***Bayero Journal of Pure and Applied Sciences, 3(1): 39 - 42***
*Received: January, 2010*
*Accepted: February, 2010*

# SEROLOGICAL EVALUATION OF PROTECTIVE IMMUNITY AGAINST TETANUS IN PREGNANT WOMEN ATTENDING SOME RURAL HOSPITALS IN KANO STATE, NORTHERN NIGERIA

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***ABSTRACT***
***A study on serological evaluation of protective immunity in pregnant women against tetanus was conducted in two rural hospitals in Kano State, northern Nigeria using immuno-electrophoresis and indirect haemmagglutination techniques. A total of sixty (thirty from each hospital) pregnant women (aged 11-45 years) were used to assess their level of protection against tetanus. Details on client's age, socio-economic status and number of tetanus toxoid injections received during antenatal visits were obtained using structured questionnaire method. Blood samples were collected and analysed for serum and anti-tetanus antibodies as well as the total protein content. The titre values obtained were statistically analysed using one-way ANOVA to see the effects of age, socio-economic status and number of tetanus toxoid injections received on the mean percentage and concentration of albumin, Ig-A, Ig-G and the total proteins of the subjects. Results obtained showed that the highest percentage and concentration of serum albumin recorded throughout the study period were 73.05 ± 9.16% and 55.30 ± 4.97 g/dl at Tiga Dam and Gaya General Hospitals respectively. The highest levels of serum Ig-A were 18.50 ± 1.53% and 13.85 ± 1.21 g/dl both at Gaya. Similarly, the highest levels of serum Ig-G were 15.21 ± 25.81% and 11.55 ± 3.57 g/dl both at Gaya while the highest value of the total protein recorded was 80.23 ± 0.83 g/dl at Tiga. Results of the indirect haemmagglutination analysis indicated that the highest and lowest titre values were 1:922 and 1:13 HU/ml respectively. It was generally observed that age, socio-economic status and number of toxoid tetanus injections demonstrated significant (P < 0.05) influence on the levels of serum albumin, Ig-A, Ig-G and the total proteins. Similarly, clients with more than three injections of tetanus toxoid were observed to elicit higher (P < 0.05) antibody response as compared to those who did not receive any injection (P > 0.05).***

***Keywords: Neonatal, tetanus, haemmagglutination, tetanus toxoid, primigravidarum, Clostridium, immunoglobulin, Kano.***

## INTRODUCTION

Tetanus is a disease caused by toxins secreted by a Gram-negative, spore-forming bacterium called *Clostridium tetani* (Jawetz *et al*., 1989) Neonatal tetanus happens as a result of the contamination of the umbilical stump with the spores of the causative organism (*C. tetani*) through unhygienic practices of delivery and cord care, or by the application of animal dung to the cut cord, traditional marks and, perhaps, other environmental conditions, which babies are exposed to at home (Richardson and Knight, 1993). Tetanus is the only vaccine-preventable disease that is not communicable, but acquired through environmental exposure to the spores of *C. tetani*. Neonatal tetanus is the most common form of tetanus in developing countries. In the past, the disease was overlooked by the health services of many developing countries. However, the extent and magnitude of the disease have become clearer and shown to be a very serious health problem. The findings of Miller (1972) have shown that case fatality rate of neonatal tetanus might be as high as 95% in untreated children and it could be dominant cause of death in the first month of life. According to Bildhaiya (1983), hospital data on neonatal tetanus in some developing countries have shown that the mortality rate was 25-37% in India (1966-1972). Nuti and Harare (1978) reported that the mortality rate in Somalia was 75% (1974-1976). Ogbeide (1966) reported a mortality rate of 87% on neonatal tetanus in Nigeria. United States Agency for International Development (USAID, 1999) reported that Kano State had the highest incidence of neonatal tetanus all over the world, with 85 deaths per every 1,000 live births. This shows that tetanus claims the lives of over a million children every year. All forms of tetanus and, especially neonatal tetanus, remain substantially under reported in many countries of the world, and routine reporting system identifies only about 2-5% of the estimated number of tetanus cases. The neonate therefore is in need of protection by the use of more reliable and accurate techniques for the assessment of risk factors associated with neonatal tetanus. It has been shown that 2-3 suitably timed injections of tetanus toxoid in pregnant women prevent the occurrence of neonatal tetanus.

It was further reported that five doses of tetanus toxoid in a pregnant woman could protect her and the baby. This could also serve for subsequent pregnancies (WHO, 1994). The present study was therefore aimed at assessing the effects of age, socio-economic status and number of toxoid tetanus injections on the levels of serum albumin, IgA, IgG, total proteins and titre values of pregnant women attending two rural hospitals in Kano State. This is with a view to suggesting ways of eliminating the menace.

## MATERIALS AND METHODS

### Study population

A total of sixty (60) human subjects were randomly selected for the study in 2002. This number was drawn from two general hospitals (Gaya and Tiga Dam) that were strategically located in the State. A group of fifty pregnant women with various vaccination histories were used as experimental subjects with ten serving as a control group. Structured questionnaires were designed and administered, sourcing information on the age, socio-economic status and number of tetanus toxoid injections received during antenatal visits.

### Sample analysis (serology)

After having informed consent, 5 ml of whole blood was taken from Brachiocephalic veins under aseptic techniques using sterile syringe and needle. The blood samples were centrifuged at 3,000 revolutions per minute (rpm) to obtain a clean serum. Sera were kept frozen in plastic test tubes at a temperature of -20°C until required for use (WHO, 1983). However, the serum samples were initially screened for total proteins, albumin as well as globulin fractions (alpha-I, alpha-II, beta and gamma) at Aminu Kano Teaching Hospital, Kano, using immuno-electrophoresis technique (Nernberg, 1972). Specific anti-tetanus antibodies were detected using an indirect haemmagglutination assay technique (Peel, 1980). Here, purified tetanus toxoid solution (32 Limes Flocculation Units/ml) coated with tannic acid treated sheep erythrocytes was used as a source of the antigen. The test was performed on micro-titre plates (Cooke's micro-titre). The international standard for tetanus anti-toxin (Staten's Serum Institute, Cophenhagen, Denmark) was used as a reference. The unknown sera were measured against the reference serum and the anti-toxin content was expressed in Haemmagglutination Units per milliliter (HU/ml), which corresponds to 1 IU of the reference serum (Hardegree *et al.*, 1970).

### Statistical analysis

The data obtained were subjected to one-way ANOVA and Duncan's multiple range test (Kleinbaun and Kupper, 1978) for comparison of antibody titres from different experimental groups.

## RESULTS AND DISCUSSION

The results of the study are presented in Tables 1-6. It was observed that the serum immunoglobulin levels varied with age, socio-economic status and vaccination as well as the geographical location of an individual. Patient's age is an essentially important factor in the interpretation of immunoglobulin levels (Richardson and Knight, 1993; Owa and Makinde, 1995). From this study, clients with age groups of 21-25 and 26-30 years had higher levels of Ig-G (Tables 1-2). This signifies that these age groups had presumably adequate protection for their babies as compared with the other age groups. Thus, it might be said that these age groups had more than two deliveries (*grand multipara*), as such they received more injections during antenatal visits as compared to those with other age groups. Generally, clients of 26–30 years of age had significantly ($P < 0.05$) demonstrated higher levels of serum albumin (Table 1) and total proteins (Table 1) while 21-25 years age group had higher levels of Ig-A and Ig-G (Table 2). The age of the clients had significant ($P < 0.05$) effect on the antibody titres. This might be due to the number of tetanus toxoid injections received during the antenatal visits (WHO, 1994; 1999).

**Table 1: Effect of age on mean percentage and concentration of serum albumin, Ig-A, Ig-G and total proteins of clients attending Gaya General Hospital, Kano**

| Age group (years) | Serum albumin | | Serum Ig-A (alpha-I and alpha II) | | Serum Ig-G (gamma) | | Total proteins (g/dl) | Mean HT (HU/ml) |
|---|---|---|---|---|---|---|---|---|
| | Percentage | Conc. (g/dl) | Percentage | Conc. (g/dl) | Percentage | Conc. (g/dl) | | |
| 11 – 15 | - | - | - | - | - | - | - | 0 |
| 16 – 20 | 67.19±3.91 | 50.8±3.42 | 16.1±2.18 | 12±1.55 | 9.91±2.18 | 6.44±1.6 | - | 1:278 |
| 21 – 25 | 62.03±3.66 | 42.5±2.97 | 18.5±1.53 | 14±1.21 | 15.2±25.8 | 11.6±3.6 | - | 1:346 |
| 26 – 30 | 72.76±5.70 | 55.3±4.97 | 10.3±2.86 | 8±2.15 | 12.5±5.57 | 9.19±3.9 | - | 1:805 |
| 31 – 35 | - | - | - | - | - | - | - | 0 |
| 36 – 40 | - | - | - | - | - | - | - | 0 |
| 41 – 45 | - | - | - | - | - | - | - | 0 |

Socio-economic status of a client (Tables 3-4) gives an indication of an individual's level of nutritional status (Richardson and Knight, 1993). In this study, it was observed that socio-economic status of the clients from the two hospitals had no effects ($P > 0.05$) on the serum albumin, Ig-A, Ig-G and the total proteins. However, it was observed that only the low-income earners were able to demonstrate high levels of serum Ig-G of 12.80 ± 1.96% and 9.11 ± 1.55 g/dl at Gaya (Table 3) and 11.37 ± 2.57% and 6.89 ± 1.70 g/dl at Tiga Dam (Table 4) while the middle-income earners had a low percentage serum.

**Table 2: Effect of age on mean percentage and concentration of serum albumin, Ig-A, Ig-G and total proteins of clients attending Tiga Dam General Hospital, Kano**

| Age group (years) | Serum albumin | | Serum Ig-A (alpha-I and alpha II) | | Serum Ig-G (gamma) | | Total proteins (g/dl) | Mean HT (HU/ml) |
|---|---|---|---|---|---|---|---|---|
| | Percentage | Conc. (g/dl) | Percentage | Conc. (g/dl) | Percentage | Conc. (g/dl) | | |
| 11 – 15 | - | - | - | - | - | - | - | 0 |
| 16 – 20 | 69.39±3.99 | 50.6±4.04 | 12.4±1.71 | 8.7±1.3 | 12.7±2.96 | 7.80±1.9 | 71.0±3.8 | 1:86 |
| 21 – 25 | 67.33±4.26 | 54.8±4.49 | 15.3±2.89 | 12±2.43 | 9.80±8.16 | 7.73±6.4 | 80.2±0.8 | 1:171 |
| 26 – 30 | 73.05±9.16 | 53.4±6.69 | 14.7±5.55 | 11±3.98 | 6.48±2.32 | 4.75±1.7 | 73.1±0.9 | 1:512 |
| 31 – 35 | - | - | - | - | - | - | - | 0 |
| 36 – 40 | 69.60±8.00 | 26.7±26.7 | 16.5±8.05 | 2.9±2.9 | 4.80±4.80 | 0.00±0.0 | 34.5±34 | 1:512 |
| 41 – 45 | - | - | - | - | - | - | - | 0 |

This can be explained by the fact that there is not much difference between the low- and the middle-income classes in terms of nutritional status. The non-recovery of these factors from the high-income earners could be explained by the fact that most of the subjects belonging to this class attend urban hospitals where more health care facilities are available. Low- and middle-income class individuals exhibited higher antibody response compared to high-income class. The reason could be due to the fact that low- and middle-income earners had more time to take care of their family when compared with their high-income earners counterparts. In addition, the ways of lives of the low- and middle-income earners could expose them to *C. tetani* regularly than the high-income earners. Their natural immunity could thus be high.

**Table 3: Effect of socio-economic status on mean percentage and concentration of serum albumin, Ig-A, Ig-G and total proteins of clients attending Gaya General Hospital, Kano**

| Socio-economic status | Serum albumin | | Serum Ig-A (alpha-I and alpha II) | | Serum Ig-G (gamma) | | Total proteins (g/dl) | Mean HT (HU/ml) |
|---|---|---|---|---|---|---|---|---|
| | % | Conc. (g/dl) | % | Conc. (g/dl) | % | Conc. (g/dl) | | |
| Low income | 66.32±2.74 | 49.1±2.45 | 15.94±1.46 | 11.86±1.06 | 12.80±1.96 | 9.11±1.55 | 74.90±0.88 | 1:44 |
| Middle income | 71.08±7.59 | 52.4±7.05 | 12.42±4.49 | 9.20±3.38 | 7.60±7.60 | 5.10±5.10 | 73.94±1.93 | 1:462 |
| High income | - | - | - | - | - | - | - | 0 |

**Table 4: Effect of socio-economic status on mean percentage and concentration of serum albumin, Ig-A, Ig-G and total proteins of clients attending Tiga Dam General Hospital, Kano**

| Socio-economic status | Serum albumin | | Serum Ig-A (alpha-I and alpha II) | | Serum Ig-G (gamma) | | Total proteins (g/dl) | Mean HT (HU/ml) |
|---|---|---|---|---|---|---|---|---|
| | % | Conc. (g/dl) | % | Conc. (g/dl) | % | Conc. (g/dl) | | |
| Low income | 69.40±3.49 | 49.04±3.91 | 13.70±1.64 | 9.11±1.27 | 11.37±2.57 | 6.89±1.70 | 68.79±4.12 | 1:187 |
| Middle income | 71.53±2.81 | 54.63±3.14 | 10.30±2.03 | 7.95±1.77 | 8.98±3.13 | 6.73±2.39 | 76.30±2.32 | 1:131 |
| High income | - | - | - | - | - | - | - | 0 |

The effects of vaccination with adsorbed tetanus toxoid injection against tetanus (Tables 5-6) were clearly demonstrated among the clients. It was observed that clients who received two injections of tetanus toxoid demonstrated a significantly ($P < 0.05$) higher levels of serum Ig-A (18.07 ± 3.41% and 13.40 ± 2.43 g/d) and Ig-G (14.95 ± 7.26% and 11.02 ± 5.45 g/dl) at Gaya (Table 5). However, at Tiga Dam, clients with more than three injections had higher levels of Ig-A and Ig-G (Table 6). These observations correspond with the WHO (1983; 1999) recommendations that three properly spaced doses of tetanus toxoid injections afford mother's protection to her neonate. From this study, it could be said that clients from Gaya hospital might be adequately immunized by demonstrating a relatively higher percentage serum Ig-G that falls within the normal range of 10.5–19.5% (WHO, 1999). On the other hand, clients from Tiga Dam (Table 6) have shown a fairly low level of Ig-G. This could be attributed to the incorrect vaccination history on the part of the clients, poor quality of vaccine and inadequate storage temperature of the vaccines. This could explain the reason for some observations where individuals with only one vaccination gave high antibody response. These observations are in support of Owa and Makinde (1995) who reported similar findings from Ile-Ife, Nigeria.

**Table 5: Effect of number of tetanus toxoid injections on mean percentage and concentration of serum albumin, Ig-A, Ig-G and total proteins of clients attending Gaya General Hospital, Kano**

| NTTI | Serum albumin | | Serum Ig-A (alpha-I and alpha II) | | Serum Ig-G (gamma) | | Total proteins (g/dl) | Mean HT (HU/ml) |
|---|---|---|---|---|---|---|---|---|
| | % | Conc. (g/dl) | % | Conc. (g/dl) | % | Conc. (g/dl) | | |
| 1 | 65.40±5.86 | 50.62±5.31 | 15.07±3.08 | 11.22±2.31 | 12.57±3.45 | 7.91±2.66 | 74.59±1.77 | 1:29 |
| 2 | 63.32±4.61 | 41.17±3.56 | 18.07±3.41 | 13.40±2.43 | 14.95±7.26 | 11.02±5.45 | 73.83±2.50 | 1:640 |
| 3 | 64.06±3.64 | 48.40±3.34 | 15.28±1.92 | 11.48±1.37 | 12.52±4.99 | 9.40±3.78 | 75.32±1.53 | 1:768 |
| >3 | 68.36±5.37 | 52.26±4.31 | 13.52±1.75 | 10.16±1.24 | 11.88±3.65 | 9.02±2.66 | 76.30±1.12 | 1:922 |
| 0 | 76.54±7.72 | 56.94±6.60 | 14.54±5.03 | 10.56±3.65 | 6.62±2.90 | 4.78±2.09 | 73.98±1.35 | 1:50 |

**Table 6: Effect of number of tetanus toxoid injections on mean percentage and concentration of serum albumin, Ig-A, Ig-G and total proteins of clients attending Tiga Dam General Hospital, Kano**

| NTTI | Serum albumin | | Serum Ig-A (alpha-I and alpha II) | | Serum Ig-G (gamma) | | Total proteins (g/dl) | Mean HT (HU/ml) |
|---|---|---|---|---|---|---|---|---|
| | % | Conc. (g/dl) | % | Conc. (g/dl) | % | Conc. (g/dl) | | |
| 1 | 63.84±7.53 | 48.79±6.07 | 16.06±2.30 | 12.16±1.54 | - | 11.66±4.21 | 76.30±2.25 | 1:13 |
| 2 | 73.50±10.25 | 52.86±14.55 | 8.40±2.80 | 4.58±2.41 | - | 2.82±1.76 | 63.78±16.21 | 1:26 |
| 3 | 76.31±5.49 | 57.30±4.18 | 11.27±3.34 | 8.53±2.53 | - | 5.46±1.54 | 74.70±0.85 | 1:330 |
| >3 | 67.13±3.53 | 40.70±8.31 | 16.65±3.09 | 9.17±2.67 | - | 5.23±3.25 | - | 1:470 |
| 0 | 67.82±7.78 | 48.48±5.56 | 12.60±4.57 | 9.18±3.38 | - | 8.14±4.72 | - | 1:90 |

Key: NTTI = Number of tetanus toxoid injections

## Conclusions and Recommendations

The elimination of neonatal tetanus is an essential and attainable goal. It may be achieved by combining two approaches: First, increasing the immunization coverage of women of child-bearing age, essentially pregnant women with tetanus toxoid and, secondly, by improving maternity care, with particular emphasis on increasing the proportions of deliveries that are attended by trained personnel. There is no doubt that training of traditional birth attendants in Kano State will assist in reducing neonatal tetanus mortality rates. Community-based surveys on neonatal tetanus should be extended in order to obtain a more global picture of neonatal tetanus so that each State or country could then decide the priority, which should be given for the elimination of this disease. The Ministry of Health in collaboration with other Health Care Agencies should embark on a serious public enlightenment campaign through radios, televisions, jingles, posters and drama among others, on the implications and magnitude of the problem. Traditional practices like circumcision, tonsillectomy and traditional marks must be discouraged as they are often carried out with unsterile instruments.

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