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IMMUNITY TO TETANUS IN MALE ADULTS IN DAR ES SALAAM, TANZANIA

S. ABOUD, E.F. LYAMUYA, E.K. KRISTOFFERSEN and R. MATRE

ABSTRACT

Objective: **To determine immunity to tetanus in male blood donors with previous diphtheria-pertussis-tetanus (DPT)/tetanus toxoid (TT) vaccination.**
Design: **A cross sectional study, conducted in September 1999.**
Setting: **Blood bank, Muhimbili Medical Centre, Dar es Salaam, Tanzania.**
Methods: **Using an antigen competition ELISA technique, serum tetanus anti-toxin levels in two hundred male blood donors were determined.**
Results: **Vaccination history was absent in 43 (21.5%) blood donors, whereas 60 (30%) and 97 (48.5%) reported childhood DPT and TT vaccination, respectively. Tetanus anti-toxin was undetectable in 47 (23.5%) blood donors and the levels were below that considered protective (≥0.1 IU/ml) in 25 (12.5%). Among those with undetectable level, 43 (91.5%) had no vaccination history. Time after last DPT/TT vaccination correlated significantly with tetanus anti-toxin levels (r^2=-0.331, p=0.001). In multivariate analysis, TT doses received and time after last vaccination explained 4.8% and 29.4%, respectively, of the variations in tetanus anti-toxin levels.**
Conclusion: **Seventy two (36%) male blood donors were susceptible to tetanus and the susceptibility was highest from 48 years. A regular TT booster dose at 10 yearly intervals is recommended to provide adequate and long lasting immunity in male adults. Proper keeping of vaccination records is emphasised.**

INTRODUCTION

Tetanus is recognised as a priority problem and is one of the six target diseases of the WHO Expanded Programme on Immunisation (EPI) since 1974(1). EPI has documented that the number of non-neonatal tetanus (NNT) cases reported to WHO from developing countries ranged from 90,335 in 1980 to 35,749 in 1990, whereas it ranged from 8,968 cases in 1980 to 4,812 cases in 1990 in industrialised countries(2). The clear fall in the reported NNT incidence in developing countries since the mid 1980s coincided with the introduction of tetanus toxoid (TT) immunisation programmes(2).

In Tanzania, immunisation has been integrated into maternal and child health (MCH) clinics since 1975. Diphtheria-pertussis-tetanus (DPT) doses are given to infants at 4, 8 and 12 weeks of age and a TT booster dose is given to the child either during outreach services or following injury/wound infection in individuals who seek treatment from hospitals. For previously un-immunised women of childbearing age, a five-dose TT schedule is administered through MCH clinics, school health programmes or special outreach services. In the early seventies tetanus had a mortality rate of 19.6%-20.6%(3-4). To date tetanus still remains a public health concern and the mortality has increased despite the country's wide immunisation programme. It is still common among residents of Dar es Salaam and it is the second commonest cause of admission (1994 – 1996 Hospital Registry) at Muhimbili Medical Centre (MMC), intensive care unit (ICU) with relatively high morbidity and mortality(5). Previous retrospective study of 106 cases of NNT treated over a three-year period in the ICU of MMC, revealed that 73 (68.9%) patients had no TT vaccination. Twenty seven (25.5%) were in the age group of 41-50 years and males were predominant (87.8%) with a mortality rate of 73.6%(5). Recent retrospective analysis of ICU admissions at MMC over a one-year period from January to December 1997, showed that NNT patients accounted for 34 (10.3%) of all admissions with a mortality rate of 76.5%(6). The reported immunisation coverage of third dose of DPT vaccine for children under five in Dar es Salaam was 88%(7). Recent studies showed that the current DPT/TT immunisation schedules provide adequate tetanus immunity for women of childbearing age(8), and children under five although about half of the 6-15-year-old children had no protection against tetanus(9). Data on immunity to tetanus following DPT/TT vaccination in male adults are still not available. The objective of this study was therefore to determine immunity to tetanus in male blood donors from MMC blood bank, Dar es Salaam, Tanzania.

MATERIALS AND METHODS

This was a cross-sectional study conducted in September 1999. All blood donors attended during that study period were included. Two hundred apparently healthy male blood donors

were examined at the time of donation from MMC blood bank, Dar es Salaam, Tanzania. Ethical clearance and informed consents were obtained before inclusion. History of vaccination with DPT and/or TT was obtained from each participant. Blood samples were drawn, serum was separated after centrifugation, inactivated at 56°C for 30 min and stored at -20°C. All sera were then transported on dry ice from Dar es Salaam to Bergen, Norway and were stored at -20°C until the time for assay.

Tetanus anti-toxin assay: Tetanus anti-toxin level was determined by an antigen competition ELISA assay as previously described(10). The assay is suitable for quantifying tetanus anti-toxin in low titer sera and the results correlate well with results of the *in vivo* neutralisation test. Tetanus anti-toxin level of 0.1 IU/ml was considered protective as documented previously(11). Briefly, TT at 0.75 LF/ml was bound to standard ELISA plates diluted in carbonate buffer pH 9.6 at 4°C overnight. The plates were stored at 4°C for up to four weeks for subsequent use.

The next day, the plate was washed three times with phosphate-buffered saline with 0.05% Tween 20 (PBST) and was blocked with 100 µl of PBST, and 2% bovine serum albumin (BSA) per well. The plate was incubated at 37°C for one hour. After washing three times with PBST, twelve two-fold serial dilutions of each test serum were prepared in PBST beginning with 1:8. In parallel, there were equivalent dilutions of the test serum diluted in PBST-BSA and 0.1 LF/ml of TT. Similar dilutions of tetanus immunoglobulin 1.1 IU/ml (WHO International Lab for Biological Standards, Copenhagen, Denmark) were added to the plate. Known positive and negative control sera were also included in every assay. The plate was incubated at 37°C for one hour. After three washings, 100 µl of horseradish peroxidase goat anti-human IgG (Dako A/S, Copenhagen, Denmark) diluted 1/1000 in PBST were added to each well and incubated at 37°C for one hour. After washing three times with PBST, 50 µl of substrate, orthophenylene diamine (Dako A/S) were added to each well. Adding 50 µl of 4 N sulphuric acid stopped the reaction. The optical density was determined at 492 nm in an ELISA plate reader (Emax™, Sunnyvale, CA, USA). A 4-parameter standard curve was constructed by plotting the average optical density of the duplicate for each dilution of the standard against its respective concentration. The lowest detection limit of the assay was 0.0005 IU/ml.

HIV-1 serology: All serum samples were screened for anti-HIV-1 antibodies by Behring Enzygnost antiHIV-1/2 plus ELISA (Behringwerke, Germany). Reactive samples were further tested by Wellcozyme anti-HIV-1 ELISA (Murex Diagnostic Ltd., Dartford, UK). Samples reactive on both assays were considered seropositive for HIV-1 antibodies and those with discordant reactivity were confirmed by a Western blot.

Statistical analysis: The data were analysed on SPSS for Windows version 9.0(12). Since the distribution of tetanus anti-toxin levels was skewed, log transformation of anti-toxin levels was performed and geometric mean levels were determined. Student's t-test was used to evaluate the differences in geometric mean tetanus anti-toxin levels with respect to age, vaccination status, TT doses received, HIV status and time after last DPT/TT vaccination. Correlation coefficient (r^2) between tetanus anti-toxin level and time after last vaccination was determined. Multiple regression analysis (linear and stepwise) to study the independent contribution of age, vaccination status, TT doses received, HIV status and time after last vaccination to the prediction of tetanus anti-toxin level was performed. The goodness of fit of the linear model was assessed and expressed as adjusted multiple coefficient of determination (R^2). The limits of the confidence in the prediction from a regression equation were determined by standard error (SE) of the estimate. The model was tested for statistical significance by F-ratio test. The effect of each independent variable was adjusted for the possibility of distorting influences from other independent variables and was expressed as standardised regression coefficient (ß). The alpha level for rejecting the null hypothesis was 0.05.

RESULTS

The median age of blood donors was 31 years (range 18-70 years). Forty three (21.5%) blood donors did not report a vaccination history. Among those who reported vaccination histories, 60 (30%) had been vaccinated with DPT vaccine during childhood, whereas 97 (48.5%) had only vaccination of 1-3 TT doses as prophylaxis following injury/wound infection. None had received both DPT and TT vaccination, and documentation/vaccination records were not available.

Table 1

Geometric mean tetanus anti-toxin levels (IU/ml) according to various characteristics among male blood donors with reported vaccination (n=157) in Dar es Salaam, Tanzania

Characteristic		No.	Protected (%)	Anti-toxin (IU/ml)	Mean difference (95% CI)	p-value
Age (years)	18-27	59	49 (83.1)	0.38		
	28-37	64	55(85.9)	0.36	0.02 (0.62;1.77)	0.877
	38-47	19	17(89.5)	0.30	0.08 (0.61;2.63)	0.521
	48+	15	7(46.7)	0.08	0.30 (0.74;3.72)	0.002
Vaccination status						
	DPT	60	40(66.7)	0.33		
	TT	97	88(90.7)	0.72	-0.39 (0.31;0.93)	<0.001
TT dose (s)	1	35	28(80.0)	0.15		
	2	46	44(95.7)	0.53	-0.38 (0.07;0.82)	0.001
	3	16	16(100)	1.95	-1.80 (0.23;2.17)	<0.001
HIV status	Positive	10	7 (70.0)	0.26		
	Negative	147	121(82.3)	0.41	-0.15 (0.21; 0.73)	0.135
Time after vaccination (years)						
	<10	123	109(88.6)	0.48		
	≥10	34	19(55.9)	0.16	0.3 (0.79;2.58)	0.001

TT=tetanus toxoid; DPT=diphtheria-pertussis-tetanus; CI=confidence interval

Table 2

Predictors for tetanus anti-toxin level among male blood donors with reported vaccination (n=157) in Dar es Salaam, Tanzania.

Independent variable	Adjusted R^2	SE	ß	p-value
Time after last vaccination	0.294	0.526	-0.352	<0.001
TT doses	0.048	0.315	0.249	0.0044

TT=tetanus toxoid; R^2=multiple coefficient of determination; SE= standard error, ß=standardised regression coefficient.

Tetanus anti-toxin was undetectable in 47 (23.5%) blood donors and the levels were below that considered protective (≥0.1 IU/ml) in 25 (12.5%). The number of blood donors and the levels of tetanus anti-toxin (IU/ml) were 2 (0.001-<0.01), 23 (0.01-<0.1), 90 (0.1-<1.0), 34 (1.0-<5.0) and 4 (≥5.0). Among those with undetectable level, 43 (91.5%) had no vaccination history. Tetanus anti-toxin level was age dependent and fell to the lowest levels in those aged ≥48 years (Table 1). Those that reported TT vaccination had significantly higher mean tetanus anti-toxin levels than those with DPT vaccination did. Individuals who had 2-3 TT doses had significantly higher anti-toxin level than those with a single TT dose did. Mean tetanus anti-toxin levels were similar in HIV-seropositive and seronegative individuals. The mean time that elapsed after last vaccination was approximately six years (range 1 month-24 years). Time after last vaccination had significant effect on tetanus anti-toxin levels. There was also significant correlation between time after last vaccination and anti-toxin levels (r^2=-0.331, p=0.001). Multiple regression analysis revealed that TT doses received and time after last vaccination predicted significantly the variations (F=34.934, p<0.001) in tetanus anti-toxin levels (Table 2).

DISCUSSION

The World Bank has reported that vaccination is the most cost-effective public health intervention for infectious diseases(13). The present study investigated immunity to tetanus in male blood donors. The study has a selection bias because self-selection of male blood donors can often mean that they are healthier than the general population.

Our findings showed that tetanus anti-toxin was undetectable in 47 (23.5%) blood donors and the levels were below 0.1 IU/ml in 25 (12.5%). This could be explained by the fact that a substantial proportion of individuals (21.5%) did not report a primary or vaccination at all, which is important in stimulating early immune response to TT. Since the introduction of TT immunisation programme in 1975, only individuals who were less than 25 years had an opportunity to get early DPT vaccination. Among those, 60 individuals reported to have been vaccinated during their childhood. Our findings do reveal a higher proportion of tetanus immunity when compared to a study done in Burma (14), but lower compared to those reported elsewhere(15-17). The higher proportions of tetanus immune persons in developed countries were most likely due to early introduction of vaccination programmes since 1940. The findings of non-protective levels with age are comparable to those from studies by other workers (18-20), which have reported increasing susceptibility to tetanus with increasing age. Younger vaccinated individuals were more likely to be protected than older people were. The predominant occurrence of clinical tetanus in the majority of male adults with no TT vaccination in Dar es Salaam(5) reflects that the affected individuals lacked immunity to tetanus. Thus, immunity to tetanus can only be achieved through vaccination with TT and this should be given to both adults and children including individuals who have suffered from clinical tetanus(11). However, some studies reported occurrence of clinical tetanus in persons with antitoxin levels higher than the arbitrary protective level(21-22). Overwhelming toxin level as well as presence of none or poorly neutralising antibody subclasses in the cases could explain the occurrence of disease in such individuals who have the so called protective anti-toxin levels. Improved vaccination programmes are required to reduce the number of non-immune persons particularly those at great risk of acquiring tetanus.

Susceptibility to tetanus as shown by non-protective levels of anti-toxin was higher (88.4% versus 18.5%) among those who did not report vaccination. However, among those who did not report vaccination, five (11.6%) were immune to tetanus. This could be due to a failure to recall the previous vaccination received, as there is no residual immunity following recovery from clinical tetanus. Without documented records, it is difficult to know whether previous vaccinations were adequate. Simonsen *et al*(23) and Yuan *et al* (16) have previously reported the association between lack of vaccination records and non-protective levels of tetanus anti-toxin. Thus, this underscores the importance of proper keeping of vaccination records. It has also been reported(11) that proper documentation of TT vaccination is essential because it is important in guiding the spacing of subsequent booster doses without hyper-immunising previously vaccinated individuals.

Our findings also showed that blood donors with TT vaccination had significantly higher mean tetanus anti-toxin levels than those with DPT vaccination did. This could be due to waning of tetanus anti-toxin levels among blood donors who had primary vaccination alone as none had secondary series of vaccination. Secondary vaccination is important in priming the primary immune response. TT doses received also predicted 4.8% of the variation in tetanus anti-toxin levels. Individuals born before the introduction of immunization programme had no opportunity to receive childhood DPT doses and they reported to have been vaccinated with 1-3 TT doses as prophylaxis following injury/wound infection instead. It was not possible to determine the intervals in which the TT doses were administered in these individuals. Roy *et*

al(24) demonstrated that the degree and duration of immunity increases with the number of TT doses given. It has also been reported(11) that proper intervals between doses of TT in the initial series increase the height and duration of the immune response.

Mean tetanus anti-toxin levels were similar in HIV-seropositive and seronegative individuals, a finding that has been reported previously(25). HIV infection interferes with antibody responses to antigen encountered after infection has occurred, but affects less severely the antibody responses of lymphocytes educated prior to infection(26). It is possible that these HIV-seropositive individuals were vaccinated before they became infected with HIV. TT as a monovalent vaccine or as a component of combined vaccines is recommended for HIV-infected adults and children regardless of the presence or absence of symptoms of AIDS.

Mean tetanus anti-toxin levels were significantly lower, ten years after last vaccination. Time after last vaccination also explained 29.4% of the variation in tetanus anti-toxin levels. Waning of tetanus anti-toxin levels could explain our findings. According to TT immunisation schedule adopted, there is no regular re-vaccination with TT after primary vaccination. Thus, none of the blood donors had received both primary and secondary vaccination. Individuals who received only three DPT doses in early infancy or in whom the interval between doses is more than 10 years could have diminished capacity to respond to a TT booster dose(11). Simonsen *et al*(27) suggested the possibility of an insufficient response to a booster dose given 17-20 years after primary vaccination. Thus, some developed countries recommend the use of tetanus diphtheria (Td) vaccine every 10 years to maintain immunity against tetanus in adults(11). Our findings support the need for a regular TT booster dose and appropriate timing of vaccination.

It is concluded that 72 (36%) male blood donors are susceptible to tetanus and the susceptibility is highest from 48 years. A regular TT booster dose at 10 yearly intervals is recommended to ensure adequate and long lasting immunity in male adults. Proper keeping of vaccination records is emphasised.

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