ORIGINAL ARTICLE

Tetanus antitoxin levels among adults over 40 years of age in Central Anatolia, Turkey

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Objective To determine tetanus antitoxin levels in adults and the aged.

Methods This study was conducted on 249 adults over 40 years of age who applied to a blood-withdrawal unit in Kayseri, Central Anatolia. Tetanus toxoid-specific antibodies were measured in serum by enzyme-linked immunosorbent assay (ELISA).

Results In the research group, only 63 persons (25.3%) were found to have protective levels (>0.1 IU/mL) of tetanus antitoxin. Antibody levels were significantly higher in educated people (30.7%), in those under 50 years of age (38.2%), in those who had been vaccinated at least twice (47.6%), and in those vaccinated not more than 10 years previously (58.3%). There was no association between antibody level and sex, occupation or place of residence.

Conclusion Our findings indicated that tetanus antibody values were under the protective level for most adults over 40 years of age, and vaccination programs directed at the adult population should be developed.

Keywords Tetanus, antitoxin, adult

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INTRODUCTION

Tetanus is not very common but is associated with a rather high mortality. In 1994 and 1995, 280 cases occurred in Turkey (the annual morbidity rates were 0.25 and 0.20/100 000, respectively), and 84 individuals died (29.8%) [1]. The fatality rate of the disease is greater in neonatals and the elderly. For example, in the USA the fatality rates are 75% in the neonatal period, around 5% in adults under 50 years of age, and 42% in adults above 50 years of age [2]. Neonatal deaths to tetanus bring this disease into the top ten, with regard to deaths from infectious diseases, in the world [3]. However, not only because of improvements in immunization during pregnancy, but also because of births taking place in safer conditions, a reduction in the number of neonatal tetanus cases has been observed recently. In spite of this, particularly in developed countries, the elderly are at greater risk of tetanus than are other groups. For instance, in the USA tetanus in those over 60 years of age occurs at a six-fold higher rate than in other age groups (0.13 versus 0.02 in 100 000) [4]. Furthermore, in a study conducted in Italy, the annual incidence among subjects over 65 years of age was found to be approximately ten times greater than that among younger individuals [5]. Forty-three of 48 annual tetanus deaths occurring in Italy (89.6%), 29 of 32 in Argentina (90.6%), and all of 12 in France and 16 in Portugal, took place in adults and the elderly over 40 years of age [6].

Deaths due to tetanus in Turkey are also occurring more frequently in the older age group. While the rate of those over 40 years of age who died of tetanus as a percentage of total tetanus deaths was 27.4% in 1989, more recently this rate ranged from 44.8% to 60.6% [7–9].

It is obvious that vaccination is the most important measure for effective and extensive protection

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against tetanus. In Turkey, vaccination against tetanus began in the mid-1960s [10], although somewhat irregularly. A systematic vaccination program, including tetanus vaccination, was started by means of a campaign implemented in 1985. At present, after a primary series of three doses of tetanus toxoid, a booster dose is administered to infants at the age of 16 months. Other boosters are administered in classes 1 and 5 of primary school and in class 1 of high school. Although, while women receive two doses of tetanus toxoid during pregnancy (29.3% of pregnant women received two doses in 1998), and men are vaccinated during their military service, there is no comprehensive tetanus vaccination program for adults.

In this study, we aimed to determine the tetanus antitoxin levels of the adult and aged populations and to suggest strategies accordingly.

MATERIALS AND METHODS

This study was conducted between 1 and 30 June 2000 with adults over 40 years of age who applied to the blood-withdrawal unit of the Medical Faculty of Erciyes University, Kayseri, Central Anatolia, for various reasons. On the assumption that 20% of the adults and elderly in this age group will have protective levels of antitoxin, the required sample size was calculated as 246 at a confidence interval of 95% and with a deviation of 5%. According to this, 249 of those who applied were included in the study, and the distribution of sex and age was taken into account. Sera separated from venous blood samples were stored at $-20\,^{\circ}C$ until the time of study. IgG class tetanus antitoxin antibodies in the sera were measured by ELISA (Virotech, Rüsselheim, Germany). The patient sera were diluted (1/100), and patient sera, standard and control sera were pipetted onto microtiter strips. Incubation was at $37\,^{\circ}C$ for 30 min. The microtiter strips were washed four times. The conjugate was pipetted, and this was followed by incubation. The microtiter strips were washed again, and the substrate was pipetted and incubated in the dark. After pipetting, stop solution extinctions were measured at 450/620 nm with a photometer.

The assessment was done according to the method used by Schroder et al. [11]; antitoxin values below 0.1 IU/mL were considered to be below the protective level, and values of 0.1 IU/mL and above were considered to be at a protective level.

The data were analyzed using SPSS 9.0 for Windows. The chi-square test was used for statistical analyses.

RESULTS

One hundred and fourteen (45.8%) of those included in the study were male, and 135 (54.2%) were female. The mean age was 56.2 ± 10.6 years (range 40–85) (male 58.2 ± 11.3, range 40–85; female 54.5 ± 9.6, range 40–80). It was observed that approximately one-third (29.3%) were illiterate or had not finished any school, 37% were primary school graduates, 8.4% were secondary school graduates, and about one-quarter (25.3%) were high school or university graduates. Approximately two-thirds of the adults included in this study (62.2%) resided in cities, 21.7% in towns, and 16.1% in villages or the country.

According to the statements of the subjects, the rate of those with up-to-date vaccinations against tetanus was 29.3%. The vaccination status of the whole group is given in Table 1.

While the tetanus vaccination status is similar in both sexes, the vaccination rate, which is 42.1% in the 40–49-years age group, decreased to 24.0% in the 50–59-years age group, and to 19.4% in the over-60 s ($\chi^2 = 13.500$; DF = 2; $P < 0.05$).

Of those who stated that they had been vaccinated against tetanus, 15.1% had been vaccinated within the last 5 years, 17.8% had been vaccinated 6–10 years ago, and 67.1% more than 10 years before.

A protective level of antitoxin (>0.1 IU/mL) was detected in 65 of the 249 individuals (25.3%) included in this study. The antitoxin levels of adults according to various factors are shown in Table 2.

Table 1 Tetanus vaccination status in adults

Vaccination status	Number	%
Not vaccinated	118	47.4
Did not remember whether or not vaccinated	58	23.3
Vaccinated	73	29.3
Vaccinated once	48	19.3
Vaccinated twice or more	21	8.4
Did not remember number of vaccinations	4	1.6
Total	249	100.0

Table 2 Antitoxin levels of the study group according to various factors

		Having protective level of antitoxin			
	Total number	**Number**	**%**	χ^2	***P***
Age groups				9.575	<0.05
40–49	76	29	38.2		
50–59	75	15	20.0		
60 +	98	19	19.4		
Total	249	63	25.3		
Residential place				0.394	>0.05
Village/town	40	11	27.5		
District center	54	12	22.2		
City center	155	40	25.8		
Total	249	63	25.3		
Sex				2.276	>0.05
Male	114	34	29.8		
Female	135	29	21.5		
Total	249	63	25.3		
Educational status				9.210	<0.05
Illiterate/did not finish school	73	9	12.3		
Primary or secondary school	113	35	31.0		
High school and above	63	19	30.2		
Total	249	63	25.3		
Occupation				5.304	>0.05
Housewife	121	23	19.0		
Official—retired	95	31	32.6		
Other	33	9	27.3		
Total	249	63	25.3		
Number of vaccinations				11.390	<0.05
Not vaccinated	118	19	16.1		
Vaccinated once	48	14	29.2		
Vaccinated twice or more	21	10	47.6		
Total[a]	187	43	23.0		
Last vaccination				6.277	<0.05
Within the last 5 years	11	7	63.6		
6–10 years ago	13	7	53.8		
More than 10 years ago	49	14	28.6		
Total[b]	73	28	38.4		

[a]One hundred and sixty-two individuals who did not remember how many times they were vaccinated were excluded from the assessment.
[b]One hundred and seventy-six individuals who had no vaccination or who did not remember whether they were vaccinated were excluded from the assessment.

Table 2 shows that no significant association was found between place of residence, sex, occupation and tetanus antitoxin levels. While the rate among women whose antitoxin levels are at a protective level is 21.5%, and this rate rises to 29.8% in males; this difference between the sexes is not statistically significant. Although the rates of protective level of antitoxin are 19.0% among housewives and 32.6% among civil servants and the retired, no statistically significant association was found with occupation.

On the other hand, the rate of protective level of antitoxin, which was 38.2% in the 40–49-years age group, decreased to approximately 20% after 50 years of age, which is a statistically significant difference. In addition, while the rate of those whose antitoxin values were at sufficient levels was 12.3% in those who had not finished any school, this rate was over 30% in those who had attended primary school and above; which constitutes a statistically significant difference.

The number of individuals who stated that they had been vaccinated one or more times was 73 (29.3%). Although 62 persons who did not remember whether they had been vaccinated or how many times they had been vaccinated were excluded, antitoxin levels were at protective levels in 16.1% of those who stated that they had never

Table 3 Protective antitoxin levels according to number of vaccinations and last vaccination time

Number of vaccinations remembered	Last vaccination time	Total number	Having protective level of antitoxin		χ^2	*P*
			Number	%		
One	Within the last 5 years	5	1	20.0	0.479	>0.05
	6–10 years before	7	3	42.9		
	More than 10 years before	36	10	27.8		
	Total	48	14	29.2		
Two or more	Within the last 5 years	4	4	100.0	6.239	<0.05
	6–10 years before	6	3	50.0		
	More than 10 years before	11	3	27.3		
	Total	21	10	47.6		

been vaccinated. This rate increased to 29.2% in those who stated that they had been vaccinated only once before, and to 47.6% in those who stated that they had been vaccinated twice or more. The difference between the groups was also found to be statistically significant.

While the rate of individuals who had protective levels of antitoxin was 28.6% in those who stated that they had been vaccinated more than 10 years ago, this rate reached 53.8% in those vaccinated 6–10 years before, and 63.6% in those vaccinated within the last 5 years. The difference between the groups is also statistically significant.

The relationship between antitoxin level and time since vaccination among persons with a similar history of vaccination is shown in Table 3.

As demonstrated by Table 3, no relationship was found between antitoxin levels and time since last vaccination in those who had been vaccinated once. However, the rate of protective antitoxin reached 100% in those who had been vaccinated twice or more, and within the last 5 years. This rate was determined as 50% in those who had been vaccinated 6–10 years before, and 27.3% in those who had been vaccinated more than 10 years before.

DISCUSSION

Tetanus is a disease that can be prevented by the antibodies that develop as a result of vaccination. In this study, it was found that 25.3% of adults over 40 years of age had protective levels of antitoxins. This rate changed depending on the developmental level of the country and the success of immunization programs. For example, in one of the two studies conducted in the USA, the rate of those who had a protective level of antibodies was 27.8% in adults over 70 years of age; in the other study, it was 50% in those individuals above 65 years of age in an urban comprehensive care geriatric center [12,13]. A study conducted in Australia was one of those studies in which a protective level of antitoxin was found to be highest in adults; it was detected in 52% of the population over 49 years of age [14]. In a study conducted in males between 21 and 54 years of age in Israel, where booster vaccinations are given to soldiers, the rate of those with a protective level of antitoxins reached 77.3% [15]. The antitoxin positivity rate was found to be only 27.7% in a group including young adults over 22 years of age in Tokyo [16]. The rate of those with protective levels of antitoxins ranged from 18.2% to 41.3% in studies conducted in different age groups in Turkey [17–19].

In our study, the rate of those with protective levels of antitoxin was approximately two-fold higher in the group under 50 years of age than in the aged. Vaccinations administered to those under 50 years of age during school, military service or pregnancy may explain this. Since the immunization program against tetanus began approximately 35 years ago in our country, neither the 50–59-years age group nor the over-60 years age group received tetanus toxoid at school. Furthermore, as the rate of non-vaccinated persons increases with age in the adults in our study, and the time since last vaccination increases with age in countries like Turkey, where immunization against tetanus cannot be carried out systematically, the association with age is to be expected. Levels of antitoxin decreased inversely proportional to age in other countries which have not yet fully established their immunization programs for adults [14,20].

On the other hand, the rate of those with a protective level of antitoxin among primary school graduates and above was seen to be higher than that among those who did not graduate from primary school and who were illiterate. Individuals who attend schools are routinely vaccinated against tetanus during their education (in classes 1 and 5 in primary schools and in class 1 in high schools in Turkey). They attend health institutions more often and consequently get more boosters during pregnancy and in cases of trauma than those who are in the illiterate group. In addition, considering that the rate of those who did not finish any school reaches 40.8% among people 60 years old and above, it can be suggested that the illiterate population is older and, accordingly, their antitoxin levels are lower. Antitoxin levels were higher in those with more education in a study conducted in the USA [12].

Although antitoxin levels in males were shown to be slightly higher than those in females in our study, the difference is not statistically significant. The antitoxin levels in males were higher in studies conducted in several countries [12–14]. This situation may be due to immunization during their military service, and to men being more subject to trauma outside and during work, and consequently being vaccinated more often. However, it is observed that tetanus vaccinations given to women, particularly during pregnancy, balance this situation.

It is known that housewives and farmers are the groups in which the disease occurs most frequently, and the groups which must be protected by providing higher levels of antibodies [4,21]. Although the difference is not statistically significant, the rate of protective levels of antitoxin was lower in housewives than in the other occupational groups in our study.

The rate of protective levels of antitoxins was found to be three-fold higher in those who stated that they had received tetanus vaccinations twice or more than in those who had never been vaccinated. It is known that booster injections followed by primary vaccination are necessary to elevate antitoxin levels to protective levels and maintain these levels. As the number of vaccinations increases, the rate of protective levels will also increase. It is suggested by the WHO that a protective level of antibodies is achieved in 80–90% of women following two doses in pregnancy, and in 95–98% following the third dose; protection lasts for 5 years and may be extended up to 10–20 years with the fourth and fifth doses [22]. However, the number of these vaccinations will be more significant if added to primary vaccination; so while there was a significant difference in terms of having protective levels of antibodies between the group whose primary vaccination was complete and the group which had never been vaccinated in a study conducted in Turkey, the antibody levels of those vaccinated once or twice and those of the non-vaccinated group were found to be similar [19].

While antitoxin values are at protective levels in 28.6% of individuals whose last vaccination was more than 10 years ago, this rate reaches 63.6% in those vaccinated within the last 5 years. In a study involving age groups ranging from 1 day old to 85 years old in Turkey, a protective level of antitoxin was detected in only 13.3% of those whose last vaccination was 10 years before, and was considerably higher in those vaccinated 2–5 years before [19]. The continuity of protection from the time of the last vaccination may vary, depending on the vaccination having been administered as a primary vaccination or series of vaccinations. Our study supported this. There was no relationship between antitoxin levels and time from last vaccination in those who stated that they had been vaccinated once. However, it was determined that the antitoxin level was higher in those who stated that they had been vaccinated twice or more and in those vaccinated within the last 5 years. The time from last vaccination may vary, depending on whether the vaccination was administered as a primary vaccination or number of vaccinations. According to the immunization program for pregnant women suggested by the WHO, a sufficient level of immunity is ensured for 20 years following the routine fifth vaccination. In our study, the reason for the lack of sufficient levels of antitoxin in around 35–45% of those vaccinated within the last 10 years may possibly be due to the incomplete vaccination of these individuals previously. In fact, while it is reported in some studies that a strong antibody response, which varies among individuals, may result from a booster dose given as late as 30 years after primary vaccination, many studies indicate that a booster dose given 15 years following the primary vaccination will have a very low probability of stimulating a response [22]. In another study, it was found that 32% of the elderly who did not have sufficient levels of antitoxins had been vaccinated within the last 10 years (21%

within the last 3–6 years); and this was attributed to the reduction of antibodies in the elderly due to the shortened immunologic memory [23].

The results obtained in the study are as follows:

- 47.4% of those included in this study stated that they had never been vaccinated against tetanus.
- Of the adults over 40 years of age, 25.3% had tetanus antitoxin protective levels.
- Tetanus antitoxin levels were much lower in those over 50 years of age who stated they had been vaccinated only once before or not at all, and who had been vaccinated more than 10 years before.
- 100.0% of those vaccinated twice or more within the last 5 years had a protective antitoxin level.

In light of these results, we believe that immunization programs for tetanus should be extended to adults and the aged in addition to children and pregnant women. Routine vaccination, which is discontinued after military service in men and pregnancy in women, should be maintained in the context of a program which should be supported by antibody studies, e.g. once every 10 years.

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