# Tetanus Seroprevalence Among Women 15-49 Years Old, in Mersin, Turkey

15-49 Yaş Arası Kadınlarda Tetanoz Seroprevelansı, Mersin, Türkiye

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Yazışma Adresi/Correspondence: Seva ÖNER, MD Mersin University School of Medicine, Department of Public Health, Mersin, TÜRKİYE/TURKEY sevaloner@yahoo.com ABSTRACT Objective: This cross-sectional study, performed in 2005 aimed to investigate the seroprevalence of tetanus among women aged 15-49 years. Material and Methods: Six hundred women recruited from 17 primary healthcare centers were included in the study. ELISA method was used for serological testing. Women with IgG titers of 0.01 IU/mL or higher were considered seropositive. Descriptive statistical analyses were made to summarize the data. Pearson's chi-square, analysis for linear trend in proportions and linear-by-linear association tests were used to compare the variables. Binary logistic regression analyses using the forward LR method and classification tree analysis were performed to examine the association between tetanus seroprevalence and the sociodemographic variables of women. Results: Of the women included in the study, 72.3% were seropositive for tetanus. In only 1.5% of seropositive women, tetanus toxoid (TT) IgG levels were 0.1 IU/mL or higher. Seropositivity for TT decreased with advancing age and over time since the last dose of TT. Women with a history of TT booster during the last 10 years had higher seropositivity rates. Classification tree analyses revealed that the most significant variable in determining seropositivity among women was the period elapsed since the last dose of the TT booster. Conclusion: We suggest that women should be questioned not only for the time of primary vaccination but also the period elapsed since the last dose.

Key Words: Tetanus; seroepidemiologic studies; women

ÖZET Amaç: Kesitsel tipte planlanan ve 2005 yılında yapılan bu çalışma ile 15-49 yaş arası kadınların tetanoz seroprevelansının araştırılması amaçlandı. Gereç ve YöntemLer: Çalışmaya 17 sağlık ocağı bölgesinden 15-49 yaş grubunda yer alan 600 kadın katıldı. Serolojik testler ELISA yöntemi ile yapıldı. Tetanoz IgG titresi 0.01 IU/mL ve üzerinde olan kadınlar seropozitif olarak kabul edildi. Verilerin özetlenmesinde tanımLayıcı istatistikler kullanıldı. Değişkenlerin karşılaştırılmasında "pearson chi-square, analysis for lineer trend in proportions ve linear-by-linear association" testleri kullanıldı. Tetanoz seroprevelansı ile kadınların sosyo-demografik özellikleri arasındaki ilişki "binary logistic regression ve classification tree analysis" testleri ile değerlendirildi. Bulgular: Çalışmaya katılan kadınların %72.3'ünde tetanoz seropozitifliği tespit edildi. Seropozitif kadınların sadece %1.5'inde tetanoz toksoid (TT) IgG düzeyi 0.1 IU/mL ve üzerindeydi. TT seropozitifliği yaş artışı ve son doz TT'den sonra geçen süre arttıkça azalmaktaydı. Son doz TT'den sonra geçen süre 0-10 yıl arasında olan kadınlarda seropozitiflik oranı daha yüksekti. Kadınların seropozitif olup olmadıklarını ayırmada en etkili değişkenin "TT'in son dozundan sonra geçen süre" olduğu "classification tree" analizi ile saptandı. Sonuç: Kadınların aşı öyküsü sorgulanırken primer aşı yanı sıra son dozdan sonra geçen sürenin de mutlaka sorulmasını öneriyoruz.

Anahtar Kelimeler: Tetanoz; seroepidemiyolojik çalışmalar; kadınlar

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etanus is an acute and fatal disease, which is caused by the exotoxin of *Clostridium tetani*. Spores of *C. tetani* are usually found in soil and in the intestinal system of both animals and humans.<sup>1</sup> Although te-

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tanus is a 100% preventable disease with vaccination, it is still the second most common cause of death due to a disease preventable by vaccination. Up to 60-90% of tetanus cases among newborns are fatal. Neonatal tetanus (NT) can result form dissection of the umbilical cord under aseptic conditions or inappropriate care of the umbilical wound. Children surviving this disease will have significant brain damage.

Since fertile women are vaccinated with TT and the labor services improved NT incidence decreased worldwide, especially in developing countries in the past decade. The number of children dying due to NT throughout the world is estimated as 200.000.<sup>4</sup> Although the World Health Organization (WHO) started a program in 1989 to eliminate NT, NT is still endemic in 90 countries with the majority of cases occurring in tropical Asian and African countries.<sup>5,6</sup>

TT vaccination was initiated in 1968 in our country and in 1980 an extended immunization program was implemented.<sup>7,8</sup> The Maternal Neonatal Tetanus Elimination Program started in our country in 1984 aiming to decrease the NT incidence below 1 per 1000 live births in all regions until 2007. At the end of 2005, 32 cases were reported and the incidence was calculated as 0.02 per 1000 live births.<sup>9</sup>

Since 2003, the adult diphtheria-tetanus toxoid (Td) vaccination scheme has been used for pregnant women. According to this scheme, pregnant women with no history of previous vaccination receive two doses of Td, which are administered one month apart after the 4<sup>th</sup> month of pregnancy. The third dose is administered at least 6 months after the second and the fourth dose, at least one year after the third. A fifth dose is also administered one year after the 4<sup>th</sup> dose.<sup>8</sup> In Turkey, the mean number of prenatal care visits is 2.4 during pregnancy and 47% of women receive two or more Td vaccine doses.<sup>9,10</sup> In addition, 16.4% of all deliveries are performed at home and this rate increases up to 49% in some regions of the country.<sup>9,10</sup>

In Turkey, NT is still a major public health problem. Determining the status of tetanus immu-

nization of fertile women and performing additional vaccination as required are among the major preventive measures. In order to investigate the level of tetanus immunity in our region, we performed a multipurpose study titled 'Tetanus and Rubella seroprevalence among women aged between 15 and 49'. The study was supported by the Research Foundation of Mersin University and was conducted in 2005. A sub-analysis of this project was published in the *Vaccine* in 2007 as 'Rubella seroprevalence in women in the reproductive period, Mersin, Turkey'.<sup>11</sup> The present study evaluated tetanus seroprevalence among women aged 15- 49 years.

### MATERIALS AND METHODS

Mersin is a province in southern Turkey that lies on the Mediterranean coast. The local ethical committee and other official institutions approved the study protocol. This project was carried out according to the Helsinki declaration principle.

The total number of women aged 15-49 years who lived in central Mersin and surrounding villages was 230.352. EPI 6 INFO program was used to calculate the minimum sample size. Considering the tetanus seroprevalence 60%, the worst acceptable ±4 and the 95% confidence interval (CI) the minimum sample size was calculated as 575. 12-19 However in order to achieve a more significant result, an increasing sample size was planned and 650 subjects were included in the study.

The region where the study was conducted includes 52 primary health care centers, 26 of which were located in villages. One third of the primary health care centers (6 out of 17 centers in villages) were included in the study. Primary health care centers were stratified into urban and rural centers and were selected by systematic sampling.

Considering the ratios in the population, the final sample size was estimated to include 550 women from urban and 100 from rural primary health care centers. The number of subjects to be sampled from each center was determined according to the ratio of 15-49 year-old women registered in that center and systematic sampling was used.

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A data form and an informed consent form were prepared in order to collect and record data. The following were questioned in the data form: residence, age, educational status, marital status, pregnancy status, verbal history of tetanus vaccination, total monthly income of the family, and status of cigarette and alcohol consumption and the status of migration to Mersin. The informed consent form included information on tetanus, NT, measures of protection and the selection criteria of the study.

The study team visited the selected primary health care centers. Women to be enrolled in the study were selected by the systematic sampling method using the household population recording slips (the standard recording form that includes the socio-demographic data about people who live in the area covered by the primary health care centers). Among the 650 women who were included in the study group, 609 were available by contact and they were asked to visit the institution. The women who visited the institution were informed about the study and were asked to read and sign the informed consent form. The data form was filled out by a face-to-face interview with 609 women who accepted to participate in the study after signing the informed consent form. Following the completion of the questions in the data form, 5-7 cm<sup>3</sup> of venous blood was drawn from each woman. Two women were excluded from the study because venous access was not possible. Since tetanus IgG enzyme-linked immunosorbent assay (ELISA) kit enabled analysis of 600 blood samples, an additional seven women were randomly excluded from the study. Blood samples were transported to the microbiology laboratory on the same day under cold chain conditions; sera were extracted and kept at-20°C until the day the tests were performed.

All serological studies were performed at the Microbiology Laboratory of Mersin University School of Medicine. Genzyme Virotech Tetanus IgG ELISA kit (Germany, Rüsselsheim, product no: EC 124.00, lot no 172-01T) was used for the ELISA test. A total of 600 patients were included in the study. The cut-off value was accepted 0.68. For values above the cut-off value, the mean dose ratios

acquired from the system, in IU/mL (international unit/mL), were determined and women with a tetanus IgG titer of 0.01 IU/mL and above were considered seropositive. <sup>1,20</sup>

Tetanus antitoxin levels were classified according to the generally adopted protective levels, <0.01 IU/mL no protection, 0.01-0.1 IU/mL weak protection, and ≥0.1 IU/mL full protection. The results of the serologic tests were reported to the participants. Women who were susceptible to tetanus (seronegative women) were advised to have TT vaccine.

#### Statistical Analysis

Descriptive statistical analyses were made to summarize the data. The Pearson's Chi-square test ( $\chi^{2P}$ ), analysis for linear trend in proportions ( $\chi^{2\text{trend}}$ ) and linear-by-linear association tests ( $\chi^{2\text{LA}}$ ) were used to compare the variables. Binary logistic regression (BLR) analyses using the forward LR method and classification tree analysis were performed to examine the association between tetanus seroprevalence and the sociodemographic variables of women.

### RESULTS

The mean age of the participants was  $30.7 \pm 9.3$  years. Of the women, 566 (94.3%) were literate, 446 (74.3%) were married, 432 (72%) were housewives and 520 (86.7%) were living in urban areas (Table 1). Of the women, 459 were married or widowed (76.5%) and the mean gravidity among this group was  $2.4 \pm 1.6$ , while the mean parity was  $2.1 \pm 1.5$ , and the mean number of living children was  $2.0 \pm 1.4$ .

Overall, 72.3% were immune to tetanus and 27.7% were not. Only 9 (1.5%) of the immune women had strong immunity (TT IgG $\geq$ 0.1 IU/mL), while 425 (70.8%) showed a weak immunity profile for tetanus (TT IgG = 0.01-0.1).

While the immunity rate in the 15-19 years age group was 87.9% it decreased to 35.5% in the 40-49 age group. There was a negative linear relation between the age group and the immunity rate (p< 0.001). Immunity rates were 41.2% and 80.9% for literate women and those who had completed 8 years of primary education, respectively. There was

TABLE 1: Demographic features of women and tetanus seropositivity.							
Variables	Total		Seropositivity		Test value	df	p value
	n	%	n	%			
Age							
15-19 years	91	15.2	80	87.9	$\chi^{2LA} = 98.2$	1	< 0.001
20-29 years	197	32.8	173	87.8			
30-39 years	179	29.8	134	74.9			
40-49 years	133	22.2	47	35.3			
Educational status							
Illiterate	34	5.7	14	41.2	$\chi^{2LA} = 34.5$	1	< 0.001
Literate	25	4.2	12	48.0			
Primary School	258	43.0	179	69.4			
Secondary School and above	283	47.2	229	80.9			
Family income							
<minimum td="" wage<=""><td>262</td><td>43.7</td><td>187</td><td>71.4</td><td><math>\chi^{2P} = 0.2</math></td><td>1</td><td>&gt; 0.05</td></minimum>	262	43.7	187	71.4	$\chi^{2P} = 0.2$	1	> 0.05
≥Minimum wage	338	56.3	247	73.1			
Employment							
Housewife	432	72.0	302	69.9	$\chi^{2P}$ = 16.9	2	< 0.001
Employed	83	13.8	55	66.3			
Student	85	14.2	77	90.6			
Marital status							
Married	446	74.3	310	69.5	$\chi^{2P} = 6.9$	1	< 0.05
Other	154	25.7	124	80.5			
Migration status							
Migrated	299	49.8	213	71.2	$\chi^{2P} = 0.4$	1	> 0.05
Original resident	301	50.2	221	73.4			
Residence							
Urban	520	86.7	380	73.1	$\chi^{2P} = 1.1$	1	> 0.05
Rural	80	13.3	54	67.5			

a positive linear relationship between TT immunity rate and the level of education (p< 0.001, Table 1).

The mean number of tetanus vaccination doses among the study population was 5.6 (ranging from 0 to 14); 75% had 4 or more vaccinations. This ratio was 89% for the 15-19 years age group, 51.1% for the 40-49 years age group, 41.2% for the illiterate group, and 86.6% for those who had completed 8 years of primary education. Of women who had a history of four or more TT vaccinations, the seropositivity rate was 81.3%, while this ratio was 50.8% for women who had 1-3 TT vaccinations and 11.5% for those who had received no vaccination. Seropositivity showed a linear increase with the number of vaccines received (p< 0.001, Table 2). While seropositivity increased during the first 4 vaccine administrations, it slowed down after additional vaccinations (Table 2).

The median time after the last TT vaccine was 5 years (ranging from 0 to 47). The seropositivity rate was 95.1% for women who received an additional vaccine 0-11 months after their last dose. This ratio was 81.1% for those who received an additional dose 5 years after the last dose, and 43.1% for the ones that received the dose after 10 years. Seropositivity showed a linear decrease with increasing time after the last TT vaccine (p< 0.05, Table 2). Figure 1 demonstrates the changes in seropositivity rates in relation with the time after the last vaccination in individuals who received 1-3 doses, 4-6 doses, and more than 6 doses of TT vaccine, respectively. In all three groups, seropositivity decreased while the time after the last dose of TT vaccine increased.

The factors that affect tetanus seropositivity were evaluated by BLR analysis, which showed

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TABLE 2: Changes in seropositivity according to the number of TT doses and the time elapsed after the last dose of TT. Seropositivity Seronegativity n n **Odds Ratio** Test value df р Number of TT doses 0 3 11.5 23 88.5 1.00 5 23.8 16 76.2 2.40 2 30 52.6 27 47.4 8.52 3 28 60.9 18 39.1 11.93  $\chi^{2trend} = 81.05$ 1 < 0.001 4 52 80.0 13 20.0 30.67 5 83.1 37.64 54 11 16.9 6 68 76.4 21 23.6 24.83 7 76 80.9 18 19.1 32.37 8< 118 86.1 19 13.9 47.61 The time elapsed after the last dose of TT (years) 0 58 3 4.9 95.1 1.00 1 61 95.3 3 4.7 1.05 2 38 92.7 3 7.3 0.66 3 49 86.0 8 14.0 0.32 37 90.2 4  $\chi^{2trend} = 113.85$ < 0.001 4 9.8 0.48 7 5 30 81.1 18.9 0.22 6 28 93.3 2 6.7 0.72

24.4

27.3

40.9

56.5

0.16

0.14

0.07

0.04

that seropositivity decreased 0.87 fold (95% CI 0.84-0.91) and 0.94 fold (%95 CI 0.91-0.96) with the increase in time after the last TT vaccine administration and with increasing age, respectively (Table 3). This confirms the decrease in seropositivity with increasing time after the last TT vaccination (Figure 1).

34

16

13

67

75.6

72.7

59.1

43.5

11

6

9

87

7

8

9

10≤

Factors affecting tetanus seropositivity also underwent evaluation with the classification tree analysis. According to this analysis, the most effective variable in determining immune and nonimmune women was the time elapsed after the last TT vaccination. Seropositivity was higher in subjects who received their last dose within the last 0-10 years. For subjects whose last vaccine dose was administered more than 10 years ago, the determining factor was the individual's age. Seropositivity was higher among women who were 33.5 years old or younger. On the other hand, in women older than 33.5 years, the number of doses was the determining factor. Seronegativity was higher among women who received 4 or less TT

**TABLE 3:** Evaluation of risk factors that affect tetanus seropositivity with BLR analyses.

Variables	Ехр (β)	95%	95% CI	
	(Odds ratio)	Lower	Upper	
The time elapsed after the last dose of TT	0.87	0.84	0.91	< 0.001
Age	0.94	0.91	0.96	< 0.001

doses. For those who had more than 4 doses, gravidity was the determining factor; when gravida was 2.5 or less, seronegativity was higher and when gravida was more than 2.5, seropositivity was higher (Figure 2).

## DISCUSSION

While seropositivity rates to tetanus vary between 28.6% and 99.4% in various countries, the rates are 35-76.6% in Turkey (Table 4). 12,13,15,16,18,19,22-24 Tetanus seropositivity rates in this study are similar to the results of previous reports from our country (except for the results of Kalyoncu et al). 12 However, our ratios are higher than those reported from

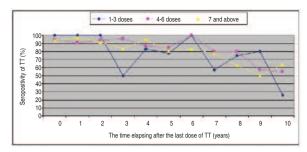


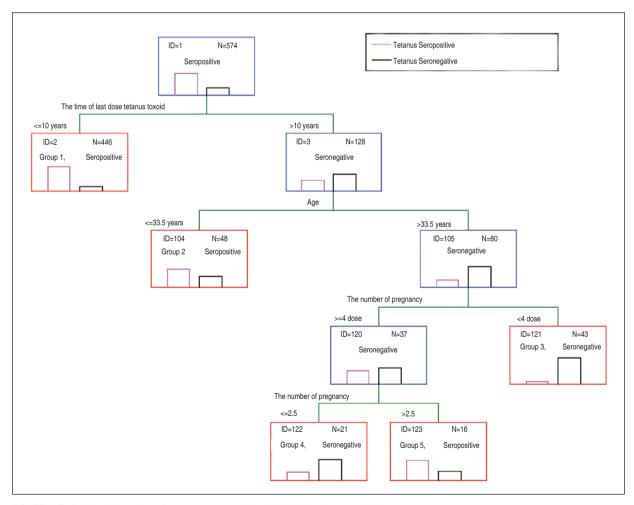
FIGURE 1: Seropositivity according to the number of TT doses and the time elapsed after the last dose of TT.

Niger and Peru and lower than those reported from England and Holland. 15,19,22,23 These differences may be attributed to different vaccination schemes. Although the seropositivity rates determined in this study seemed to be higher than those reported in other studies, the immunity levels in our study we-

re relatively lower and this finding suggests the need for an additional booster dose of TT for women in our region.

Studies from our country and others report that tetanus immunity decreases with increasing age. <sup>12,15,16,22,25</sup> Karabay, Tuncer Ertem and Öztürk reported tetanus immunity rates for individuals aged 40, 50 and 60 years and older as 25.3%, 20.2% and 15.7%, respectively. <sup>26-28</sup> Our study also supports this finding with the demonstration of decreased tetanus seropositivity with increasing age. This result may be related to the absence of TT vaccination in the advanced age group or due to lack of additional booster doses.

Our study revealed that increased educational level led to increased immunity. This result is also



**FIGURE 2:** Evaluation of factors that affect tetanus seropositivity with classification tree analysis. ID: Number of node.

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TABLE 4: Similar studies conducted in Turkey and other countries.							
Author	Country/Province	Study Group	TT Seropositivity (%)				
Maple PAC et al <sup>22</sup>	England	15-49 years	90+				
De Melker HE et al <sup>15</sup>	Netherlands	15-49 years	72.4-99.4				
Vernacchio L et al <sup>19</sup>	Peru	Pregnants	61.2				
Okafor GO et al <sup>23</sup>	Nigeria	Pregnants	28.6				
Kalyoncu et al <sup>12</sup>	Turkey/Eskişehir	20-49 years	76.6				
Ergönül et al <sup>16</sup>	/Ankara	18 years above	66.7				
Kalaça et al <sup>13</sup>	/İstanbul	Women after childbirth	64.8				
Sagsöz et al <sup>17</sup>	/Kırıkkale	Pregnants	64.6				
Bozkurt et al <sup>18</sup>	/Van	Pregnants	53				
Akyol et al <sup>24</sup>	/Konya	Women after childbirth	35				

concordant with the results reported by Kalaça et al.<sup>13</sup> This can be attributed to the increased ability of educated women to seek health care and to more systematic vaccination of younger people in the recent years according to the national vaccination scheme.

Kalaça et al and Koeing et al reported increased immunity with increasing number of TT doses and Kurtoglu et al and Öztürk et al reported decreasing immunity with increasing time after the last TT vaccination. 13,25,28,29 Our study also demonstrated a strong relationship between seropositivity and the number of TT doses as well as the time passed after the last dose of TT. Individuals who received vaccination within the previous 10 or more years demonstrated significantly decreased seropositivity. In addition, no significant changes were detected in tetanus seropositivity in cases who received 4 or more doses of TT and the time after the last dose had a significantly greater effect on seropositivity. This was an expected result, since it takes 3-4 TT vaccines and an additional dose 10 years after the last dose to establish and maintain an effective immunity against tetanus.1

Classification tree analysis also demonstrated that the time after the last dose was the most significant factor in immunity. Women who were older than 33.5 years and who had their last dose more than 10 years ago, demonstrated decreased immunity. Reports of Okada et al. and Atabey et al. also support these findings.<sup>30,31</sup>

# CONCLUSION

Consequently, our study demonstrated that although 72.3% of the participants were immune against tetanus, an additional booster of TT was required for almost all women in order to maintain adequate immunity. It is obvious for our country, where highrisk pregnancies are still an important problem, that immunity ratios should be increased. For this purpose: I) all pregnant women should be reached by health professionals, II) all these pregnant women should receive qualified prenatal care including tetanus immunization, III) deliveries should be performed under appropriate, healthy conditions, IV) an additional booster dose of TT should be administered every 10 years following the last dose, V) seronegative women should be determined and they should receive primary vaccination.

Variables acquired after classification tree analysis, i.e. the time after the last TT dose, age of the woman, number of TT vaccines and the number of gravidities of the woman should be considered while evaluating seronegative women.

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