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Tetanus Antibody Status in Mothers and their Infants

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Summary

Awotedu, K. O., Williams, A. I. O., Salimonu, L. S., Akinkugbe, F. M. and Osunkoya, B. O. (1979). *Nigerian Journal of Paediatrics*, **6(2) 34. Tetanus Antibody status in Nigerian Mothers and their Infants.** An indirect haemagglutination method using glutaraldehyde treated sheep red blood cells (SRBC) was adapted for the assay of tetanus antibody levels in the sera of 35 Nigerian mothers and their respective infants before and after a tetanus immunization schedule. Sixty per cent of the children's tetanus antibody levels before immunization were closely related to the initial antitoxin level in their respective mothers. Eighty six per cent of the infants showed progressive increase in antitoxin level after successive immunizations. Children who initially had very low antibody titres eventually had high titres.

For more than half a century, it has been known that tetanus antibody crosses through the placenta from mother to foetus (Ten Broeck and Bauer, 1922; Schofield, Turker and Westbook, 1961). Suggestions have been made that newborn infants might be passively protected against neonatal tetanus antigen by active immunizations of their mothers (Nattan-Larrier, Ramon and Grasset, 1927; Bull. Wld. Hlth. Org., 1950). Not all Nigerian mothers have the advantage of being immunized although tetanus toxoid is now becoming generally available. Thus, Paediatricians and Obstetricians have recently been recommending routine preventive immunization of pregnant women and also ensuring that infants receive complete courses of triple vaccination (Diphtheria, Tetanus and Pertussis vaccines).

In order to provide practical guidance to immunization policy for infants in paediatric clinics in countries where tetanus is common, several methods have been used by investigators for measuring tetanus antitoxin levels since Stavitsky (1954) applied Boyden's haemagglutination method for the titration of antitoxins (1951). Peebles *et al.* (1962) have used the mice neutralization test for measuring tetanus antibody titres in some paediatric patients. Sgouris (1972) has also detected and quantitated diphtheria

and tetanus antibodies in sera using counter-immunoelectrophoresis. Formalinized sheep erythrocytes have been used by Czimas (1960) for passive haemagglutination test.

In the present study, attempts were made to measure the antibody levels of mothers (some of whom were not immunized during pregnancy) and their respective infants before and after a course of tetanus immunization. Sheep red blood cells were treated with glutaraldehyde before being used for passive haemagglutination assay on the sera.

Materials and Methods

Patients' Population

Tests were carried out on sera obtained from 35 apparently normal mothers and their respective last born infants, attending the Institute of Child Health Clinic, University of Ibadan, Nigeria. The ages of the children at the time the specimens were collected ranged between three months and one year. Twenty-one of the 35 children had completed three tetanus toxoid immunizations at 3, 4 and 5 months of age while one started at one year. A further ten of the thirty-five children had only two immunizations while four had only one immunization. Of the thirty-five children, one had an exchange blood transfusion during the period of study. Medical records of each patient were screened to exclude laceration or injury requiring the administration of tetanus toxoid.

During the first visit to the clinic, 5ml of blood was collected from both mother and child. On the second and third visits to the clinic, blood was collected only from the children before immunizations. Serum samples were obtained by centrifugation after the clot had retracted at room temperature and were thereafter stored at −20°C.

Antigen

Glutaraldehyde–treated sheep erythrocytes tanned with tannic acid were coated with commercially prepared toxoid (Lister Institute, Elstree, England). A checker board titration of different concentrations of both the antigen and antitoxin was carried out to find the concentration of antigen which was most suitable. It was found that a 1:16 dilution of toxoid was most suitable.

Standard Antitoxin

Tetanus Antitoxin 1500 I.U. in 1ml. (Hoechst Pharmaceuticals Ltd., Lagos) was diluted down to 5 units/ml with normal rabbit serum in Phosphate Buffered Saline (PBS), pH 7.2.

Buffered Saline and Diluent Solution

PBS solutions were used at pH 7.2 and pH 5.5. The diluent solution consisted of 1 per cent rabbit serum in PBS pH 7.2. Few drops of sodium azide were added as a preservative.

Treatment of sheep erythrocytes (RBC) with glutaraldehyde

Sheep erythrocytes in Alsever solution was centrifuged at 3000 r.p.m. for 20 minutes at room temperature and washed thrice with 0.15M. NaCl. After the last wash, the packed cells were chilled at 4°C in an ice bath.

Twenty-five per cent glutaraldehyde was diluted to one per cent with a solution containing one volume of 0.15M PBS pH 7.2, nine volumes of normal saline, and five volumes of distilled water. Known volumes of the chilled glutaraldehyde salt solution were used to suspend the packed red cells and the mixture of cells and glutaraldehyde was incubated for 30 minutes at 4°C with occasional mixing. The glutaraldehyde-treated cells were collected at room temperature and washed five times with normal saline as well as five times in distilled water. The cells were resuspended to a concentration of 30 per cent in distilled water. Few drops of one per cent sodium azide were added as a preservative. The cells were stored at 4°C.

Passive haemagglutination test

A 30 per cent (V/V) stock suspension of glutaraldehyde-treated sheep RBC was prepared and the same stock was used throughout the study. Sensitized cells were prepared daily for each test as follows: 0.5ml of the glutaraldehyde-treated sheep cells was washed twice in PBS pH 7.2. The treated cells were suspended in 15mls of 1:20,000 tannic acid and the mixture was left for 20 minutes at room temperature with occasional mixing. The cells were then washed three times in PBS pH 7.2 after centrifugation. 2.5 per cent suspension of the cells were made in PBS pH. 7.2

0.5ml of tanned sheep rbc and 2.5mls of 1:6 dilution of tetanus toxoid were made up to 10mls with PBS pH 5.5. The mixture was incubated at 37°C for 60 minutes with occasional shaking. The cells were then washed 3 times in one per cent normal rabbit serum (NRS), centrifuged and diluted to a one per cent suspension in NRS. A non-sensitized cell (negative control) was prepared in the same manner except that saline replaced toxoid.

Determination of the titre

Serial ten-fold dilutions of the patients' sera and the standard antitoxin were made in NRS in microtitre plates. To each well containing two drops (0.05ml) diluted serum, 0.05ml of the suspension of toxoid-sensitized cells was added. The first six of the eight rows of wells were occupied by test sera. The seventh row contained the sensitized cells plus antitoxin whilst the last row had non-sensitized cells plus antitoxin. The plates were incubated at room temperature (22°C) overnight. The highest serum dilution that showed a carpet of cells with end folding was recorded as the end point. A negative reaction was recorded when the cells appeared in a ring at the bottom of the tube.

Results

The data in the Table show that eight (23 per ent) of the mothers had haemagglutination titres below 400, seventeen (49 per cent) had titres between 1,600 and 6,400 ,while only two (5 per cent) had titres of 25,600. Twenty-one of the children received the three immunizations, eight missed the second immunization while ten missed the third one. Before being immunized, twelve (35 per cent) of the children showed titres below 400 while only one (3 per cent) showed a titre of 25,600. The rest fell in the range, 400–6,400. Of the children who turned up for the second immunization, none had titres below 400. Eighteen (65 per cent) had titres between 1,600-6,400 while only one (3 per cent) had a titre of 25,000. Fifteen (65 per cent) of the children who turned up for the third immunization had titres ranging between 1,600 and 6,400 while four (16 per cent) had a titre of 25,000. Three children (12 per cent) were in the range of 400–800 and another three (12 per cent) also had a titre of 12,800.

Eighteen of the 22 children who completed their immunization showed progressive and appreciable increase in the antibody titres with successive immunization. Nine of the ten who had only two immunizations showed increase in antibody titre. One of the 21 children who completed the immunization programme initially had a very high antibody titre (25,600) and did not show any change in antibody level after immunization. Two children showed a slight decrease in antibody levels after immunization.

Discussion

The present results show that generally, the antibody level to tetanus is high in the mothers and their corresponding infants. This is to be expected as most of the mothers had tetanus vaccination during pregnancy. Vaillard (1892) clearly demonstrated that when pregnant female animals were immunized with detoxified tetanus toxoid, their young ones were highly immuned against tetanus organism and the protection persisted for several weeks. Later studies by Nattan-Larrier, Ramon and Grassets (1927)

TABLE

Haemagglutination Titres and Distribution of Mothers and their Immunized Infants

Haemagglutination Titres	*< 400*	*400–800*	*1600–6400*	*12800*	*25600*	*Total*
No. of mothers	8(23)	8(23)	17(49)	0	2(5)	35
No. of infants before 1st immunization	12(35)	11(31)	11(31)	0	1(3)	35
No. of infants before 2nd immunization	0	9(32)	18(65)	0	1(3)	28
No. of infants before 3rd immunization	0	3(12)	15(65)	3(12)	4(16)	25

Figures in parenthesis are per cent of total

confirmed this observation and also showed that the transmission of tetanus immunity may be through the colostrum and milk (horses, cattles, pigs) or through the placenta (human, apes, rodents) or in those cases where very high levels of maternal antibodies existed, transfer may occur through both ways.

The present study has shown that antibody levels in children are closely related to those of their mothers. This highlights the importance of effective immunization of pregnant mothers so that the children might possess at birth and in early infancy enough passively acquired anti-tetanus antibodies before they are subsequently actively immunized. The study also showed that most of the children responded to immunization by increased titres of antitoxin after successive immunizations. In three cases however, there was no increase in antibody titres after two successive immunizations. It is possible that these children did not respond to the tetanus toxoid and may belong to the group of 'non-responders' as postulated by White *et al.*, (1969).

Kryl, Prasilova and Neubertore (1964) compared the response to tetanus immunisation by twelve infants born to tetanus-immunized mothers and eight infants born to non-immunized mothers. The average titre produced after two toxoid immunisations (at 2–4 months of age) was almost identical in the two groups. They also noted that three babies with the highest titres of antibody at the time of the first innoculation failed to show any increase in titres, 9–10 weeks later during the third innoculation. In the present study, children who started with lower antibody titres responded better to immunization than those with higher antibody titres. This may be due to the 'mopping up' of the tetanus antigen by the pre-existing antibody in the latter groups of children resulting in immune complex formation and subsequent poor response to the toxoid stimulation.

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