograms and 2.0 micrograms following a 0 and 28, or a 0, 7 and 28 schedule) in 25 adult subjects prompted subsequent studies.

Lyons et al. reported the results of a phase II study, conducted from 2001 to 2003, which enrolled a total of 94 subjects. The vaccine was administered in two or three intramuscular doses (6.0 or 12.0 micrograms each) with observation over 8 weeks. No serious adverse reactions were observed. The vaccine was well tolerated and conferred high seroconversion rates [day 56 (primary endpoint): 95-100%] and induced persisting immune responses up to 2 years after vaccination [28].

Subsequent phase III trials were conducted. In a multicenter, observer-blinded, randomized controlled phase III trial, 867 adult volunteers received either two intramuscular doses the JEV test vaccine (on days 0 and 28; n = 430) or the licensed vaccine (JE-VAX®) subcutaneously according to its recommended three-dose schedule (on days 0.7, and 28; n = 437). The safety profile of the test vaccine was good, and its local tolerability profile was more favorable than that of the licensed vaccine. The frequency of adverse events was similar in both treatment groups and adverse events were generally mild. The seroconversion rate of the test vaccine was 98%, compared with 95% for the licensed vaccine, on day 56. The geometric mean titer in recipients of the test vaccine was 244, compared with 102 for the licensed vaccine [ratio 2.3 (95% CI 1.967-2.75)] [29].

Later, the same research group published the results of a randomized multi-center phase III trial. Healthy subjects were randomized to receive 2 doses of IC51 vaccine (n = 2012) or placebo (n = 663) four weeks apart. Adverse events were documented over a period of 2 months. The rate of severe events was similar in both the IC51 group (0.5%) and the placebo group (0.9%) demonstrating a good safety profile of IC51 vaccine. These data, together with the immunogenicity data from the previous phase III clinical trial, were the basis for the authorization of the IC51 vaccine [29, 30].

In another study, adult subjects were followed up in order to compare the immunogenicity of the IC51 vaccine and the vaccine JE-VAX®. At 6 months, immunogenicity was higher with the IC51 vaccine (seroconversion rate [SCR]: 95%; geometric mean titer [GMT]: 84) than with JE-VAX® (SCR: 74%; GMT: 34). With regard to IC51, the SCR at 12 months was 83% and the GMT re-

mained above the protective titer of 1:10. This phase III follow-up study confirmed that the immune response following IC51 vaccination was more robust than the immune response following JE-VAX® [31].

The standard administration of IC51 is of 2 doses of 6 micrograms 28 days apart. However, one study investigated the immunogenicity of a single-shot, high-dose regimen (1 x 12 micrograms) in comparison with the 2-injection, standard regimen. The single-shot, high-dose regimen resulted in about 60% SCR 10 days after administration, but did not reach the almost 100% SCR achieved by the 2-dose standard administration on day 35. The standard regimen conferred essentially 100% seroconversion 7 days after the second injection [32].

On December 18, 2008, the European Committee for Medicinal Products for Human Use (CHMP) recommended granting marketing authorization for the IC51 vaccine (IXIARO®) in the 27 countries of the European Union and in Norway and Iceland. In the US, IXIARO® was authorized and recommended for use in persons aged ≥ 17 years in 2009 [33]. The product is also approved and available in Canada, Switzerland and Australia.

In the first 12 months after licensing, the safety profile of IXIARO® was reviewed on the basis of clinical trial results and post-marketing safety data. The clinical safety profile was derived from a pooled analysis that included safety data from 10 phase III trials carried out in 4,043 subjects who had received at least one dose of IXIARO® and were followed up for 3 years after the primary immunization. The local and systemic tolerability of IXIARO® was similar to that seen in the previous evaluation at the time of licensure of the vaccine. On post-marketing surveillance, no serious allergic reactions were observed. This comprehensive safety review confirmed the good safety profile of IXIARO® in clinical and post-marketing use [34].

A phase III study investigated the immunogenicity and safety of IC51 and HAVRIX1440 (hepatitis A vaccine) when administered alone or concomitantly to healthy subjects. The immune response elicited by single administration was compared with that of concomitant vaccination in terms of GMT and SCR on days 28 and 56. The results indicated that the immunogenicity of the two vaccines was comparable, whether they were administered together or separately. On the basis of these data, the authors affirmed that travelers to endemic regions could receive both vaccines concomitantly [35].

Eder S. et al. assessed the effect of a booster dose on neutralizing JE antibody titers up to 12 months after boosting. The booster dose of IXIARO® was administered 15 months after the primary immunization to 198 subjects who had previously been immunized in a randomized trial [36]. Neutralizing antibody titers were assessed by means of the plaque-reduction neutralization test (PRNT). Prior to the booster dose, 69.2% (137/198) of subjects had PRNT50 titers ≥ 1:10. One month after the booster, the rate of subjects with PRNT50 ≥ 1:10 (recognized as a protective titer) had increased to 100%. The evaluation of PRNT50 showed a high rate (98.5%) at 6 and 12 months. GMTs were 22.5 before the booster and 900, 487 and 361 at 1, 6 and 12 months, respectively, after the booster. The booster dose of IXIARO® 15 months after primary immunization proved highly immunogenic; GMTs were > 5-fold higher than those seen immediately after primary immunization, and remained at high levels for at least 12 months after the booster [37].

IXIARO® IN CHILDREN

In 2009, phase III studies to evaluate the immunogenicity and safety of administering the IC51 vaccine to children began. A study involving 60 healthy Indian children aged between 1 and 3 years evaluated the immunogenicity and safety of 3 and 6 micrograms of IXIARO® in comparison with the licensed vaccine JenceVac. Immunogenicity was assessed by measuring antibodies at the baseline and 28 days after the first and second administrations. On day 56, SCRs in the 3- and 6-microgram IXIARO® groups and the JenceVac group were 95.7%, 95.2% and 90.9%, respectively, and GMTs were 201, 218 and 230, respectively. Local and systemic tolerability was registered in a diary 7 days post-vaccination. No apparent difference was seen in the safety profiles of the two vaccines. These first immunogenicity and safety data in children supported the use of a 3-microgram dose in children under 3 years of age [38].

A very recent uncontrolled, open-label, phase III trial was carried out in order to assess the safety and immunogenicity of IXIARO® in a population of previously JEV-unexposed travelers to JE-endemic regions [39]. The vaccine was administered to 100 travelers aged ≥ 2 months to < 18 years. Solicited adverse events were observed for 7 days after each injection, and unsolicited adverse events for a total of 7 months. Furthermore, JE neutralizing antibodies were investigated in 64 subjects. Two different vaccine doses were studied: 0.25 and 0.50 ml IXIARO®. The most common solicited local adverse events were redness, induration and tenderness with 0.25 ml IXIARO®, and tenderness and pain with 0.5 ml IXIARO®. Common solicited systemic adverse events were diarrhea and loss of appetite with 0.25 ml IXIARO® and muscle pain and excessive fatigue with 0.5 ml IXIARO®. In total, up to day 56, adverse events were reported by 83.3% of subjects who had received the 0.25 ml dose and 76.1% of those vaccinated with the 0.5 ml dose. All subjects had developed protective levels of JE neutralizing antibodies by day 56, and 91.2% retained protective titers at month 7. IXIARO® was generally well tolerated in children, with an overall safety profile similar to that seen in adults. Moreover, it was highly immunogenic in both dose-groups.

Another recent study [40] monitored the safety profile of IXIARO® in a pediatric population of 1869 children aged between 2 months and 17 years, who were recruited and randomized in an age-stratified manner to receive IXIARO® or one of the control vaccines: Prevenar® and HAVRIX®. Adverse events, serious adverse events and medically attended adverse events were assessed up to day 56 and month 7 after the first dose. This study showed

that incidences of adverse events, serious adverse events or medically attended adverse events did not differ significantly between the groups. Adverse events were most frequent in children < 1 year of age and decreased with age. Adverse events of special interest, such as hypersensitivity/allergy or neurological disorders up to day 56, were reported in 4.6% (IXIARO®) versus 6.3% (Prevenar®) in the ≥ 2 months to < 1 year age-group and in 3.4% (IXIARO®) versus 3.3% (HAVRIX®) in the ≥ 1 to < 18 years age-group. Fever, the most frequent systemic reaction, was observed in 23.7% of infants and 3.8% of adolescents, and decreased with age. The authors concluded that the safety profile of IXIARO® was comparable to that of the control vaccines.

Another study also evaluated the immunogenicity of IXIARO®. Age-stratified cohorts of children between 2 months and 17 years of age received 2 doses of IXIARO® administered 28 days apart [< 3 years, 0.25 mL (half adult dose); ≥ 3 years, 0.5 mL (full adult dose)]. The endpoints were seroconversion rate, 4-fold increase in JE neutralizing antibody titer and geometric mean titer assessed 56 days and 7 months after the first administration. A total of 496 subjects were enrolled and the immune response to JE virus at both time-points was also analyzed according to pre-vaccination JE virus and dengue virus serostatus. On day 56, seroconversion had been obtained in ≥ 99.2% of subjects, 4-fold increases in titer were reported in 77.4-100% in various age-groups, and geometric mean titers ranged from 176 to 687, with younger children displaying the greatest immune response. At month 7, seroconversion was maintained in 85.5-100% of subjects. Pre-existing JE virus immunity did not impact on the immune response at day 56; however, it yielded better persistence of protective antibody titers at month 7 [41]. The above findings revealed that IXIARO® was highly immunogenic in the pediatric population at both doses tested, eliciting protective antibody titers by day 56 in > 99% of subjects who received the age-appropriate dose.

In May 2013, the Food and Drug Administration (FDA) licensed IXIARO® for use in children aged 2 months through 16 years [42], and in the same year the vaccine was also indicated for children by the European Medicines Agency (EMA) [43].

IXIARO® in the elderly

The safety and immunogenicity of IXIARO® were also evaluated in a clinical trial involving elderly subjects. An open-label, single-arm, multi-center study was conducted in 200 healthy subjects aged 64-83 years, including those with adequately controlled chronic conditions, who received two doses of IXIARO® 28 days apart. Antibody levels were tested 42 days after the second dose. Systemic and local adverse events were monitored for 7 days after each dose, and unsolicited adverse events were recorded up to day 70 and subsequently at month 7. Although 19% of subjects had serious or medically attended adverse events up to day 70 (primary endpoint), none of these was associated with the vaccine. Solicited local adverse events were reported by 33.5% of participants, the most common being local tenderness; solicited systemic adverse events were reported by 27% of participants, the most common being headache. A seroprotection rate of 65%, with a GMT of 37, was found. Subjects who had received a tick-borne encephalitis (TBE) vaccine in the previous 5 years had an SCR of 90% and GMT of 65. The authors concluded that IXIARO® was generally well tolerated in the elderly, and that its safety profile was largely comparable to that seen in younger adults. While SCR and GMT were lower than in younger adults, SCR was in the range reported in the elderly for other vaccines, such as TBE, hepatitis-A virus (HAV)/hepatitis-B virus (HBV), and influenza vaccines. Another aspect to be taken into account was the duration of protection, which is uncertain in the elderly. The authors therefore suggested that a booster dose (third dose) should be considered before any further exposure to JEV [44].

Dosage, administration and schedule of IXIARO®

IXIARO® is injected into a muscle, preferably the shoulder muscle, or, in young children, into the thigh muscle. In adults, including those aged over 65 years, and children aged three years and older, a full dose of IXIARO® (0.5 ml) should be administered, and an additional 0.5 ml dose should be given four weeks later. In adults aged 18-65 years, a rapid vaccination course may also be implemented, in which the second dose is given seven days after the first. In children aged between two months and three years, half the adult dose of IXIARO® (0.25 ml) should be given, and an additional 0.25 ml dose should be given four weeks later. It is recommended that individuals who receive the first dose of IXIARO® should receive both doses, and that the second dose be given at least one week before potential exposure to the virus. In adults, the second dose can be given up to 11 months after the first. Adults aged 18-65 years who are likely to be exposed to the JEV again, or who are at continuous risk of the disease, should receive a booster dose of IXIARO® one to two years later and a second booster dose 10 years after the first booster. Children and adolescents may also receive a booster dose one to two years after the initial vaccination. A booster dose should also be considered for adults aged over 65 years before any further exposure to JEV. IXIARO® can be injected under the skin in people who have a bleeding disorder, such as low blood platelet counts or hemophilia [43].

A randomized, observer-blind, phase 3 study evaluated the immunogenicity of IXIARO® administered according to a rapid schedule [43]. Two cohorts were recruited: 217 subjects aged 18-65 years received IXIARO® together with rabies vaccine in a rapid immunization schedule (0-7 days); 56 subjects received IXIARO® according to the common immunization schedule (0-28 days). Seroconversion was similar in both cohorts and seroconversion rates and antibody titers remained high in both groups up to 12 months after immunization. With the rapid schedule, seroconversion rates on days 14 and 21 and after one year were 99%, 100% and 94%, respectively; at the same time-points, GMT values were 715, 1255

and 117, respectively. A third dose (first booster dose) was given within the second year (12-24 months) after the primary vaccination course. Long-term seroprotection data following a first booster dose suggested that a second booster should be given 10 years after the first, prior to potential exposure to JEV.
Subsequently, antibody persistence after booster immunization in adults was assessed in an uncontrolled, open-label extension, in which 67 subjects were followed up to determine JEV neutralizing antibody titers approximately 6 years after a booster dose. In 96% of subjects (64/67), protective antibody levels (PRNT50 ≥ 1:10) persisted, with a GMT of 148 (95% CI:107-207). Mathematical modeling was applied to evaluate the average duration of protection. On the basis of this model, it was estimated that the average duration of protection would be 14 years and that 75% of vaccinees would retain protective antibody levels (PRNT50 ≥ 1:10) for 10 years. A second booster should therefore be given 10 years after the first, prior to potential exposure to JEV [43]. Some authors have speculated that the protection conferred by the booster dose could last for a lifetime.

Conclusions

JE is the main cause of viral encephalitis in many Asian countries, and causes significant morbidity and mortality in autochthonous populations. Survivors often suffer permanent severe neurological sequelae that require lifelong support and care. The burden of the disease is heavy not only in terms of its clinical impact, but also on account of its socio-economic consequences in endemic areas. JE epidemics have also been documented, such as the outbreaks in India, which caused about 400 cases in 2014 and 1700 deaths in 2005 [45, 46].
As mentioned above, JE virus transmission is seasonal. However, climate changes and increasingly extreme weather conditions could lengthen the infective period of the JE virus. Moreover, the areas affected by JE have gradually expanded; indeed, growing numbers of JE cases have been observed further north in Asia, owing to the rise in global temperatures. For example, cases have spread to Nepal from Northern India, and the disease has now become widespread in hill and mountain districts [47]. JE cases have also been associated with increasingly frequent floods in China [48].
Travelers to endemic areas are exposed to the risk of acquiring a serious JE infection, and the rise in international travel to Southeast Asia has exacerbated this risk. Published recommendations for travelers underscore the importance of considering the travel destination, the high morbidity and mortality rates of JE, the lack of available treatment, travel-related factors (itinerary, duration, season and activity) and the benefits of JE vaccination [26, 33]. With regard to the risk of JE infection, the activity of the traveler is more significant than the length of the stay. Indeed, activities such as camping, trekking, biking, fishing and hunting increase the risk of exposure to the mosquitoes that transmit the JE virus. The fact that holiday-makers are now more inclined to engage in outdoor activities and to stay in unconventional tourist lodgings may have contributed to the recent increase in cases among travelers [49].
Travelers should be aware of the risk of contracting JE and how it can be prevented by vaccination, as this disease can have devastating effects. IXIARO® can be administered to children, adults and the elderly. It is safe, well tolerated and efficacious. The vaccination schedule involves 2 administering doses four weeks apart. For adults, a rapid schedule (0-7 days) is available, which could greatly enhance the feasibility of its use.
Healthcare workers should inform both short- and long-term travelers of the risk of JE in each period of the year and recommend vaccination. Indeed, it has been shown that short-term travelers are also at risk, not only in rural environments, but also in cities and coastal towns, especially in tourist localities where excursions to country areas are organized. In rural areas, the risk is linked to potential contact with poultry.

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Author contributions

DA and DP conceived and designed the overview. FZ, PLL and MI performed a search of the literature on epidemiology of Japanese encephalitis. DA carried out a search of literature on the immunogenicity, efficacy and safety of the IC51 vaccine (IXIARO®). All authors contributed to the draft of the article. DA, PLL and DP revised critically the manuscript. All authors read and approved the final version of the manuscript.

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