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# *Placental Transfer of Tetanus Toxoid Antibodies: is there an Impairment in Nigeria?*

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## Summary

**Chan MCK, Hart CA, Igbagiri FP and Oruamabo RS. Placental Transfer of Tetanus Toxoid Antibodies: is there an Impairment in Nigeria?** *Nigerian Journal of Paediatrics 1994;* **21**: 94. In a joint study undertaken at the University of Port Harcourt Teaching Hospital, Nigeria and at the University of Liverpool and School of Tropical Medicine, UK, antitetanus antibody levels were determined in 20 paired mother-baby with tetanus as well as in 10 paired mother-baby controls, using the ELISA test. Maternal tetanus antibody concentration and that of their babies with tetanus ranged from 0.420 to 8.115 IU/ml, and from 0.038 to 6.404 IU/ml, respectively. In the controls, maternal antibody concentration ranged from 4.794 to 9.560 IU/ml while that of the babies ranged from 3.574 IU/ml to 9.841 IU/ml. The mean sequestration index (SI) was lower ($P<0.001$) in the study group than in the controls. Mothers of babies with tetanus were younger ($p<0.02$), had lower level of education ($P = 0.0133$) and their tetanus toxoid coverage was lower ($P<0.001$) than those of the controls. With the mean SI of 0.91 for the controls in the present study being close to 0.98 found in Gabonese women and about 2.5 times lower than that of French women, it is concluded that there was an impairment in the transfer of antitetanus toxoid antibodies across the placenta among Port Harcourt women.


## Introduction

OVER five years after the Expanded Programme on Immunization (EPI) was launched in Nigeria, neonatal tetanus has remained a common problem in the country; in some studies, the disease accounts for over 30 percent of total neonatal admissions. [1,2] The condition has been reported even in babies whose mothers had received antitetanus vaccination during pregnancy. [3] Placental transfer of tetanus toxoid antibodies in African ba-

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bies in Gabon has recently been reported to be limited as compared with the unlimited transfer in French babies; [4] yet, the level of antitetanus antibodies in either the Gabonese, or the French babies, was not lower than the protective concentration of 0.01 IU/ml. [5 6] The mean sequestration index (SI), a ratio of cord blood level to maternal blood antibody level, obtained for the French infants was 2.52 compared with 0.98 for the Gabonese babies.[4] The above findings thus suggested that there was a block in the placental transfer of antitetanus antibodies in Gabonese women. The aim of the present study was therefore, to undertake a study of antitetanus toxoid antibodies levels in mothers and their babies with neonatal tetanus and to compare these with those of controls. The study also aimed at examining such factors as may limit transplacental transfer of antibodies.

## Subjects and Methods

The study was jointly carried out by us at the University of Port Harcourt Teaching Hospital (UPHTH), Nigeria and by a team of workers from Liverpool School of Tropical Medicine, United Kingdom (UK). Sera from babies admitted into the department of Paediatrics, UPHTH, with neonatal tetanus and from their mothers, were obtained on admission, between February and September 1992; the sera were stored at -20$^0$C in the Microbiology laboratory. Over the same period, maternal and cord blood sera, to serve as controls, were obtained from newly delivered mothers in the labour ward of UPHTH and similarly stored. The mother-baby controls were followed up at home for a period of 28 days to observe any symptoms and signs of tetanus. Information recorded on each case and control, included mother's vaccination record during the present and previous pregnancies, weight at presentation or birthweight for controls, height and mothers' level of education. Other recorded data were the age of the patients at presentation, admission weight, gestational age according to maternal dates and date of discharge or death. The sera were transported to Liverpool in an insulated container with two dry ice-packs (GIOSTYLE) which were maintained at -20$^0$C for 48 hours prior to transportation.

### *Antibody Assay*

Enzyme-linked immunosorbent assay (ELISA) was used to determine antitetanus IgG antibodies in the sera. Absorbed tetanus toxoid *(Pasteur Merieus, Lyon)* diluted 1 in 100 in carbonate-bicarbonate buffer, pH 9.6, was used to coat a microtitre assay plate *(Dynatech, Sussex)* with 100ul per well and incubated for two hours at room temperature. The plate was washed four times and the test and reference samples diluted 1 in 400 in PBS Tween (0.05 percent) and containing one percent bovine serum albumin, were subsequently added in 100ul volumes to duplicate wells and incubated overnight at 4$^0$C. After being re-washed, alkaline phosphatase conjugated rabbit anti-human IgG specific for gamma chains *(Dako, Glostrup)* was freshly diluted 1 in 1000 and PBS Tween was added in 100ul volumes per well. The plate was incubated

at room temperature for four hours. After another series of washings, 100ul of a substrate solution (p-nitrophenol phosphate, 1mg/ml in 10 percent diethanolamine buffer, pH 9.8) was added to each well and incubated in the dark at room temperature for 30 minutes. The reaction was stopped by adding 50ul of 3M NaOH to each well and the plate was read on a MR710 spectrophotometer *(Dynatech, Sussex)* set at 405nm wavelength. The standard curve obtained by the nonlinear regression of six dilutions of a reference serum of a known antitetanus antibody concentration (22 IU/ml) was used to calculate the concentrations of the test samples in IU/ml through interpolation of the OD obtained.

Data obtained for the tetanus mother-baby pairs and controls were arranged in two sets. Comparison of associations was carried our statistically, using the Chi-square test with correction for continuity where relevant and Fisher's exact probability test where numbers in cells were too few; the Student's 't' test was used to compare means of normally distributed data. Approval for the study was obtained from the Ethical Committee, UPHTH; informed consent was also obtained from each mother before enrolment in the study.

## Results

Twenty paired mother-baby with neonatal tetanus and 18 paired mother-baby controls were initially recruited into the study, but analysis of the data on the controls was carried out in 10 because sera from four pairs were discarded for being unsuitable for assay, while four mothers withdrew from the study. Table I summarizes the data on the 20 paired mother-baby with tetanus. As can be seen, maternal tetanus antibody concentration ranged from 0.420 to 8.115 IU/ml (mean $\pm$ SD = 1.9 $\pm$ 2.1 IU/ml) and that of the babies ranged from 0.038 to 6.404 IU/ml (mean $\pm$ SD = 0.78 $\pm$ 1.3 IU/ml). The sequestration index, (SI), ranged from 0.03 to 0.91 (mean $\pm$ SD = 0.45 $\pm$ 0.27). In Table II, summarizing the data on the 10 paired mother-baby controls, it will be observed that the antibody concentration of the mothers ranged from 4.794 to 9.560 IU/ml (mean $\pm$ SD = 7.4 $\pm$ 1.7 IU/ml), while that of the babies ranged from 3.574 to 9.841 IU/ml (mean $\pm$ SD = 6.81 $\pm$ 2.3 IU/ml). SI ranged from 0.57 to 1.08 (mean $\pm$ SD = 091 $\pm$ 0.17). It should be further observed that of the 20 mothers of babies with tetanus, only six had been immunized during the pregnancy (Table I), while all, except one mother, in the controls had been immunized (Table II). Table III contains the features and characteristics of maternal age, parity, tetanus toxoid coverage (TTC) during pregnancy and antibody concentration in the study group and controls. It is evident that mothers of babies with tetanus were significantly younger ($P<0.02$) than those of the controls; completed tetanus toxoid coverage in the mothers of the controls was higher ($P<0.001$) than that in the study group; the educational levels of the study group was lower ($P<0.0133$) than that of the controls; the subjects were also less well-nourished, as indicated by the quetelet's index as well as by the height ($P<0.01$), than the controls. Antibody concentrations in the mothers, in the babies with

tetanus and the SI in this study group were significantly lower ($P<0.001$, $P<0.01$, $P<0.01$, respectively) than those of the controls.

## Discussion

The present findings have confirmed those reported by Gendrel *et al* [4] among Gabonese women. The SI of 0.91 for the controls in the present study was close to 0.98 found in the Gabonese mothers and about 2.5 times lower than the SI in Parisian mothers.[4] It is therefore evident that there was an impairment in the trans-

TABLE I

*Serial Numbers and Data on 20 Paired Mother-baby with Neonatal Tetanus*

| *Number* | *Data* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J |
| 1 | 0.514 | 0.274 | 0.53 | 35 | 1.65 | 24 | Y | 3 | 7 | 5 |
| 2 | 0.703 | 0.269 | 0.38 | 42 | 2.8 | 29 | N | - | - | 8 |
| 3 | - | 0.797 | - | 35 | 2.3 | 24 | Y | 3 | 7 | 11 |
| 4 | 1.423 | 0.334 | 0.23 | 40 | 3.0 | ? | N | - | - | 4 |
| 5 | 0.763 | 0.325 | 0.43 | 40 | 3.55 | 19 | N | - | - | 5 |
| 6 | 0.891 | 0.587 | 0.66 | 37 | 2.8 | 22 | N | - | - | 7 |
| 7 | 1.067 | 0.480 | 0.45 | 40 | 2.5 | 25 | N | - | - | 8 |
| 8 | 0.831 | 0.179 | 0.22 | 40 | 2.8 | 23 | N | - | - | 6 |
| 9 | 2.938 | 0.686 | 0.23 | 38 | 2.9 | 22 | N | - | - | 7 |
| 10 | 1.402 | 1.179 | 0.84 | 40 | 3.2 | 24 | N | - | - | 5 |
| 11 | 1.213 | 0.280 | 0.23 | 40 | 2.7 | 18 | N | - | - | 5 |
| 12 | 0.484 | 0.368 | 0.76 | 44 | 3.4 | 28 | Y | 1 | 7 | 7 |
| 13 | 1.247 | 0.038 | 0.03 | 40 | 2.2 | 37 | Y | 2 | 5 | 6 |
| 14 | 1.380 | 0.681 | 0.49 | 40 | 2.9 | 28 | N | - | - | 7 |
| 15 | 0.420 | 0.368 | 0.88 | 40 | 3.0 | 15 | N | - | - | 11 |
| 16 | 1.732 | 0.844 | 0.49 | 40 | 3.0 | 25 | Y | 4 | 8 | 4 |
| 17 | 1.861 | 0.847 | 0.45 | 50 | 2.65 | ? | N | - | - | 10 |
| 18 | 7.060 | 6.404 | 0.91 | 40 | 2.9 | 24 | Y | 3 | 9 | 8 |
| 19 | 1.123 | 0.227 | 0.20 | 40 | 3.3 | 25 | N | - | - | 8 |
| 20 | 8.115 | 0.497 | 0.06 | 35 | 2.7 | 16 | N | - | - | 8 |

A = Maternal anti TT(IU/ml)
B = Neonatal anti TT (IU/ml)
C = Sequestration index
D = Gestational age (weeks)
E = Admission weight (kg)
F = Maternal age (years)
G = Immunization status during present pregnancy (Y = Yes, N = No)
H = Number of immunizations received
I = Month of last immunization
J = Age (days) of baby at onset of tetanus

fer of antitetanus toxoid antibodies across the placenta among mothers in Port Harcourt. There is no ready explanation at the moment for this observed impairment. Transplacental transfer of antibodies is related mainly to gestational age and foetal growth, [4] but in the present study, this relationship was not established as the babies in the study group and controls were full term babies with the mean gestational age of 39.44 and 39 weeks, respectively. It has been postulated that the impairment of specific, or total IgG placental transport was a consequence of the high IgG levels in the sera of African mothers.[4] This postulate was however, not supported by the present findings; it would, therefore, be valuable to pursue this in future studies. The fact that the SI was significantly higher in the controls than in the study group and mothers in the control group were significantly better educated and nourished than the tetanus counterparts would suggest a possible role of social and environmental influences in the impairment of placental transfer of tetanus antibodies in African women.

Vaccination of pregnant women with tetanus toxoid has been shown to decrease the incidence of neonatal tetanus in many developing countries.[7] Nevertheless, there have been reports of neonatal tetanus in babies born of immunized mothers.[3 8] It is noteworthy that in the present study, all the babies with

TABLE II

*Serial Numbers and Data on 10 paired Mother-baby Controls*

| *Number* | *Data* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J |
| 1 | 6.220 | 3.574 | 0.57 | 40 | 3.7 | 32 | Y | 2 | 5 | 4 |
| 2 | 5.846 | 3.797 | 0.65 | 32 | 1.4 | 21 | Y | 2 | 8 | 1 |
| 3 | 9.951 | 9.841 | 0.99 | 41 | 4.0 | 33 | Y | 2 | 7 | >5 |
| 4 | 8.146 | 7.460 | 0.92 | 40 | 3.3 | 37 | Y | 1 | 7 | >5 |
| 5 | 8.828 | 8.710 | 0.99 | 40 | 3.2 | 32 | Y | 2 | 6 | 1 |
| 6 | 5.867 | 5.736 | 0.98 | 40 | 3.5 | 18 | Y | 2 | 8 | 1 |
| 7 | 4.794 | 4.327 | 0.90 | 40 | ? | 37 | N | - | - | >5 |
| 8 | 7.297 | 7.900 | 1.08 | 66 | 3.25 | 32 | Y | 1 | 6 | 2 |
| 9 | 9.560 | 9.572 | 1.00 | 39 | 3.35 | 22 | Y | 2 | 8.5 | 1 |
| 10 | 7.271 | 7.288 | 1.00 | 38 | 2.7 | 40 | Y | 1 | 7.5 | >5 |

A = Maternal anti TT (IU/ml) B = Neonatal anti TT (IU/ml)
C = Sequestration index (B/A) D = Gestational age (weeks)
E = Birthweight (Kg) F = Maternal age
G = Immunization status during present pregnancy (Y = Yes, No = No)
H = Number of immunizations received
I = Month of last immunization J = Parity

TABLE III

*Characteristics of Study Group and Controls*

| *Characteristic* | *Paired Mother-Baby study group* | *Paired Mother-Baby controls* | *P value* |
|---|---|---|---|
| *Maternal age (yrs.)* | | | <0.02* |
| <20 | 4(22.2) | 1(10) | |
| 20 - 25 | 10(55.6) | 2(20) | |
| >25 | 4(22.2) | 7(70) | |
| *Parity* | | | NS |
| <3 | 9(45) | 5(50) | |
| 3 - 5 | 8(40) | 1(10) | |
| >5 | 3(15) | 4(40) | |
| *Tetanus toxoid coverage during pregnancy* | | | <0.001** |
| Complete | 6(30) | 10(90.9) | |
| None | 14(70) | 1( 9.1) | |
| *Maternal level of education* | | | = 0.0133*** |
| None | 5(25) | 0 | |
| Primary | 11(55) | 4(30.8) | |
| Secondary | 4(20) | 6(46.1) | |
| Tertiary | 0 | 3(23.1) | |
| *Quetelet's Index ($W/H^2$)* | n = 20 | n = 12 | <0.001+ |
| Mean ± SD | 21.8 ± 2.83 | 28.6 ± 4.4 | |
| *Height* | n = 20 | n = 13 | <0.01+ |
| Mean ± SD | 154.5 ± 6.6 | 161.8 ± 4.6 | |
| *Maternal antibody concentration* | n = 19 | n = 10 | <0.001+ |
| Mean ± SD | 1.9 ± 2.1 | 7.4 ± 1.7 | |
| *Baby antibody concentration* | n = 20 | n = 10 | <0.001+ |
| Mean ± SD | 0.78 ± 1.4 | 6.81 ± 2.3 | |
| *Sequestration index* | n = 19 | n = 10 | <0.001+ |
| Mean ± SD | 0.45 ± 0.27 | 0.91 ± 0.17 | |

NS = Not significant
SD = 1 standard deviation
W = Weight (Kg)
H = Height (metres)
* = Chi square test
** = Chi square test with correction for continuity
*** = Fisher's exact probability test
\+ = Student's 't' test
Figures in parentheses represent percent of total

tetanus had antitetanus antibodies above the presumed protective value of 0.01 IU/ml,[5 6] including the six infants whose mothers had been fully immunized during current pregnancy. Neonatal tetanus is characterized by its severity and portal of entry. It is therefore, possible that in neonatal tetanus, a high toxin production, acting on the newborn immune system and gaining a direct access to a large vascular bed, would require a higher specific antibody level to provide protection. A similar situation has in fact, been demonstrated in adults with severe tetanus.[9] It must be admitted that it is difficult to test this hypothesis, because of ethical considerations, but it is strongly advocated that, in addition to increasing the vaccination coverage of pregnant women, measures aimed at improving their living standards should be vigorously pursued.

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