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INFECTIOUS DISEASES

Serological survey of immunity to tetanus in adult population of Northern Halkidiki, Greece

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Abstract. *Background*: Despite the implementation of the obligatory anti-tetanus vaccination, the tetanus cases in Greece are not eliminated. Because of the increased possibility of *Clostridium tetani* infection of the Northern Halkidiki population – like any other rural population – the evaluation of the immunity to tetanus in the area is considered necessary. *Methods*: The tetanus antitoxin levels were determined using the enzyme-linked immunosorbent assay (ELISA) in 405 healthy adult individuals attending the health center for routine laboratory tests. *Results*: 64.4% of the studied population was found protected (tetanus antitoxin levels $\geqslant 0.1$ IU/ml). The percentage of protected people decreased as age increased from 83.3% in the 21–30 to 51.2% in the >60 age group. 82.1% of the tested males and 52.6% of the tested females had tetanus antitoxin levels $\geqslant 0.1$ IU/ml ($p < 0.0001$). The percentages of immune men (100–66.2% in various age groups) were found higher than those of women (80.8–35.5% in the respective age groups). The geometrical mean titres (GMTs) were 0.44 in all of 261 immune people, 0.53 in 133 immune men and 0.37 in 128 immune women ($p = 0.0021$). *Conclusion*: The proportion of protection among men over 60 and women over 30 years old is inadequate, the levels of tetanus antitoxin decline with age and a significant difference was found between the proportion of protection of males and females.

Key words: Immunity, Tetanus, Tetanus antitoxin, Vaccination

Introduction

The national policy for immunization against tetanus in Greece includes three doses of tetanus toxoid given at 2, 4, 6 months of age (combined vaccine against diphtheria, tetanus and pertussis [DTP]), followed by a fourth dose at 18 months and a booster dose at school entry, between 4 and 6 years of age. It has been recommended that a booster dose using the adult vaccine (Td) should be administered every 10 years, but it is not always performed. The tetanus vaccine is also administered to recruits in the armed forces and to people at risk of *Clostridium tetani* infection like workers in big industrial units and certain public services. The local policy of tetanus vaccination after injury in health centers and industrial units recommends a single booster dose in case of prior vaccination and three doses (0, 1, 6–12 months) in case of lack of vaccination.

Today tetanus is still a serious public health problem in the world. The true incidence of tetanus is not known and although there is an effective vaccine available, is estimated to be between half to one million cases per year worldwide [1, 2]. The majority of cases occur in developing countries, with 50% of those occurring in neonates. In contrast, tetanus is uncommon in developed countries, with most cases occurring in older adults [3–5].

In the United States, in the 50's approximately 500 cases per year had been reported, in the 1965–1974 period just 176 cases per year (mostly cases of non-immunized or partially immunized people) and from 1975 and on the reported cases were fewer than 100 per year [6–8]. From 1991 to 1994, 54% of the 188 patients were age 60 years and older, 5% were age 20 years and younger [9], reflecting the low prevalence of protective antibody titers among elderly population [10, 11]. Between 1995 and 1997, in the United States, there were 124 cases of tetanus, with a male to female ratio of 3:2. The incidence was 36–48 cases per year or 0, 15 cases per million annually. This rate has remained relatively stable since the late 1980s [12]. Among those cases only one occurred in a neonate, while in 1997, neonatal tetanus accounted for an estimated 277,400 deaths worldwide [13]. In the US between 1992 and 2000, 15 cases were reported in

children under 15 years of age in 11 states [14]. In the Marches region of Italy, during the 1992–1995 period 36 cases of tetanus were reported (7 males and 29 females with mean age 73.5 years) [15]. In the same area between 1996 and 1999, 32 cases of tetanus were reported (3 males and 29 females with mean age 74.65 years) [16]. In the referral hospital of Leuven, Belgium, 27 cases of tetanus were reported during the 1983–1993 period, 23 men and 14 women with mean age of 68.5 years [17]. The number of tetanus cases reported in Turkey was 42 in 1996 and 51 in 1997 [18]. A gradual decrease in the numbers of reported cases of tetanus was observed in Poland. The number of cases reported was 37 in 1997, 22 in 1998 and 21 in 1999. In 1997, 70% of the cases occurred in persons aged >60, while in 1998–1999, all those cases were among people aged >40 [19–21].

In a 14-year study in a hospital in Bombay (India), 8697 cases of tetanus were analyzed. The incidence was higher in the first decade of life, the mortality in the neonatal group was 86.38%, while in the non-neonatal group 40.18%. The disease was seen more frequently in male than in female [22]. In Taiwan, during the 1991–1999 period, 20 tetanus patients were treated in a university hospital, 18 adults and just 2 children [23]. In a 10-year retrospective study in Malaysia, a total of 22 cases were reported (15 male and 7 female) with most cases occurring in the 60–69 age group [24].

Since the implementation of the vaccination program in Greece in the 40's and 50's, the number of tetanus cases and deaths declined from an average of 90.7 cases and 28.7 deaths per year in the 70's to 40.4 cases and 8.3 deaths per year in the 80's. In the 1990–1995 period, 36.3 cases and 3.1 deaths were reported per year. 82% of the tetanus cases occurred among people over the age of 14 in the 70's, 96% in the 80's and 97.2% in the 1990–1995 period. In 1996 and 1997, seven and two cases of tetanus were reported respectively. The neonatal cases were eliminated with the last case reported in the 80's [25]. According to data provided by the Hellenic center for infectious diseases control, during the 1998–2001 period 28 cases of tetanus were reported among which 19 were people over the age of 50, in eight cases the age is not reported and one case occurred in the 1–4 age group. According to the same source, in the province of Halkidiki during the 1998–2001 period no cases of tetanus were reported.

The Health Center of Paleohori is situated in a rural area of Northern Halkidiki, a province of Northern Greece. As part of the National Health System of Greece, it provides primary health care services to a population of roughly 25,000. The population under study, like any other rural population, has an increased possibility of *Clostridium tetani* infection. For this reason the evaluation of the immunity rates to tetanus in this area is considered necessary.

Materials and methods

Study population

The population under study consisted of 405 healthy adult individuals, 162 males and 243 females. The age of the studied population ranged from 21 to 80 years. Blood samples were taken from randomly selected adults among those attending the Health Center for routine laboratory tests. Individuals who had recently received blood or blood products, including immunoglobulins, were excluded from the study. The sera were transported in special containers to the laboratory of microbiology of the Aristotelian University of Thessaloniki, stored at -20 °C and used only once after thawing. Each serum titre was determined in duplicate. Specific data on vaccination history were not obtained.

Detection of tetanus antitoxin

The serum levels of tetanus antitoxin was determined by IgG-specific enzyme-linked immunosorbent assay (ELISA) of Virotech System-Diagnostika GmbH. The assay accurately discriminates tetanus antitoxin levels above or equal to 0.1 IU/ml, while lower values cannot be defined, according to the manufacturer. It is suggested that values of <0.1 IU/ml are considered indicative of susceptibility (subjects needing immunization). Tetanus antitoxin concentration of $\geqslant 0.1$ IU/ml is indicated as immune protection [26] or safe immune protection [27, 28]. Others describe the antitoxin-antibody concentration of >0.6 IU/ml as sufficient vaccination protection [26].

Statistical analysis

The χ^2 test was used to evaluate statistical significance in the comparison of the prevalence of tetanus antitoxin between different age groups and between males and females. The Mann–Whitney two samples *T*-test was used to compare the levels of tetanus antitoxin (IU/ml) between males and females both in total and in the different age groups. The chosen significance level was 5%.

Results

Presentation of the study group by age is shown in Table 1. 261 (64.4%) people of the 405 individuals of the studied population were found protected (tetanus antitoxin levels $\geqslant 0.1$ IU/ml). The distribution of people according to the level of tetanus antitoxin and the age demonstrates that as age increases, the percentage of people with protective levels of tetanus antitoxin decreases, from 83.3% in the 21–30 age group to 51.2% in the >60 age group, without statistically significant difference between successive age

Table 1. Distribution of tetanus immune people and antitoxin levels in age-stratified Northern Halkidiki population

				Tetanus antitoxin levels	
Age group (years)	Tested no.	Protected no. (%)	GMTs (IU/ml)	0.1–0.5 IU/ml no. (%)	>0.5 IU/ml no. (%)
21–30	60	50 (83.3)	0.59	17 (34)	33 (66)
31–40	67	47 (70.1)	0.36	31 (66)	16 (34)
41–50	74	53 (71.6)	0.52	28 (52.8)	25 (47.2)
51–60	77	46 (59.7)	0.52	22 (47.8)	24 (52.2)
>60	127	65 (51.2)	0.31	49 (75.4)	16 (24.6)
	405	261 (64.4)	0.44	147 (56.3)	114 (43.7)

GMTs: geometric mean titres.

groups ($p > 0.1$). Comparison of the protection proportion of the 21–30 group and each one of the 31–40 and 41–50 groups did not give statistically significant difference ($p > 0.05$, $p > 0.1$ respectively). Between 21–30 and 51–60 and >60 groups, a statistically significant difference was found ($p < 0.01$, $p < 0.001$ respectively).

Table 1 also shows the grouping of the protected individuals into two categories, according to the levels of tetanus antitoxin. Most of the protected individuals (56.3%) had antitoxin levels of 0.1–0.5 IU/ml, which is considered as adequate protection, but a booster dose is demanded [26]. The other 43.7% had antitoxin level >0.5 IU/ml and will demand a booster dose after 2 years or more depending on the level of tetanus antitoxin [26].

The distribution of tetanus-immune people and antitoxin levels by sex is shown in Table 2. 82.1% of the tested males and 52.67% of the tested females had tetanus antitoxin levels $\geqslant$0.1 IU/ml. A significant difference in the proportion of protection between males and females was found ($p < 0.0001$). As shown in Table 2 and Figure 1, the protection rate did not differ significantly between males and females of the 21–30 age group ($p = 0.174$), while in the remaining age groups a significant difference was found ($p = 0.0008$, $p < 0.0001$, $p < 0.001$ and $p = 0.0005$). In Tables 1 and 2 are displayed the geometrical mean titres (GMTs) according to age and sex. The statistical analysis of the tetanus antitoxin levels (IU/ml) have shown that between the total of males and females there is significant difference ($p = 0.021$), while between males and females in the various age groups there is statistical difference between 31–40 ($p = 0.0001$), 41–50 ($p = 0.0192$) and 51–60 ($p = 0.0101$) age groups but that doesnot occur in the 21–30 ($p = 0.1349$) and the >60 ($p = 0.4795$) age groups.

Discussion

Our results showed that only 64.4% of the population under study is protected from tetanus and that the proportion of protected people decreases as age increases (from 83.3 to 51.2%). A similar study was performed by the same laboratory in 1980 [29] in which children 1–15 years old were found protected against tetanus in percentages varying from 96.61 to 100% in different age groups. In the 16–20 age group 85.24% of the people are protected and beyond that age the proportion of protection also decreases as age increases (from 87.2 to 27.4%). Several similar studies were performed in various places; in the Netherlands, more than 95% of those born after the introduction of the routine vaccination had tetanus antitoxin levels above the minimum protective level [30]. In the UK, only 50% of the elderly (over 65 years) were found protected from tetanus and low levels of protection were particularly prevalent in the $\geqslant$80 age group [31]. Another study which took place in England and Wales showed that while more than 80% of those aged 4–39 years had protective

Table 2. Distribution of tetanus immune people by age and sex in Northern Halkidiki population

	Males			Females		
Age group (years)	Tested no.	Protected no. (%)	GMTs (IU/ml)	Tested no.	Protected no. (%)	GMTs (IU/ml)
21–30	8	8 (100)	0.90	52	42 (80.8)	0.56
31–40	19	19 (100)	0.70	48	28 (58.3)	0.23
41–50	31	30 (96.8)	0.68	43	23 (53.5)	0.37
51–60	39	33 (84.6)	0.69	38	13 (32.4)	0.26
>60	65	43 (66.2)	0.29	62	22 (35.5)	0.37
	162	133 (82.1)	0.51	243	128 (52.67)	0.37

GMTs: geometric mean titres.

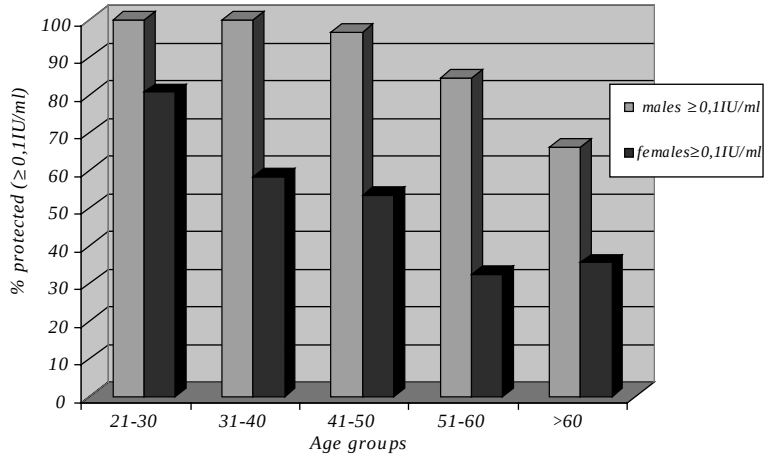

Figure 1. Distribution of Northern Halkidiki population protected against tetanus by age groups and sex.

antitoxin levels, 53% of those aged 60 and over were protected against tetanus [32]. In Australia, 52% of the adult population ($\geqslant$49 years) of the study had protective levels of tetanus antitoxin ($>$0.15 IU/ml). A significant decline in the prevalence of immunity with increasing age was found [33]. Only 12.3% (40/324) of the adult population ($>$49 years) in Guipuzcoa (Spain) is protected against tetanus (17.3% of those in the 50–59 age group vs. 7.7% of those in the over 70 age group). This is in contrast with the situation observed in the infant and adolescent populations [34]. In the USA, 69.7% of the Americans over 6 years of age have protective levels of tetanus antibodies ($>$0.15 IU/ml). The rate decreased from 87.7% in the 6–11 age group to 27.8% among those 70 years of age and older. Most of the 107 cases of tetanus (with 20 deaths) reported in 1989 and 1990 occurred in people 60 years of age and older [7]. In Berlin, among 2079 blood donors, 72% were found protected against tetanus, while the immunity levels were higher among males and decreasing by age [35].

In Toronto, also in a study among blood donors, 17.5% of the participants had a tetanus antitoxin level that was non-protective ($<$0.15 IU/ml) [36]. In Graz, Austria, among 218 subjects who were hospitalized in a dermatology unit 63% were found to be adequately protected ($\geqslant$0.1 IU/ml), 56% showed high antibody concentration above 0.5 IU/ml [37]. In Ankara, Turkey, among 100 individuals over the age of 18, 68% were found to have protective levels of tetanus antitoxin ($>$0.01 IU/ml) without significant difference between males and females while only 35.7% of the individuals over 50 years of age were found protected against tetanus [38].

The distribution of the tetanus-immune people according to sex in our study shows that more males are protected than females (82.1 vs. 52.67%), which can also be demonstrated by the levels of immunity between the various age groups of males and females. This can be explained by two facts: firstly, men receive a booster dose of the vaccine during their obligatory military service and secondly, men of rural areas, as they are more engaged in agricultural activities than women, are more prone to injuries, which means increased possibility of receiving a booster dose of tetanus vaccine in the health center. Men who work in the local mines also receive a booster dose of the tetanus vaccine regularly so they are more protected (all 19 men in our study who work in the mines had $\geqslant$0.1 IU/ml of tetanus antitoxin). In the USA, Gergen et al. [7] also supports that more men are immune than women (79.0 vs. 62.4%), and Alagappan et al. [11] reported similar results (73.0 vs. 41%). In Germany, Klouche et al. [39] found a 15.5% of inadequate protection of which 70% were females. In Spain, Cilla et al. [34] reports that women are less protected from tetanus than men (5.4% of women protected vs. 17% of men) and Ortega et al. [40] reported that the prevalence of antibodies vs. tetanus was significantly greater in males than in females.

The effectiveness of the vaccine is well known. According to a study conducted by our laboratory, children who were vaccinated by receiving 4–5 doses of the vaccine, after 1–9 years from the last dose were found 100% immune. Adults up to the age of 24 and $\geqslant$10 years since the last dose of the vaccine were found 78% immune [41]. In Romania, among young recruits to whom a DT vaccine was administered 5–7 years before, 97.26% were found immune [42]. Alagappan et al. [43] have shown that the immunologic response to tetanus toxoid in the elderly is adequate. From 35 people $\geqslant$65 years old, 86% had protective titers after 2 months and 82% had protective titers after 1 year.

In conclusion, in Northern Halkidiki, the proportion of tetanus-immune people is inadequate (64.4%), the levels of tetanus antitoxin decline with age and there is a statistically significant difference ($p < 0.0001$) in the proportion of protection between males and females. As shown in Table 2 the proportion of immune men until the age of 60 is relatively satisfactory (100–84.6%) and no booster dose is needed. Taking under consideration the fact that if

the percentage of protected people is $<70\%$ a booster vaccination is indicated, a booster dose to men over the age of 60 and women over 30 is recommended. So our results, in accordance with other studies [40, 44] and the guidelines of the Immunization Practices Advisory Committee suggest that routine booster immunization should be administered every 10 years and booster dose after trauma depending on the case [45].

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