Original Article

Short-term Persistence of Protective Maternally Acquired Immunity in Neonates Delivered by Primiparous Women in Ibadan, Nigeria

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ABSTRACT

BACKGROUND: Unresolved questions remain concerning the protective effect and duration of immunity acquired from mothers. This study investigated persistence of immunity against tetanus in the first two weeks of life among neonates in Nigeria.

METHODS: In a longitudinal study, 244 primiparous mothers and their newborns were consecutively recruited at 16 selected Primary Healthcare Centres in Ibadan, Nigeria. All the newborns were tested for protection against tetanus using a validated rapid diagnostic, “Tetanos Quick Sticks” (TQS) on days 1, 7 and 14. Persistent immunity was defined as positive TQS result on day-14. Data were analysed using descriptive statistics, Chi-square and logistic regression at p = 0.05.

RESULTS: There were 137(56.1%) male neonates; 87.7% were delivered at ≥37 weeks of gestation. The prevalence of protective immunity against tetanus (PIaT) among neonates on day-1 was 63.5%; 119 out of 153 neonates remained positive to TQS test by day-14, giving a persistence rate of 77.8%. Independent predictors of persistent PIaT were residence in urban area (OR = 9.66; 95% CI = 2.42-38.45), maternal age (OR = 2.06; 95% CI = 1.49-2.85) and gestational age (OR = 1.84; 95% CI = 1.23-2.74).

CONCLUSION: Protective immunity against tetanus waned in some neonates over the first two weeks of life, and this decline was inversely related to maternal and gestational ages.

KEYWORDS: Protective immunity, Primiparous women, Tetanos Quick Sticks, Neonatal tetanus

INTRODUCTION

Tetanus is one of the three leading causes of neonatal deaths in resource-limited countries worldwide. It contributes about 36% of deaths due to infections in this age group (1) and killed 34,000 newborns in 2015 alone (2). The disease primarily affects skeletal muscles and manifests as “spasms and trismus” (3). Infection usually occurs through contaminated unhealed umbilical stump, particularly when the stump is cut with a non-sterile instrument. Infants who have acquired sufficient passive immunity from the mother are at low risk. Thus, it is possible to prevent neonatal tetanus through...
vaccination, adoption of cheap and effective measures such as hand washing and the use of clean materials for treatment of the neonates’ umbilical stump. In 2016, approximately 96% reduction in mortality from neonatal tetanus was achieved through universal vaccination of pregnant mothers with tetanus toxoid which started in 1988 (2). Nigeria remains among 18 countries that still have not eliminated the disease in spite of the ongoing efforts to actively vaccinate pregnant women during antenatal care visits (2). Many Nigerian women seldom receive the recommended booster doses of tetanus toxoid after the routine immunisation in infancy (4). Even when the recommended two or more tetanus toxoid injections are given during pregnancy, the length of the protection offered by the tetanus vaccine in the neonates is not known. To this end, there have been reports of neonatal tetanus despite maternal vaccination during pregnancy (5). Therefore, the persistence of tetanus as a cause of morbidity and deaths among Nigerian children constitutes a matter of special concern. Most infants present with tetanus at about 8 to 14 days after birth, suggesting that the infection occurs in the first week of life, but it is not known whether the immunity transferred from mother to foetus sufficiently lasts till 14 days after birth. It is therefore necessary to identify potential risk factors for non-protective immunity against tetanus in order to provide guides for making immediate decision to treat or take definitive steps towards prevention of tetanus in the newborn.

A recent publication showed that serological monitoring of immunity against tetanus is critical to maternal and neonatal tetanus elimination initiative in countries with high burden (6). However, it is apparent that such important information needed for policy review is lacking in Nigeria, in order to direct policy. Also, community-specific information is needed on factors that negatively impact on immunisation practices and its effectiveness in order to expedite tetanus eradication campaign in Nigeria. It was therefore necessary to investigate potential risk factors for non-protective immunity against tetanus among women and their neonates in Nigeria. This article presents the findings of the follow-up phase of a longitudinal study from which the baseline findings were earlier published (7). The aim of this aspect of the research was to find out whether protection offered by transplacentally-transferred antibodies against tetanus in neonates of primiparous mothers lasts till day-14. We also made attempt to address the question of whether neonates given anti-tetanus serum injection at birth have protective immunity by day 14 compared with those not given.

METHODS

Study design, study area and study setting: This research adopted a longitudinal design. Data were gathered for the same neonates repeatedly: within 24 hours after birth, days 7 and 14 of neonatal life. The research was carried out in 16 Primary Healthcare Centres (PHC) located in Ibadan, Nigeria. These primary healthcare facilities were located within the communities and at readily accessible distances in order to serve as the first “point-of-call” for healthcare services. Ibadan has 11 local governments areas (LGAs), made up of five within the metropolis and six at the periphery of the metropolis. Officially, the five LGA within the metropolis are regarded as “urban LGAs” while those at the periphery of the metropolis are “rural LGAs” (8). As at the time of planning the study, 148 PHC were actively providing maternity services in the study area. Nurses/midwives and community health officers were responsible for day-to-day provision of healthcare at the PHCs level.

Study population and sample size: This study focused on newborns of primiparous women who delivered their first pregnancy at any of the primary health and maternity centres located in the eight selected LGAs in Ibadan. The choice of this study population was based on an anecdotal observation by the researchers, which suggested that the majority of the cases of neonatal tetanus admitted at the University College Hospital were children of primiparous women (Unpublished data). Assuming the seroprevalence of non-
protective immunity against tetanus as 50%, a minimum detectable difference of 10% between hypothesised and true value, we estimated that 196 primiparous mothers would be required for the study at 95% level of confidence. This sample size was obtained using the formula for estimating minimum size for single proportion (9). Since this study involved follow-up, it was anticipated that about 10% of the enrolled participants might be lost to follow-up or refuse follow-up data collection giving a minimum of 218 neonates required for the study.

**Sampling technique:** The final sampling unit for this study was mother-baby pair. A three-stage sampling technique was employed; to select local government areas (LGAs) in Ibadan, to select two primary health centres from the list of those providing maternity services in each LGA, and to consecutively recruit mothers and their newborns as they deliver in health centres on daily basis. Those who refused to provide informed consent, those who gave family history of allergy to any form of immunoglobulin and those who, in the judgement of the investigators, were not likely to comply with study protocol were not recruited. During the 20-month study period, there were 263 livebirths given by primiparous women, but 244 (92.8%) neonates participated in the study. Of the 244 mother-neonate pairs, four declined participation on day 14 of follow-up visits giving 240 neonates.

**Data collection:** All eligible primiparous mothers were asked to participate in the study within 24 hours after delivery. Mothers’ consent was sought soon after they had verbally expressed their intention to participate in the study. Data were collected using an interviewer-administered semi-structured questionnaire. The items in the questionnaire were adapted from those used for three previously published studies (10-12), and the questionnaire was described in an earlier publication (7). The investigator and 16 trained-nurses (assistants) collected data on day 1 on both mothers and neonates, and subsequently two times on days 7 and 14 on neonates only. A rapid diagnostic kit known as ‘Tetanos Quick Stick’ (TQS) (Gamma, Angleur, Belgium) was used to check for protection against tetanus as previously described (10). Also, anthropometry (weight, OFC and length) and physical examinations were carried out for neonates.

Positive and negative TQS results were interpreted as Protective Immunity against Tetanus (PlaT) and Non-Protective Immunity against Tetanus (NPlaT), respectively. Persistent PlaT was defined as positive TQS result on day-14 among those who had positive TQS results on day-1. Neonates who tested negative to TQS on day 1 (that is NPlaT) were given human antitetanus serum (ATS) immunisation injection 500 IU (Vins Bioproducts Limited, India) once after a sensitivity test was carried out by injecting 0.1 ml serum in 1:10 dilution subcutaneously and observing for half an hour for any reactions of local or general.

**Data analysis:** The main outcome variables measured were test results for TQS at birth (for mothers and neonates) and at 7 days as well as 14 days after birth (for neonates only). A positive and negative tests were defined as protective and non-protection tetanus immunity against tetanus, respectively. A positive and negative tests were defined as protective and non-protective tetanus immunity against tetanus, respectively. Independent variables measured included: demographic variables such as age, gender, socioeconomic class and obstetric history. The family socio-economic background was determined using the classification proposed by Oyedeji (13). According to Oyedeji (13), socioeconomic index score was awarded based on the occupations and educational attainment of the neonate’s father and mother. In this study, average score of 1 was assigned class 1 (upper class), scores of 2 and 3 were assigned class 2 (middle class) while scores of 4 and 5 were assigned class 3 (lower class). Chi-square test, Student’s t test and logistic regression analyses were performed. Level of statistical significance was set at p = 0.05. All data were analysed using SPSS for Windows 18.0 (SPSS Inc., IL, USA).

**Ethical considerations:** Participation in the study was completely voluntary and based on written informed consent. The study proposal was reviewed and approved by the Oyo State Ethical Review Committee. Eligible mothers were
informed that they were free to withdraw their consent at any time. Neonates who tested negative to TQS received care according to standard protocol. Privacy of research participants was ensured using a serial number on the information collected, rather than a name or hospital number. The results of TQS test in this study were made available to the health workers in their respective centres and the participants did not pay for any of the procedures and treatments. Mothers who did not have protective antitoxin were offered tetanus toxoid.

RESULTS

Characteristics of participants: The primiparous mothers’ age ranged from 20 to 33 years with a mean of 27.9±3.4 years. Some of the characteristics of the women, antenatal booking and immunisation status as well as the baseline characteristics of neonates were described in an earlier publication (7). All the four neonates who did not participate in the study on day 14 were All the four neonates who did not participate in the study on day 14 were females, term and weighed 2600 to 3300 g. Only 8.2% of fathers and 7.4% of the mothers had at least university education, and 17.6% of the fathers and 27.0% of mothers of had no formal education. The most frequent occupational class was class III and class IV for the fathers and mothers, respectively. Of the 244 parents (fathers and mothers) who participated in the study, 64(26.2%) had the same levels of education while 46(18.9%) of the fathers and mothers were in the same occupational class.

Table 1: Immunity against tetanus on Day 14 among 153 neonates who tested positive to TQS on Day 1

| Characteristics                        | Not protected | Protected | OR (95% CI) | P     |
|----------------------------------------|---------------|-----------|-------------|-------|
| **Gender**                             |               |           |             |       |
| Female                                 | 21            | 44        | 2.75 (1.26, 6.04) | 0.010 |
| Male*                                  | 13            | 75        | 1           |       |
| **Gestational age category**           |               |           |             |       |
| Preterm (<37 weeks)                    | 14            | 6         | 13.18 (4.53, 38.36) | <0.001 |
| Term (≥37 weeks)*                      | 20            | 113       | 1           |       |
| **Birthweight category (g)**           |               |           |             |       |
| ≥2500 (Normal)                         | 27            | 113       | 0.21 (0.06, 0.66) | 0.004 |
| <2500 (Low)*                           | 7             | 6         | 1           |       |
| **Mothers’ place of residence**        |               |           |             |       |
| Rural LGA                              | 28            | 42        | 8.56 (3.28, 22.31) | <0.001 |
| Urban LGA*                             | 6             | 77        | 1           |       |

*Reference category, OR = Odds Ratio (for being negative), CI = Confidence Interval; 
*All those in the <2500 g birth weight category were also Preterm (<37 weeks)

Persistence of protective immunity against tetanus: At baseline, 155 were tested positive to TQS test, giving a seroprevalence of protective immunity against tetanus (PIaT) of 63.5%, as earlier reported (7). Table 1 shows that, among 155 neonates who tested positive to TQS on day 1, there were significant associations between immunity against tetanus on day 14 and gender, gestational age category, birthweight category and mothers’ residence. The prevalence and odds of PIaT on day 14 was significantly higher among males (85.2%) than females (67.7%); p = 0.010. Also, prevalence and odds of PIaT on day-14 was significantly higher among term (30.0%) more
than preterm (85.0%) neonates, those who weighed ≥2500 g (80.7%) than <2500 g (46.2%) and among neonates of mothers from urban (92.8%) than rural (60.0%) LGAs. However, there was no significant association between immunity against tetanus on day 14 and mothers’ level of education as shown in Table 2 ($\chi^2 = 1.541; df = 4; p = 0.819$). Similarly, there was no significant association between immunity against tetanus on day 14 and family social class as shown in Table 3 ($\chi^2 = 1.541; df = 4; p = 0.819$).

Table 2: Immunity against tetanus on Day 14 among 153 neonates who tested positive to TQS on Day 1 by mothers’ level of education

| Mothers’ Level of Education | Tetanus immunity in Neonates on Day 14 | OR (95% CI) | P |
|-----------------------------|--------------------------------------|-------------|---|
|                             | Not protected                        | Protected   |   |
|                             | n   | %  | n   | %  |            |  |
| University graduates*       | 7   | 28.0 | 18 | 72.0 | 1           | - |
| Post-Secondary certificate not university | 3 | 21.4 | 11 | 78.6 | 0.70 (0.23, 0.50) | 0.721 |
| Secondary School or Grade II certificate University | 8 | 18.6 | 35 | 81.4 | 0.59 (0.18, 1.88) | 0.381 |
| Modern 3 and Primary 6 Certificate | 8 | 19.0 | 34 | 81.0 | 0.61 (0.19, 1.94) | 0.546 |
| No formal education         | 8   | 27.6 | 21 | 72.4 | 0.98 (0.30, 3.23) | 0.786 |

*Reference category, OR = Odds Ratio, CI = Confidence Interval

Table 3: Immunity against tetanus on Day 14 among 153 neonates who tested positive to TQS on day family social class

| Family Social Class | Tetanus immunity in Neonates on Day 14 | OR (95% CI) | P |
|---------------------|--------------------------------------|-------------|---|
|                     | Not protected                        | Protected   |   |
|                     | n   | %  | n   | %  |            |  |
| I (Highest)         | 0   | 0.0 | 0   | 0.0 | -           | - |
| II*                 | 3   | 37.5 | 5  | 62.5 | 1           | - |
| III                 | 15  | 27.8 | 39 | 72.2 | 0.64 (0.14, 3.02) | 0.681 |
| IV                  | 12  | 18.2 | 54 | 81.8 | 0.37 (0.08, 1.77) | 0.347 |
| V (Lowest)          | 4   | 16.0 | 21 | 84.0 | 0.32 (0.05, 1.90) |   |

*Reference category, OR = Odds Ratio, CI = Confidence Interval

Predictive factors for persistence of protective immunity against tetanus: Table 4 shows the estimates for the predictor-model to identify independent predictors for PlaT on day 7 among neonates. Maternal age (p<0.0001), mothers’ residence (p < 0.0001) and administration of 2 doses of tetanus toxoid injection (p = 0.021) were the important independent predictors of PlaT on day 7. Since maternal age and gestational age are numerical variables, an increase in one year in maternal age has a 118% (95% CI 50% to 217%) increase in odds of having PlaT on day 7. A neonate of mothers from urban compared to a rural LGA is 6.82 (95% CI = 3.81 to 8.13) times more likely to have PlaT on day 7. However, the Nagelkerke R Square (0.864) shows that about 86.4% of the variation in the immunity against tetanus on day 7 is explained by this logistic model.
Table 4: Predictive factors for persistence of protective immunity against tetanus till Day 7 among neonates who tested positive on Day 1

| B           | S.E. | Wald | df | p     | OR   | 95% CI for OR |
|-------------|------|------|----|-------|------|---------------|
| Maternal age in years | 0.78 | 0.19 | 16.66 | 1 | <0.0001 | 2.18 | 1.50, 3.17 |
| Mothers’ place of residence (Urban) | 1.92 | 1.18 | 12.91 | 1 | <0.0001 | 6.82 | 3.81, 8.13 |
| Gestational age in weeks | 0.15 | 0.29 | 0.27 | 1 | 0.601 | 1.17 | 0.66, 2.07 |
| Mothers received 2 doses of tetanus toxoid injection (Yes) | 1.25 | 1.54 | 5.35 | 1 | 0.021 | 3.48 | 1.72, 7.06 |
| Birthweight (≥2500 g) | 1.09 | 1.55 | 0.49 | 1 | 0.483 | 2.97 | 0.14, 62.2 |
| Neonate’s gender (female) | 1.03 | 1.44 | 0.51 | 1 | 0.472 | 2.81 | 0.17, 4.78 |
| Constant | -    | 14.3 | 4.72 | 1 | 0.030 | 0.00 | - |

Wald estimates give the “importance” of the contribution of each variable in the model.

The estimates for the predictor-model to identify independent predictors for PlaT on day 14 among neonates were as shown in Table 5. Maternal age (p<0.0001), mothers’ residence (p = 0.001) and gestational age in weeks (p = 0.003) were the important independent predictors for PlaT on day 14. Since maternal age and gestational age are numerical variables, an increase in one year in maternal age and one week in gestational age has a 106% (95% CI 49% to 185%) and 84% (95% CI 23% to 174%) increase in odds of having PlaT on day-14, respectively. A neonate of mothers from urban compared to a rural LGA is 9.7 (95% CI 2.43 to 38.45) times more likely to have PlaT on day14. However, the Nagelkerke R Square (0.664) suggests that about 66.4% of the variation in the immunity against tetanus on day 14 is explained by this logistic model.

**DISCUSSION**

This study revealed that persistence rate of protective immunity against tetanus till day 7 and day 14 was moderately high. Only a few neonates (17.4%) who had protective immunity against tetanus lost it by day 7. Female gender, residence in rural local government areas and low birth weight were risk factors for non-protective immunity against tetanus among neonates of primiparous mothers on day 14. The fact that lack of protection against tetanus could still occur among newborns of women who were vaccinated and that those who acquired immunity may lose it within 7-14 days should prompt health workers to continue to pay attention to identified risk factors. This aspect of clinical practice is critical, in order to provide guides for making immediate decision to treat or take definitive steps towards prevention of tetanus in the newborn.

Prior to the conduct of this study, to our knowledge, the roles of gender of neonates and place of residence in determining the level of immunity against tetanus in neonatal age group were not found in literature. Though reasons for the significant gender difference in non-protective immunity against tetanus are not clear from our data, it is likely that the gender difference observed may have resulted from differences in immunisation status of mothers in the two groups. However, this reason was not further explored. On the contrary, the higher prevalence of non-protective immunity on day 14 among neonates who had protective immunity on day 1 in rural area (40.0%) than urban (7.2%) area was expected. This finding corroborates the fact that the tetanus immunisation coverage among mothers was also poorer in the same area as shown in our data.

The identification of low birth weight as a risk factor for lack of persistence in the maternally acquired immunity till day 14 of life may be partly explained by the fact that all the neonates...
who were in this category were delivered before term. Studies have shown that the immunised mother transfer antibodies against tetanus across the placenta to the foetus in form IgG antibodies (14,15).

This is expected to provide passive protection of the newborn against tetanus in the first two weeks, but gradually wanes over time. However, the influences of the timing of vaccination, history of previous tetanus vaccination and maternal biological characteristics such as immune status on the attainment of protective level of immunity in the newborn are unclear from this data. Nevertheless, all the neonates who tested negative to TQS and were given the ATS injection remained protected against tetanus beyond day 7 of the follow-up.

Within the limit of the study design, our data defined potential maternal and newborn risk factors for low tetanus immunity. This information can assist healthcare providers in objectively deciding who should be given the ATS injection before discharge from the delivery centres. The much desired evidence to support the continued use of ATS injections for the purpose of protecting neonates whose mothers received less than two doses or no tetanus toxoid injection before the third trimester in pregnancy has been corroborated by our findings. Also, we have provided more information on the possibility of utilising the validated Immunochromatographic Rapid Diagnostic Test (IRDT) kit for testing protection against tetanus in mothers and neonates, especially in the first 14 days of life.

Our data are unique in that, within the limits of accessible literature, it appears to be the first attempt at investigating the persistence of immunity against tetanus in Nigerian neonates. We have brought to the fore the importance of socio-demographic characteristics in guiding tetanus immunisation policy. For instance, it is now clear that efforts towards the attainment of the WHO-recommended 80% coverage among pregnant women need to be scaled up in the rural areas in Nigeria. However, it is important to identify the reasons why neonates continued to present with tetanus in the hospital despite routine maternal vaccination which could not be explained from our data. At a time when the global focus has shifted to curbing neonatal mortality, it is fitting that research be intensified into the prevention of mortality among neonates.

There are two points which validate and support the generalisability of our data. First, this study included preterm and term neonates, and the percentage of neonates lost to follow-up was considerably lower than the 10% allowed for in the study design. Second, we chose a representative sample of the study area, across all socio-economic strata, and we used validated instrument and test kit to check for protection against tetanus immunity. However, it remains unknown whether detection of maternally acquired antibody against tetanus can be determined beyond day 14 in Nigeria neonates. It is worthy of note that previous studies alluded to the fact that HIV might be playing important roles in the transfer of antibodies against tetanus from mothers to foetus (16,17). Though attempts were made to find out about the HIV status during pregnancy, only four mothers could provide proof of being seronegative to HIV test. It was practically impossible to assess the impact of HIV infection on the protective immunity against tetanus among the cohort of women who took part in the study.

In conclusion, immunity against tetanus among neonates of primiparous women in Ibadan waned in some neonates within two weeks of life. Health workers should be aware of risk factors associated with declining immunity and continue to consider administration of tetanus immunoglobulin to neonates whose mothers did not receive complete tetanus toxoid injections during pregnancy. A more conscientious enforcement of routine tetanus prevention practices is recommended, especially in the rural areas and for neonates with low birth weight and prematurity.

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