

Prevalence of tetanus immunity in the Egyptian population

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Abstract. Our aim was to determine the level of tetanus immunity in the general Egyptian population. The surveyed population consisted of 709 healthy subjects from five regions of Egypt, 2 month to 105 years of age. We used an ELISA assay to determine titers of anti-tetanus IgG. According to the widely used criteria, 31.7% of the total population was susceptible to tetanus (IgG level < 0.15 IU/ml), 15.7% had basic protection (0.15–1.0 IU/ml), and 52.6% had full protection (> 1.0 IU/ml). The results revealed that the majority (68.3%) of the population from 2 months to 50 years had a protective level of IgG against tetanus. The level of susceptibility increases with age until it reaches 90.3% in females who are over than 60 years olds. Significantly, more males (23.7%) than females (41.3%) were unprotected against tetanus. Our results suggest that monitoring immunization status and administering the tetanus vaccine as required are essential to insure adequate and long-lasting antibody levels.

1. Introduction

Tetanus is a serious disease with high mortality, and although it is very difficult to treat, it is easily prevented by vaccination [1]. Tetanus is acquired through inoculation of wounds with spores of *Clostridium tetanus* [2]. Tetanus is not directly communicable between hosts, therefore, vaccination cannot confer herd immunity. Effective control requires immunizing every individual by vaccination. Although some have reported natural immunity, it is believed that immunity to tetanus toxin is induced only by vaccination [1,3].

In order to achieve adequate levels of herd immunity and to prevent outbreaks, it is necessary to monitor the immunity levels of the general population and to identify and vaccinate insufficiently protected groups. The regular vaccination was introduced into Egypt in the beginning of 1970. Children are vaccinated at 2, 4, 6, and 18 month of age with a Diphtheria, Tetanus and Pertussis “whole cell” (DPT).

The object of this study was to evaluate the prevalence of tetanus immunity in Egypt. For tetanus, the preliminary data [4] indicate that the tetanus cases decreased from 790 cases in 1995 to 383 cases in 1999.

2. Material and methods

2.1. Sample

In the period from Jan 1988–Nov 2000, a total of 709 apparently healthy male and female subjects with age range 0.2–105 years were collected. The samples correspond to approximately 0.002% of the Egyptian population. They were randomly selected from those applying to analytical laboratories for blood chemistry tests in five cities representative of north, central and south Egypt (Alexandria, Kalioubia, Cairo-Giza, Sohag, and Kena). All the subjects were residents in these cities or relevant suburbs and were organized in the study according to sex and age, representing all sectors of the general population. Samples were sorted by age and sex into the following age groups: 0–10 years, 11–20 years, 21–30 years, 31–40 years, 41–50 years, 51–60 years, and > 60 years old (Table 1), serum samples were frozen and stored at -75°C until antibody testing was performed.

Table 1
Population studied according to age and sex

Age group (years)	Males	Females	Total	Mean age (years)	Standard deviation of mean (years)
0-10	78	94	172	2.35	2.23
11-20	28	38	66	15.72	2.97
21-30	81	54	135	26.03	2.92
31-40	79	47	126	35.69	2.79
41-50	64	28	92	45.28	3.03
51-60	37	28	65	55.25	3.19
> 60	22	31	53	72.39	11.29
Total	389	320	709	29.56	21.21

Table 2
Tetanus immunity by age for both sexes

Age group (years)	Number of subjects	Subjects with antitoxin level TT								
		Susceptible (< 0.15 IU/ml)			Basic (0.15-1.0 IU/ml)			Full (> 1 IU/ml)		
		No.	%	CI95%	No.	%	CI95%	No.	%	CI95%
0-10	172	64	37.2	0.01-0.02	36	20.9	0.66-0.75	72	41.9	2.03-3.0
11-21	66	16	24.2	0.008-0.01	18	27.3	0.33-0.60	33	50.0	1.62-3.0
21-30	135	25	18.5	0.007-0.02	18	13.3	0.65-0.73	92	68.1	1.91-2.71
31-40	126	28	22.2	0.011-0.03	20	15.9	0.64-0.76	78	61.9	2.23-3.22
41-50	92	30	32.6	0.006-0.01	9	9.8	0.52-0.81	53	57.6	1.87-3.0
51-60	65	26	40.0	0.005-0.02	8	12.3	0.61-0.81	30	46.2	1.61-3.3
> 60	53	36	67.9	0.004-0.01	2	3.8	0.56-0.79	15	28.3	1.7-4.21
Total	709	225	31.7	0.01-0.033	111	15.7	0.35-0.85	373	52.6	1.5-3.11

Table 3

Age specific prevalence of subjects lacks a protective tetanus antitoxin level (< 0.15 IU/ml) according to sex. The overall prevalence was 23.7% in males and 41.3% in females. CI is the confidence interval

Age group (years)	Males				Females			
	Total	No. susceptible	%	CI95%	Total	No. susceptible	%	CI95%
0-10	78	23	29.5	0.01-0.03	94	41	43.6	0.01-0.02
11-21	28	6	21.7	0.004-0.02	38	10	26.3	0.005-0.02
21-30	81	8	9.9	0.005-0.04	54	17	31.5	0.001-0.02
31-40	79	15	18.9	0.004-0.03	47	13	27.7	0.008-0.040
41-50	64	21	32.8	0.006-0.01	28	9	32.1	0.004-0.009
51-60	37	11	29.7	0.004-0.009	28	14	50.0	0.005-0.037
> 60	22	8	36.4	0.002-0.005	31	28	90.3	0.004-0.008
Total	389	92	23.7	0.01-0.030	320	132	41.3	0.01-0.041

2.2. ELISA test

Serum samples were tested for tetanus toxoid immunoglobulins G (IgG) by enzyme linked immunosorbent assay (ELISA) according to [2,5-8].

The microtiter plates (Corning-Costar, UK) were coated with purified tetanus toxoid (TT) 3 µg/ml (VAC-SERA, Agouza-Cairo, Egypt), in 0.05 M carbonate/bicarbonate buffer pH 9.6. The plates were incubated for 3 hours at room temperature and then at 4 °C overnight. The TT solution was removed from the plates, and then the plates were sealed and stored at 4 °C. The plates were used over test period of 5 months without loss of sensitivity. The plates were washed with PBS pH 7.2 containing 0.05% tween 20, and then

the plates were blocked with 1% BSA-0.05% tween 20 in PBS for 1 hour at 37 °C. The diluted serum (1:100 in PBS-0.5% BSA-0.05% tween 20) was distributed in triplicate aliquots of 100 µl/well and incubated for 1 hour at 37 °C. After incubation, peroxidase-labelled goat anti-human IgG (H+L) conjugate (KPL, Gaithersburg, MD, USA) diluted 1:1500 in PBS-BSA-tween 20 buffer was added. Plates were incubated for 1 hour at 37 °C, washed with PBS-BSA-tween 20, then with PBS only. The ready-made TMB peroxidase substrate (Sigma, MO, USA) was added to the plates. The reaction was blocked after 10 minutes by adding 0.5 M HCl. The absorbance values of the diluted sera were measured at 405 nm using automatic multiscan plate reader (iEMS Labsystem Analyzer, MD, USA). After reading,

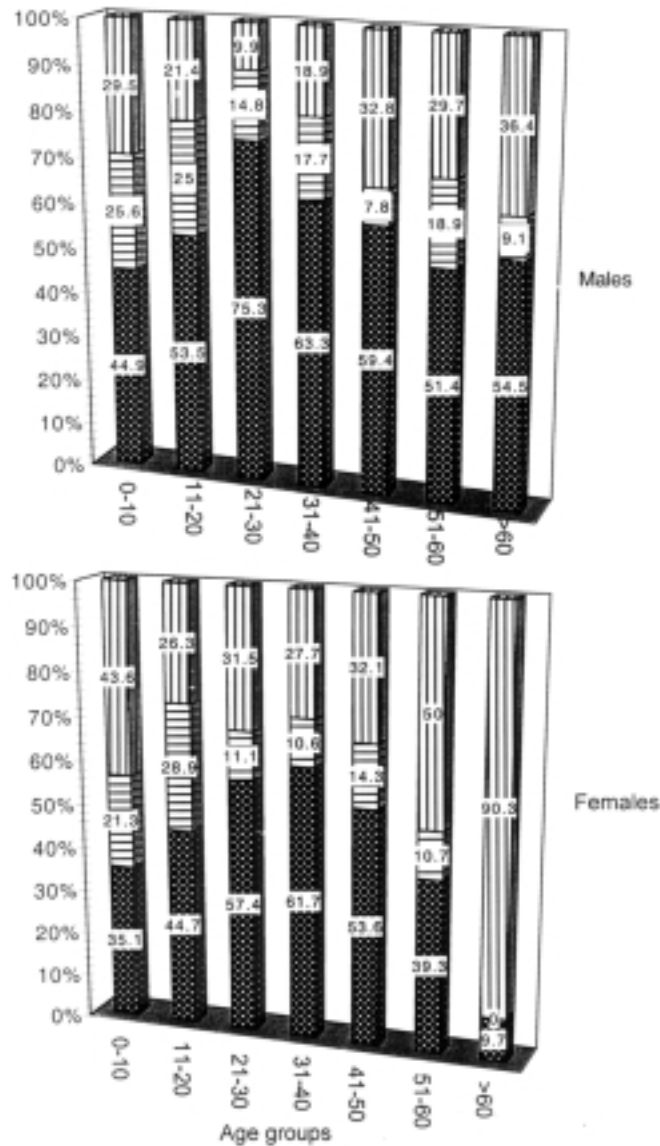


Fig. 1. Age specific prevalence (%) of tetanus antitoxin levels in males and females. Full protection (white dots), basic protection (horizontal lines) and susceptible (vertical lines).

the absorbance were converted to antitoxin titer in IU ml⁻¹ using a six point calibration curve (0.01, 0.05, 0.1, 0.5, 1.0, 1.5 IU ml⁻¹), prepared by using WHO tetanus anti-toxin standard serum.

Although the ELISA is specific, sensitive, and considerably more accurate than the neutralization assay, the two assays yield different results at antibody level below 0.2 IU/ml [6]. In addition, there are not established criteria for interpreting antitoxin levels. We have chosen levels of below 0.15 IU/ml as susceptible, levels 0.15–1.0 IU/ml as a basic protection and levels more

than 1.0 IU/ml as a full protection [2,6,8]. Differences in proportions were evaluated using the student-*t* test. A *p* value < 0.05 was considered to be significant.

3. Results

Tables 2 and 3 show the distribution of tetanus immunity levels by age groups, 31.7% of the population studied was susceptible to tetanus, 15.7% had basic immunity and 52.6% were fully protected. More than

81% of those aged between 0–30 years had sufficient levels of immunity against tetanus, while there is some decline in protective immunity, from 77.8% in 31–40 year age group to 67.4%, 58.5% and 32.1% in the age groups of 41–50, 51–60, and more than 60 years, respectively. The unexpected result was in children of age 0–10 years, 62.8% had sufficient protection, while 29.5% of males were significantly ($p < 0.05$) less susceptible than females (43.6%) for the disease.

The prevalence of tetanus antitoxin levels varied by sex (Fig. 1). The full protection was found in 59.1% of the males, and in 43.4% of the females ($p < 0.05$). Significance ($p < 0.05$) differences based on sex were observed throughout all age groups, except age group of 41–50. In the age group > 60 years the difference between the males and the females was highly significant ($p < 0.005$), with 36.4% of males and 90.3% females susceptible.

4. Discussion

The lack of indirect protection for tetanus, together with ubiquity of tetanus spores means that every individual without tetanus protection is at risk [1]. Our study shows that the status of tetanus immunity in the Egyptian population, 31.7% susceptible, 15.7% basic protection, and 52.6% fully protected is comparable to other countries [1]. These results show that the currently used vaccination program in Egypt is effective, but more effective vaccination policy for both adults and especially the young would be beneficial. Vaccination against tetanus and diphtheria should be mandatory for admission to school and upon graduation.

We have found that there is insufficient protection against tetanus among the older participants, 67.9% of subjects greater than 60 years of age. This may explain the 383 tetanus cases reported in 1999 [4]. However, the low immunity levels 37.2% (29.5% males, and 43.6% females) of the age 0–10 years, as in diphtheria (38.5% males, and 47.9% females) [10], may be explained through the socioeconomic dimension of the population. Some population sectors need public campaigns including mass audio-visual media to increase awareness for the necessary vaccination schedules.

We show that the tetanus immunity levels decrease with increasing age. More than 67.9% of the population tested aged above 60 years is susceptible for the disease (Table 2). This pattern of tetanus immunity is consistent with reports from other countries [1,6,9, 11–19].

Gender was independently associated with immunity status against tetanus as in diphtheria [10]. This effect was observed in females that were significantly less likely being to protected (Fig. 1). Other studies have also shown significant differences between tetanus antitoxin levels in men and women [2].

In conclusion, the immunity prevalence against tetanus observed in Egypt is concerning, and our results are in agreement with reports from other countries [2,6,8,20–23]. These studies also recommended a booster immunization for elderly individuals who had already completed a primary vaccination regimen and a primary series of tetanus toxoid vaccination for unvaccinated individuals. Further, vaccination is important in developing countries where vaccination efforts are hindered by cold-chain problems, sub optimum application practice, and high prevalence of concomitant infection.

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