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ANTI-TETANUS IMMUNOGLOBULIN ISOTYPES IN WOMEN OF CHILDBEARING AGE

K. AHMADI AND M.B. ESLAMI*

*From the Department of Immunology, Baghiatollah University of Medical Sciences, Tehran, and the *School of Public Health, Tehran University of Medical Sciences, Tehran, I.R. Iran.*

ABSTRACT

Neonatal tetanus (NT) is a leading cause of neonatal mortality in many parts of the world. Tetanus is a vaccine-preventable disease and is second only to measles worldwide as a cause of childhood mortality. In this study the various immunoglobulin classes of anti-tetanus antibody in the sera of 105 Iranian women of childbearing age (13-45 years) were titrated by enzyme linked immunosorbent assay (ELISA). Our results demonstrate that the majority of women (96.29%) had protective anti-tetanus toxoid antibody. All women were negative for IgE. 92.39%, 91.43% and 80.96% of women were found to be positive for IgG, IgA and IgM, respectively. Therefore, with respect to lack of protective anti-tetanus antibody in a minority of women, our results suggest that this group might be at risk of tetanus disease and if they get pregnant, need to be vaccinated against tetanus in order to produce sufficient Ab for their own protection and to provide their fetuses with anti-tetanus antibody.

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INTRODUCTION

Tetanus remains a fatal disease despite the advancement which has taken place in the treatment of infectious diseases. The disease is due to the exotoxins of *Clostridium tetani* and specific antibody against the major exotoxin of this organism, tetanospasmin, has the ability to neutralize it. Immunization of mothers during pregnancy would protect their infants through the transplacental passage of maternal anti-tetanus antibody.[1] Due to marked differences in the biological activity of immunoglobulin (Ig) subclasses, it has been found that the human antibody class of importance in neutralizing tetanus toxin in mice is IgG.[2,3] Human IgM and IgA classes appeared to neutralize tetanus toxin at a higher concentration than IgG.[4] It has also been reported that neonatal tetanus (NT) is a leading cause of neonatal mortality in many parts of the world. This vaccine-preventable disease is second only to measles worldwide as a cause of childhood mortality.

MATERIALS AND METHODS

Collection of sera

Volunteer woman subjects with age ranging from 13 to 45 years were randomly chosen and divided into two main groups. The members of the first and second groups received one or two injections respectively of 1070 Lf commercial aluminium-phosphate-adsorbed tetanus toxoid (Razi Institute, Iran). Blood for serological study was collected and after clotting, the serum was separated by centrifugation and stored at -70°C until analysis.

Solid phase ELISA

Goat IgG antihuman IgG/ peroxidase conjugate (γ-chain specific), goat IgG anti-human IgM/peroxidase conjugate (μ-chain specific), goat IgG anti-human IgA peroxidase conjugate (α-chain specific), goat IgG anti-human IgE/peroxidase conjugate (ε-chain specific), goat anti-human polyvalent immunoglobulin/peroxidase

Table I. Distribution of all vaccinated women according to Ab class.

Results		Positive		Negative	
Row	Class of Ab	Number	Percentage	Number	Percentage
1	Total Ab	101	96.19	4	3.81
2	IgM	85	80.96	20	19.04
3	IgG	97	92.39	8	7.61
4	IgA	96	91.43	9	8.57

conjugate and the substrate 2,2' azino-bis 3-ethyl benz-thiazolin-6-sulphonic acid (ABTS) M.W.= 548.7 were all purchased from Sigma Co.

ELISA technique was used to titrate the anti-tetanus serum antibody level.[5-7] Wells of microtiter plates (Costar Co.) were coated with tetanus toxoid (5 µg/0.1 mL PBS) under different test conditions. Many variables were tested in order to establish a satisfactory procedure. In this report only the optimized assay will be described. The method that was finally chosen was incubation at 37°C for 3h and then at 4°C overnight. Plates were sealed and stored at 4°C. Briefly the antigen-coated plates were washed and incubated with 100 µL (0.1%) of bovine serum albumin (BSA) in phosphate buffer saline (PBS). After washing, 100 µL of serum samples, standards and negative controls were diluted in 0.1% PBS and added to microplate wells. After incubation and washing, 100 µL peroxidase-conjugated antibody (diluted in 0.05% Tween 20 in PBS) directed against the class of immunoglobulin under test was added and the reaction revealed with ABTS substrate. Finally the reaction was stopped wtih 0.1 mol hydrofluoric acid (HF) and the optical density (OD) measured by a multiscan ELISA reader (Multiscan microtitre plate-reader) at 492 nm. The end point in all assays was the last dilution giving an OD of greater than 0.1 in comparison to negative control serum.[5]

Statistical analysis

The results were expressed as the mean of experiment ± standard error of mean (SEM) or ± standard deviation (SD). Unpaired t-test was used to determine the probability (p) of two sets of data from experiments being different from each other.

RESULTS

Table I shows the total population who received one or two doses of tetanus toxoid. 3.81% had no anti-tetanus antibody in their sera. In the first group 5 out of 53 and in the second group, 3 out of 52 mothers were negative for IgG antibody (Tables II, III). Tables II and III give corresponding data for mean titers of anti-tetanus antibody. The 16.98% and 15.38% of women with one and two injections had the highest reciprocal titers of antibody (14.96 $\log_2$), respectively (Tables II, III). High numbers of both groups of women produced anti-tetanus antibody. 79.24% of women in the first group and 80.76% of women in the second group produced antibody with reciprocal titers ranging from 6.64 to 13.96. A second injection of tetanus toxoid increased the percentage of women with antibody titers above 14.96 (Tables II and III). Figs. 1 and 2 show that there are no significant differences in duration of having protective antibody in the serum in women who received either one or two doses of tetanus toxoid. Therefore in both

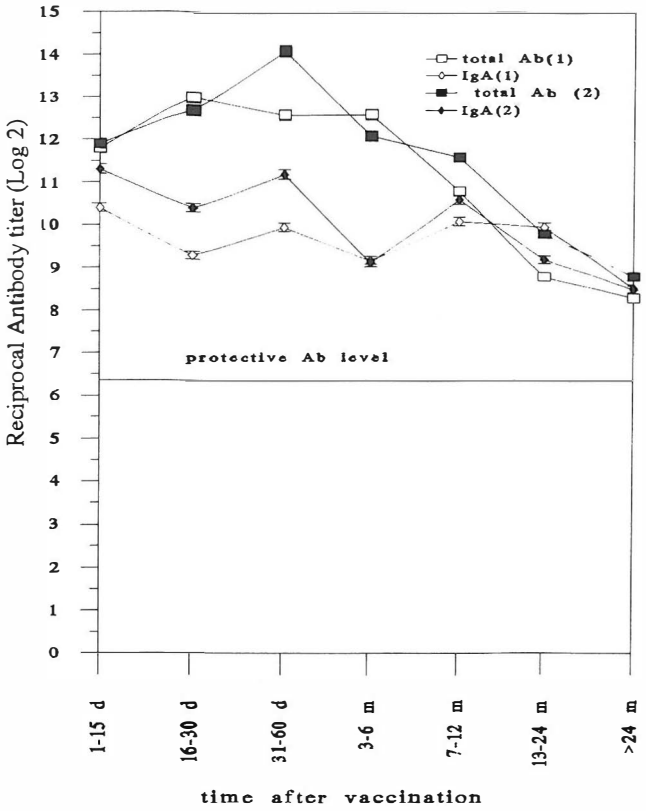

Fig. 1. Antibody (Ab) titer (total and IgA) in women after the first and second vaccination.
d: day m: month.

Table II. Antibody titers of women who received a single injection of tetanus toxoid.

Row	Ab titer	Class of Ab							
		Total Ab		IgM		IgG		Ig A	
	Log_2	No.	%	No.	%	No.	%	No.	%
1	Negative	2	3.7	5	9.4	5	9.4	4	7.5
2	6.64	4	7.5	14	26.4	5	9.4	12	22.6
3	8.96	7	13.2	10	18.8	9	16.9	11	20.7
4	9.96	4	7.5	3	5.6	8	15	7	13.2
5	10.96	7	13.2	11	20.7	6	11.3	12	22.6
6	11.96	10	18.8	4	7.5	8	15	-	0
7	12.96	7	13.2	3	5.6	7	13.2	1	1.8
8	13.96	3	5.6	2	3.7	4	7.5	2	3.7
9	14.96	9	16.9	1	1.8	1	1.8	4	7.5

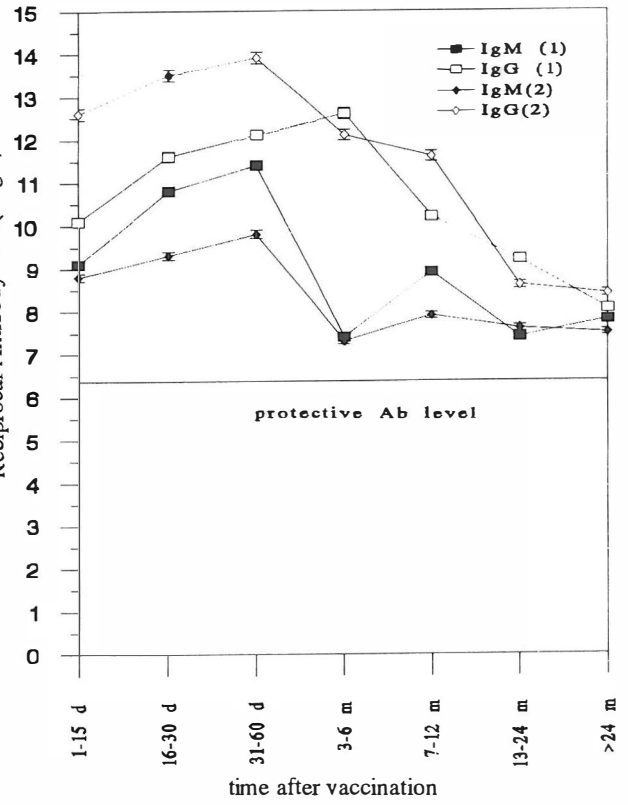

Fig. 2. Antibody (Ab) titer (IgM and IgG) in women after the first and second vaccination.
d: day m: month.

groups sufficient antibody levels were maintained up to 12 months.

DISCUSSION

Neonatal tetanus (NT) has been recognized by WHO as one of the most fatal diseases to be under control and TT vaccination in women of child-bearing age should be considered as the major strategy for NT prevention.[6-8] According to data provided in this research project, women who received two doses of tetanus toxoid produced significantly higher antibody titers up to 60 days postvaccination. Moreover, in both groups more than 90% of women had sufficient anti-tetanus anitibody levels up to 24 months postvaccination. These findings correspond well to the results reported by others.[1,9] Abacioglu et al.[9] reported that mothers immunized during pregnancy as well as their newborns had significantly higher antibody concentrations than mothers immunized at least a year before their last pregnancies.

In this investigation we found a high level of IgM and IgA antibodies in 80.96% and 91.43% of women respectively. These results are in agreement with those of Engstrom[10] who reported the presence of IgA antibody against tetanus toxoid. It has also been reported that human IgM and IgA show little, if any, neutralization of tetanus toxin as compared with human IgG.[4] Therefore, the presence of these two classes of antibody in vaccinated women may not be able to protect women from tetanus. Ourth[4] believed that IgM and IgA may be mainly directed towards toxoid sites which result in a non-neutralizing antibody response. Despite the lack of detectable antibody in a minority of vaccinated women, nonetheless vaccination is considered to be the best measure for preventing mothers and infants from developing tetanus.[11,12] The type of nutrition and HLA

Table III. Antibody titers of women who received two injections of tetanus toxoid.

Row	Ab titer	Class of Ab							
		Total Ab		IgM		IgG		IgA	
	Log_2	No.	%	No.	%	No.	%	No.	%
1	Negative	2	3.8	15	28.8	3	5.7	5	9.6
2	6.64	7	13.4	19	36.5	7	13.4	10	19.2
3	8.96	6	11.5	11	21.1	9	17.3	10	19.2
4	9.96	4	7.7	3	5.7	8	15.3	9	17.3
5	10.96	9	17.3	2	3.8	5	9.6	8	15.3
6	11.96	5	9.6	2	3.8	3	5.7	2	3.8
7	12.96	10	19.2	-	-	6	11.5	2	3.8
8	13.96	1	1.9	-	-	8	15.3	2	3.8
9	14.96	8	15.3	-	-	3	5.7	4	7.7

haplotype might be responsible for unresponsiveness and low responsiveness to tetanus toxoid.[13,14] Moreover, it is not surprising if a minority of the population in this study show no detectable anti-tetanus antibody. Supporting our data, Gergen[15] has recently reported that a group of American people did not maintain a protective antibody against tetanus toxoid despite undergoing vaccination. Finally, in contrast to lack of detectable IgE antibody in the present investigation, others reported the presence of specific IgE antibody after booster immunization with tetanus toxoid.[2,16-18] The reason that IgE was not detected in this investigation might be related to the vaccine used. The vaccine used in this study only contained tetanus toxoid, while others used a combination of diphtheria-pertussis (DP) or diphtheria-pertussis-tetanus (DPT) vaccine.

Taken together, it can be concluded that with respect to the lack of detectable IgG antibody in a minority of women, some mothers and their infants are at risk of acquiring tetanus. Therefore it is recommended that obstetricians and gynecologists encourage pregnant women to become fully vaccinated against tetanus. This study also suggests that measurement of Ig isotypes might be important in determining the immune status of individuals receiving different vaccine preparations.

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