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Original Article

Prevalence of Tetanus Immunity in the Kocaeli Region, Turkey

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SUMMARY: We assessed the antibody levels and risk factors for tetanus in an adult population in the Kocaeli Region of Turkey. In 595 individuals over 20 years of age, serum concentrations of anti-tetanus antibodies were detected by enzyme-linked immunosorbent assay and a brief questionnaire was administered. The overall immunity rate was approximately 80% and there was a progressive decline in protection with increasing age. Antibody levels were significantly higher in educated people (80.1%), in employed people (90.3%), in those under 40 years of age (95.0%) and in those who stated that they had received a previous tetanus vaccination (87.9%). There was no association between antibody level and sex, residence, socioeconomic status, military status or history of injury. These data indicate that a vigorous post-injury prophylaxy with antitoxin and antisera should be put into practice, especially for older people, and that immunization programs applied every 10 years must be strengthened.

INTRODUCTION

Tetanus still remains a significant health problem in developing countries, although it is a very rare in developed countries. The global incidence of tetanus is thought to be about one million annually (1). Tetanus is a serious disease with a high mortality rate, but it is easily prevented by vaccination (1). Most cases of tetanus occur in adults who are not immunized or who are inadequately immunized. In developing countries, neonatal tetanus may be responsible for 20-30% of cases and 70% of deaths (2). In 2001, 56 tetanus cases, of which 32 (57%) were neonatal tetanus, were recorded by the Turkish Ministry of Health across the country (unpublished data given with permission). However, in developed countries, the highest incidence and death rates occur in elderly age groups (1-3).

The most effective way of preventing tetanus is by antenatal and childhood immunization. In Turkey, immunization as part of the diphtheria-tetanus-pertussis (DTP) vaccine begins at the 2nd month of life and consists of 3 doses at intervals of 4 weeks. Booster doses include one DTP in the 18th month, one DT in the 7th year (first class of primary school), one Td (tetanus-adult diphtheria) in the 12th year (5th class of primary school), and another Td in the 15th year of life (first class of high school). Additionally, a booster dose is given to men when they start their military service. The Turkish Ministry of Health recommends two injections of tetanus toxoid for every pregnant woman without regard to vaccination history, and three injections for unimmunized pregnant women. A booster dose every 10 years is recommended by health authorities but is not usually administered. Although an antenatal immunization program is used against neonatal tetanus in Turkey, there is no program available for the elderly.

The aim of this study was to determine the antibody levels and risk factors for tetanus in the adult population in the Kocaeli Region and to determine the necessity of establishing immunization program for the elderly.

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MATERIALS AND METHODS

Study population: The present study was conducted at Kocaeli University Hospital. Kocaeli is an industrial region which is representative of the general population of Turkey in terms of its socioeconomic, cultural and ethnic diversity. Kocaeli Province is also the biggest area affected in the earthquake of 17 August 1999, in which more than 16,000 people died and 500,000 were left homeless.

A total of 595 blood samples were collected randomly from outpatients over 20 years of age in April of 2002. Three hundred seventy-nine females and 216 males were studied. After having received their informed consent, 5 ml of blood was taken from patients and a questionnaire was administered to obtain data on the patients' educational and socioeconomic status, residence, history of military service, pregnancy, prior vaccination and history of serious injury. The serum samples were stored at –20°C until tested.

Laboratory methods: Serum concentrations of anti-tetanus antibodies were detected by enzyme-linked immunosorbent assay (ELISA) (Genzyme Virotech GmbH, Rüsselsheim, Germany).

The procedure was performed following the manufacturer's instructions. Patient sera were diluted 1:100 in PBS dilution buffer and 100-μl samples were pipetted into microtiter wells previously coated with tetanus toxin. Standards (0.001 IU/ml, 0.002 IU/ml, 0.01 IU/ml, 0.02 IU/ml and 0.05 IU/ml IgG anti-tetanus antibodies) were tested at the same time as positive controls and dilution buffer alone was also tested as a negative control. Absorbance at 450/620 nm was read using a microtiter plate reader and results were expressed in units based on a standard curve drawn using control sera. Although the accepted threshold value is 0.03 IU/ml, the results were evaluated as follows: <0.15 IU/ml = "susceptible", 0.15-0.99 IU/ml = "basic" protection, ≥1.0 IU/ml = "full" protection (4-7).

Statistical analysis: Age was considered to be a numeric variable, while gender, residence, education, occupation, military service, history of injuery and economic status were

considered to be categorical variables. The categorical variables were shown as frequencies and percentages, the only numerical variable, "age", was shown as an arithmetical mean and standard deviation. The categorical variables were tested with the chi-square test and ordinal variables such as "grouped age" with the Mantel-Haenszel test for linear association. The significance level was set at 0.05. Statistical analysis was carried out with the Epi Info Ver. 6.0 statistical program (Centers for Disease Control and Prevention, Atlanta, Ga., USA).

RESULTS

A total of 595 subjects over 20 years of age were included in the study. Of these, 379 (63.7%) were female and 216 (36.3%) were male. The mean age was 46.66 ± 14.71 years (male, 49.03 ± 16.35, range 20 - 82; female, 45.31 ± 13.53, range 20 - 99). Table 1 shows the age and gender distribution of the study population.

Overall, 26.1% of the population were found to have full protective levels of tetanus antibodies (≥1.0 IU/ml), however, the full protection rate dropped with increasing age. In the <40, 40 - 60 and >60 age groups, the full protection rates were 54.0, 15.3 and 3.4%, respectively (Table 2).

In 54.5% of the total subjects, antibody levels were detected between 0.15 and 0.99 IU/ml, which is accepted to be basic protection. In the <40, 40 - 60 and >60 age groups, the basic protection rates were 41.1, 60.9 and 62.2%, respectively (Table 2).

In 19.5% of the full study group, antibody levels were <0.15 IU/ml, which is defined as susceptible and at risk. In the <40, 40 - 60 and >60 age groups, the susceptibility rates were 5.0, 23.7 and 34.5%, respectively (Table 2). Susceptibility was also found to be significantly increased in subjects older than 40 years of age (Mantel-Haenszel χ^2, $P < 0.05$).

When the age groups were compared according to their immune status, differences were significant and showed a trend (Mantel-Haenszel test for linear association). Immune status was found to be full until age 40, than it gradually shifts to basic between ages 41 and 60, and finally, over the age of 60, it changes from basic to susceptible.

Full protection, basic protection and susceptibility rates were similar in the male and female populations in all age groups ($P > 0.05$) (Table 3).

Table 4 shows associations between antibody levels and various factors when ≥0.15 IU/ml is accepted as a protective level.

The rate of protective levels of antitoxin, which was 95.0% in the <40 age group, decreased to 76.3% in the 40 - 60 age group and to 65.5% in the >60 age group, which is a statistically significant difference ($P < 0.05$). In total, 80.5% of our subjects showed protective levels (≥0.15 IU/ml) of anti-tetanus antibodies.

While the rate of those whose antitoxin values were at sufficient levels was 67.9% in those who had not finished any school, the rate was over 75% in those who had completed primary school or further education, which constitutes a statistically significant difference ($P < 0.05$). A progressive increase in antitoxin values was also detected with increasing educational status.

In addition, while 90.3% of employed people had protective levels of antitoxin, the protective rate was 78.9% in unemployed subjects and 70.0% in retired people ($P < 0.05$).

The rate of individuals who had protective levels of antitoxin was 87.9% in those who stated that they had been vaccinated with tetanus toxoid. This rate declined to 68.4% in those who stated that they had never been vaccinated ($P < 0.05$).

While the protectivity rate in women who had been vaccinated during pregnancy was 97.7%, this rate was 68.5% in nonvaccinated women ($P < 0.05$).

Table 1. Population studied according to age and sex

Age group (years)	Male	Female	Total
<40	69	133	202
40 - 60	86	188	274
>60	61	58	119
Total	216	379	595

Table 2. Tetanus immunity by age for both sex

Age group (years)	No. of subjects	Subjects with antitoxin level of tetanus toxoid*								
		Susceptible (<0.15 IU/ml)			Basic (0.15 - 0.99 IU/ml)			Full (≥1.0 IU/ml)		
		n	%	CI95%**	*n*	%	CI95%	*n*	%	CI95%
<40	202	10	5.0	0.102 - 0.146	83	41.1	0.379 - 0.471	109	54.0	2.615 - 3.441
40-60	274	65	23.7	0.111 - 0.122	167	60.9	0.338 - 0.391	42	15.3	1.962 - 2.661
>60	119	41	34.5	0.102 - 0.119	74	62.2	0.301 - 0.366	4	3.4	0.767 - 4.252
Total	595	116	19.5	0.110 - 0.119	324	54.5	0.352 - 0.392	155	26.1	2.512 - 3.129

*Mantel-Haenszel test for linear association, $P < 0.001$.
**CI, confidence interval.

Table 3. Tetanus immunity by age and gender

Age group (years)	Total	Male				Female			
		No. of susceptible (%)	No. of basic (%)	No. of full (%)	Total	No. of susceptible (%)	No. of basic (%)	No. of full (%)	Total
<40	202	3 (4.3)	26 (37.7)	40 (58.0)	69	7 (5.2)	57 (42.6)	69 (52.2)	133
40 - 60	274	16 (18.6)	53 (61.6)	17 (19.8)	86	49 (26.1)	114 (60.5)	25 (13.3)	188
>60	119	18 (29.5)	42 (68.9)	1 (1.6)	61	23 (39.7)	32 (55.2)	3 (5.2)	58
Total	595	37 (17.1)	121 (56.0)	58 (26.9)	216	79 (21.0)	203 (53.3)	97 (25.7)	379

Table 4. Antitoxin protective level of the study group according to various factors

	Total	Subjects with protective level (≥0.15 IU/ml)		
		n (%)	χ^2	*P*
*Age groups			47.313	<0.05
<40	202	192 (95.0)		
40-60	274	209 (76.3)		
>60	119	78 (65.5)		
Total	595	479 (80.5)		
Gender			1.243	>0.05
Male	216	179 (82.9)		
Female	378	299 (79.1)		
Total	594	478 (80.5)		
Residence			0.006	>0.05
Rural	35	28 (80.0)		
Urban	560	451 (80.5)		
Total	595	479 (80.5)		
*Education			12.424	<0.05
Did not finish school	56	38 (67.9)		
Primary school	187	142 (75.9)		
Secondary school	46	40 (87.0)		
High school	151	129 (85.4)		
University	150	125 (83.3)		
Total	590	474 (80.3)		
Occupation			21.048	<0.05
Employed	186	168 (90.3)		
Unemployed	279	220 (78.9)		
Retired	130	91 (70.0)		
Total	595	479 (80.5)		
Vaccination history			27.037	<0.05
Yes	215	147 (87.9)		
No	257	226 (68.4)		
Total	472	373 (79.0)		
Vaccination history in pregnancy			28.986	<0.05
Yes	168	115 (97.7)		
No	87	85 (68.5)		
Total	255	200 (78.4)		
Military service			2.556	>0.05
Yes	21	20 (95.2)		
No	193	157 (81.3)		
Total	214	177 (82.7)		
Injury history			0.138	>0.05
Yes	178	145 (81.5)		
No	418	335 (80.1)		
Total	596	480 (80.5)		

*Mantel-Haenszel test for linear association.

Antibody levels did not vary by gender, residence, history of military service, injury or socioeconomic status (data not shown) ($P > 0.05$) (Table 4).

DISCUSSION

Tetanus is a prime example of a serious but preventable disease. Effective control requires immunizing every individual by vaccination. Measurement of serum levels of antibody is used to determine protectivity rates.

Although 0.01 IU/ml is considered to be the minimum protective level, this level is usually used when antibody levels are determined in animals by neutralization assay. Enzyme immunoassays and neutralization assays yield different results at antibody levels below 0.2 IU per mililiter, probably because the enzyme immunoassay quantitates antibodies with low levels of neutralizing activity (5,8,9). For this reason, antibody levels above 0.15 IU/ml on enzyme immunoassay can be assumed with reasonable confidence to be protective. Thus, we used this value as our cutoff level (4,5).

The overall immunity rate was approximately 80% in the present study. In studies conducted with different methods and cutoff levels, the status of immunity to tetanus differs between countries. In studies carried out with ELISA testing and a 0.1 IU/ml cutoff level, as in the present study, immunity rates were 72% in Germany (10), 70 and 72% in the USA (5,11) and 68% in Egypt (7). In a study performed in the USA, hemagglutination assay was used with a 0.01-IU/ml cutoff level and tetanus immunity was found to be 46% (12). In two studies performed in Holland, toxin binding inhibition (ToBI) assay was used with a 0.01- IU/ml cutoff level and tetanus immunity was found to be 72 and 86% (13,14). Studies performed in Turkey have also shown different results: 25% (15), 26% (6) and 59.9 - 75.0% (16); in these studies, the same test procedure and cutoff level were used as in our study, however, the immunity rate we detected is higher than that obtained in the other studies. This high result is probably due to the mass vaccination program carried out after the earthquake in 1999.

In the present study, we found a progressive decline in protection with increasing age. This result is consistent with that found in other studies (5-7,10,12-17). Vaccinations administered to younger people during school, military service or pregnancy may explain this result.

In 19.5% of the full study group and 26.9% of subjects over 40 years of age, antibody levels were <0.15 IU/ml, which is defined as susceptible.

Although antitoxin levels in males were shown to be slightly higher than those in females (84.9 and 79%, respectively) in our study, the difference is not statistically significant. Antitoxin levels in males have been found to be higher than in females in studies conducted in several different countries (5,7,10-13,17), but in studies conducted in Turkey, no significant differences have been detected between males and females (15,16). Soyletir et al. detected significantly higher results only in men aged 40 - 60 years (6), and we detected no differences between the sexes. This result may be due to the effective program for vaccination during pregnancy in Turkey. In India, where tetanus toxoid is given to women of childbearing age and especially to pregnant women to prevent neonatal tetanus, the immunity status of women is better than that of men (8).

The rate of those with a protective level of antitoxin among subjects with at least a primary school education was seen to be higher than that among those who did not graduate from primary school (80.1 and 67.9%, respectively, $P < 0.05$), probably because individuals who attend schools are routinely vaccinated against tetanus during their education. Antitoxin levels have been found to be higher in those with more education in several studies (5,12,15).

The immunity rate was higher in employed people than in retired or unemployed people (90.3, 70.0 and 78.9%, respectively, $P < 0.05$), probably due to their increased vaccination rates, which reflect their increased risk of injury. Additionally, retired people fall into the older age group and immunity rates are known to decline with age.

The rate of protective levels of antitoxins was found to be higher in those who stated that they had received a tetanus vaccination at some time than in those who had never been

vaccinated (87.9 and 68.4%, respectively, $P < 0.05$). This is an expected result and is consistent with results obtained in other studies (10,15-16).

Tetanus immunity was also associated with vaccination during pregnancy. The protectivity rate in women who had been vaccinated during pregnancy was higher than in nonvaccinated women (97.7 and 68.5%, respectively, $P < 0.05$).

No significant differences in antitoxin levels were found between men who had and had not completed their military service. Althoug men are vaccinated during their military service and would therefore be expected to show higher protective levels, men who have not yet done their military service are younger than those who have, and the higher levels associated with younger age may account for this lack of association of prective levels with military status.

The lack of association of antitoxin levels with socio-economic status and residence suggests that the existing vaccination program sucessfully reaches different parts of the population equally well, at least in the western part of Turkey.

The present data show that a vigorous post-injury prophylaxy with antitoxin and antisera should be put into practice, especially for older people, and that immunization programs applied every 10 years must be strengthened.

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