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SERUM TOTAL IgG AND TETANUS SPECIFIC IgG IN NIGERIAN HUMAN IMMUNODEFICIENCY VIRUS INFECTED PRIMIGRAVIDAE AND THE CORD BLOOD OF THEIR BABIES AT BIRTH

M.O. Fatokun[1], O.O. Enabor[1], F.A. Bello[1], O.A. Adesina[1] and G.O. Arinola[2]

1. Department of Obstetrics and Gynaecology, University College Hospital, Ibadan, Nigeria
2. Department of Chemical Pathology, Immunology Unit, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Correspondence:
Dr. F.A. Bello
Dept. of Obs. and Gynae,
University College Hospital,
Ibadan, Nigeria.
E-mail: dr.nikebello@yahoo.com.

ABSTRACT

Background: **HIV infection affects millions of women and children, particularly in sub-Saharan Africa. Tetanus also causes significant maternal and neonatal morbidity and mortality in developing countries. Since the main effect of HIV is immunosuppression, there is potential for a negative influence on the host immune response to tetanus in women with HIV.**

Objective: **This case-control study evaluated the effect of HIV infection on maternal tetanus antibody production and neonatal tetanus antibody levels.**

Methods: **Thirty registered primigravidae were recruited from the clinic;15 were HIV positive and 15 were HIV negative. Serum samples of maternal and cord blood were obtained from both groups at delivery. Maternal total IgG and cord blood tetanus-specific antibody were estimated by Enzyme Linked Immunosorbent Assay.**

Results: **There was no significant difference in the total IgG level of HIV positive mothers compared with HIV negative mothers. No significant difference in the tetanus-specific IgG level in the cord blood of babies of HIV positive mothers compared with cord blood of babies of the HIV negative mothers.**

Conclusion: **HIV infection did not significantly reduce total IgG production in Nigerian primigravidae. Tetanus-specific IgG levels were above protective levels in neonates of HIV positive mothers suggesting adequate protection.**

Keywords: Tetanus, Antibody, Pregnancy, Immunisation, HIV.

INTRODUCTION

The devastation of Human Immunodeficiency Virus (HIV) infection remains pronounced globally, particularly in sub-Saharan Africa where an estimated 25.8 million people including pregnant women are affected[1]. In 2010, antenatal client prevalence of HIV in Nigeria was 4.1%[2]. It was reported that 90% of paediatric HIV cases were due to mother-to-child transmission (MTCT) of HIV[3].

Literature reports that the phenomena of inflammation and immunomodulation are involved either in HIV infection[4] or during pregnancy[5], which can affect vaccine response. Studies have highlighted the involvement of abnormal cellular and humoral immune responses during HIV infection. This includes abnormal pattern of serum protein electrophoresis, polyclonal hypergammaglobulinaemia, hyperproteinemia and plasma cell dyscrasias[6,7]. In addition, impaired phagocytosis, reduced number and functions of T-lymphocytes were reported during pregnancy[8]. Despite maternal hypergammaglobulinaemia in pregnant HIV infected mothers, reduced IgG transplacental transference to the foetus has been reported[6,9,10], which in turn, may influence vaccine response and neonatal immunity.

For the protection of mothers and babies, pregnant women in developing countries are usually vaccinated against tetanus. While no vaccine has yet been licenced for the prevention of HIV, maternal and neonatal tetanus prophylaxis has actively been pursued by the World Health Organization (WHO) since 1989[11,12]. Newborn babies are protected from neonatal tetanus by maternal anti-tetanus antibody of the IgG class which is transplacentally transferred from third trimester of gestation following tetanus toxoid vaccination in pregnant women.

Immunization for pregnant women with tetanus toxoid vaccine is the single most effective strategy independent of other interventions in eliminating neonatal tetanus[11,12]. However, there are conflicting reports of the influence of maternal HIV infection on anti-tetanus antibody production by the mothers as

well as its transfer through the placenta to their unborn babies. For instance, lower anti-tetanus antibody levels in HIV infected women were reported from Senegal[13] and Brazil[14], but not in The Gambia[15]. Cumberland *et al.*[6] found reduced transplacental transfer of tetanus antibody, and about 50% lower antibody levels in cord serum. In contrast, De Moraes-Pinto *et al.* from Malawi found that maternal HIV infection had no effect on cord anti-tetanus IgG levels as well as transplacental transfer of anti-tetanus antibody[10]. In addition, seroprevalence studies have suggested that HIV-infected patients are less likely to have adequate anti-tetanus antibodies. The progressive decline of CD4 levels in HIV-infected individuals could potentially lead to lost immunity to tetanus[16,17].

With the potential for HIV infection among women in the reproductive age group, and specifically pregnant women, it is necessary to evaluate the effect of HIV infection on the efficacy of tetanus toxoid in this group of people where tetanus toxoid vaccination is recommended. It is hypothesized that passively transferred immunity to the developing foetus by pregnant mothers will be affected by HIV infection. This study assessed tetanus-specific IgG levels of cord blood from babies born to HIV positive and negative primigravidae who received tetanus immunization.

MATERIALS AND METHODS

This was a case-control study conducted over four months at the labour ward of the University College Hospital (UCH), Ibadan, Nigeria. Ethical approval was obtained from the UI/UCH Ethical Committee (UI/EC/11/0128). The study group consisted of 15 HIV-infected pregnant mothers, while the control group consisted of age-matched HIV-negative pregnant mothers. Maternal HIV status is routinely determined at the antenatal booking visit. Following counselling, HIV infection is diagnosed using rapid screening tests and confirmed by Western Blot at the Virology Laboratory, College of Medicine, University of Ibadan, Ibadan, Nigeria. Only clients who had registered for antenatal care were recruited to ensure that their HIV status was known. All HIV positive clients are routinely placed on antiretroviral therapy (ARV) as part of antenatal care, in line with the protocol for prevention of maternal to child transmission of HIV. Patients were placed on Truvada (emtricitabine and tenofovir disoproxil fumarate) and efavirenz as from first presentation after 14 weeks of gestation.

Compliance was determined by the attending physician enquiring from the patient how many doses had been missed in the past one week. Patients who missed more than one dose were referred to adherence counsellors. The adherence counsellors would attempt to identify any reason for non-adherence. The patient would be assisted to develop a plan to improve adherence and compliance. Challenges and successes with plan would be reviewed periodically.

Consenting HIV-positive clients were recruited as participants, when admitted in active labour. On account of the anticipated small population, a convenience sample of consecutive clients were counselled until the sample size was complete. The next consenting, age-matched, HIV-negative client who presented in active labour (after each study group participant), was recruited as a control. Sample size was calculated based on the difference in antibody transfer between HIV-positive and -negative women obtained from a similar study[18]. Participants were enrolled based on documentation in the medical records of having received two doses of tetanus toxoid, the second one being at least four weeks before delivery.

A sample of 5 ml of maternal venous blood was obtained in a universal specimen bottle without anticoagulant. After delivery, the baby's blood was collected after clamping and cutting the umbilical cord. Maternal and baby sera were separated from the whole blood after centrifugation and stored at -20^{0}C.

A data collection tool was used to collect information from the participants and their medical records. The following sections were included: bio-demographic data, HIV status, history of index pregnancy, labour, delivery parameters and history of tetanus toxoid administration. The babies' Apgar scores, birth weights and other anthropometric parameters were also obtained.

Cord blood tetanus specific IgG and maternal total IgG were measured using Enzyme Linked Immunosorbent Assay (ELISA) method with EUROIMMUN (GMbh, Leubeck, Germany). A fixed volume per well of appropriate sample dilution buffer, antigen standard cocktail or an experimental sample was pipetted into microtitre plates. This sample was incubated at room temperature (25^{0}C-27^{0}C). The ELISA immunoplate was washed 3 times with 350µl/well of washing buffer. A concentration of 100µl of diluted Avidin-HRP conjugate was added, after which the plate was incubated for 30 minutes in darkness. The plate was washed four times and 100 µl per well of developing solution was added. The reaction was stopped with 100µl per well of stop solution and the Optical Density (OD) was read at a specific wavelength within 30 minutes of addition of stop solution. The average absorbance of each OD was plotted against corresponding cytokine values to create a standard

curve. The average absorbance of each serum sample was used to determine corresponding cytokine values by interpolating from the curve.

The main outcome variable was the antenatal tetanus IgG production and transfer to babies of the participants. All data were collected, cleaned and manually entered into a computer. Analysis was done using the Statistical Package for Social Sciences (SPSS) version 17. Proportions between groups were compared using the ÷-square test with Fisher's exact correction. Continuous variables were presented as mean±SD and differences between means compared using the students' T-test. The level of significance was P<0.05.

RESULTS

The mean ages of HIV positive women (study group) and the HIV negative women (control group) were comparable: 28.27 ± 3.9years versus 27.32 ± 4.1years (p = 0.688). Table 1 highlights differences in socio-demographic variables between study and control groups. All participants were married. Both groups were similar in age group, occupation and educational level.

Among the patients' spouses, 11 (73.3%) in the study group and 13 (86.7%) in the control group had tertiary education (p=0.505). Eleven husbands (73.3%) were skilled in the study group while 13(86.7%) were skilled in the control group (p=0.174).

Tetanus immunization profile is presented in Table 2 while delivery and neonatal parameters are highlighted in Table 3. A greater proportion of HIV-negative women had their second dose in the 2nd trimester [80% v. 33%, (p=0.341)]. There was no report of adverse effects following immunization in either group. The mean birth weight was 2.99 ± 3.12kg in the study group and 2.89 ± 4.08kg in the control group (p=0.807). There were no statistically significant relationships between tetanus immunization profile and delivery parameters.

Table 4 highlights the mean total IgG and tetanus specific IgG for both groups (mothers and babies).

Table 1: Comparison of socio-demographic characteristics of HIV infected pregnant mothers compared with HIV uninfected mothers

Variable	Study (n, %) *n=15*	Control (n, %) *n=15*	p (Chi-square)
Age group(years)			
21-30	8(53.3)	10(66.7)	0.456(0.556)
31-40	7(46.7)	5(33.3)	
Occupation			
Skilled	4(26.7)	11(73.3)	0.125(7.219)
Unskilled	11(73.3)	4(26.7)	
Religion			
Christianity	10(66.7)	12(80.0)	0.409(0.682)
Islam	5(33.3)	3(20.0)	
Tribe			
Yoruba	12(80.0)	14(93.3)	0.125(4.154)
Others	3(20.0)	1(6.7)	
Educational level			
<Tertiary	4(26.7)	2(13.3)	0.505(1.367)
Tertiary	11(73.3)	13(86.7)	

Table 2: Tetanus immunization record of the participants

Variable	Study (n, %) *n=15*	Control (n, %) n=15	P (Chi-square)
First dose			
2nd trimester	13 (86.7)	11 (73.3)	0.361 (0.833)
3rd trimester	2 (13.3)	4 (26.7)	
Second dose			
2nd trimester	5 (33.3)	12 (80.0)	0.341 (0.682)
3rd trimester	10 (66.7)	3 (20.0)	

Table 3: Comparison of delivery mode and neonatal parameters of the study participants

	Study (n, %)	Control (n, %)	p (Chi-square)
Delivery mode			
SVD	4 (26.7)	7 (46.7)	0.067 (5.418)
CS	11 (73.3)	8 (53.3)	
Gender			
Male	6 (40)	8 (53.3)	0.464 (0.536)
Female	9 (60)	7 (46.7)	
Birth Weight			
<2.5kg	1 (6.7)	2 (13.3)	0.543 (0.370)
≥2.5kg	14 (93.3)	13 (86.7)	
OFC			
<35cm	5 (33.3)	5 (33.3)	1.000 (0.000)
≥35cm	10 (66.7)	10 (66.7)	
Length			
<50cm	4 (26.7)	2 (13.3)	0.361 (0.833)
≥50cm	11 (73.3)	13 (86.7)	
Apgar(1minute)			
5-6	2 (13.3)	1 (6.7)	0.543(0.370)
7-10	13 (86.7)	14 (93.3)	

Mean total IgG was not significantly different in maternal and neonatal sera of HIV positive women ($p = 0.601$ and $p = 0.187$ respectively) compared with HIV non-infected controls. Mean maternal tetanus specific IgG and cord blood tetanus specific IgG were not significantly lower in HIV positive women when compared with controls ($p = 0.251$ and $p = 0.484$ respectively).

naemia as a result of polyclonal B lymphocyte action by HIV as previously reported[4,7].

The authors of this study inferred that compliance with antiretroviral therapy, personal hygiene and regular antenatal care might explain the similarity in the levels of total IgG of HIV-infected mothers and controls, since the study participants were registered antenatal clients and will likely comply with instructions.

Table 4: Serum levels of total IgG and tetanus specific IgG in pregnant mothers infected with HIV and cord blood of their babies compared with controls

Variable	HIV infected mothers	Control	P value
Total IgG(g/dl)			
Mothers	18.54±5.62	14.36±5.57	0.601
Babies	15.13±11.6	14.36±2.10	0.187
Tetanus IgG(IU/ml)			
Mothers	0.71±0.13	0.77±0.12	0.251
Babies	0.72±0.12	0.76±0.16	0.484

DISCUSSION

The influence of maternal HIV on the production and placenta transfer of maternal total IgG and tetanus-specific IgG to the foetus was evaluated in this research. Total maternal IgG values were higher (though not significantly) among HIV positive women than controls in our study. This is in support of hypergammaglobuli-

Mean maternal tetanus-specific IgG values were not significantly reduced in the HIV infected pregnant mothers as found elsewhere[7]. In the present study, a 5% reduction from anti-tetanus IgG values in neonates from HIV negative mothers was observed while a study in Brazil reported a 33% reduction[9]. Nigerian

studies have suggested a reduction in trans-placental transfer of tetanus antibody by placenta malaria parasites[19] and that pregnant women are reservoirs of *Plasmodium* malaria parasites especially in the placenta[20]. Therefore, the presence of malaria parasites during pregnancy might have affected the production of anti-tetanus IgG among Nigerian subjects compared with Brazilians, though malaria was not tested in the index study.

Lower level of tetanus-specific antibodies among HIV-infected women may be due to increased lymphocyte apoptosis and HIV-induced loss of memory T- and B-lymphocyte functions. Furthermore, placental abnormalities and saturation of active transport receptors associated with HIV infection may also result in inefficient transplacental antibody transfer to fetuses of HIV-infected women[21]. This may be compounded by increased pro-inflammatory cytokines secretion and reduced thymic sizes and lower CD4+ T-lymphocyte counts. Our proposition is supported by the finding that *in-utero* antiretroviral therapy exposure has been associated with mitochondrial toxicity, lower numbers of circulating T-cell lymphocytes and neutrophils in young infants. Nonetheless, the slightly lower levels of anti-tetanus antibodies in this study were not significant.

The level of anti-tetanus antibody considered protective by WHO guideline is >0.01U/ml [6]. Our findings indicate that antibody levels were protective in both mother and baby pairs considered for the study. Though majority of HIV positive women received the 2nd tetanus immunization dose in the third trimester, it was not likely that this significantly influenced maternal total IgG or tetanus specific IgG. Moreover, most viral infections affecting the mothers were found not to cause congenital foetal infection suggesting that the placenta may play an important role as a potent immune-regulatory interface protecting the foetus from systemic infection. This modulatory effect of the placenta might also explain non-significant differences in the levels of total IgG and tetanus antibody in HIV infected pregnant mothers compared with HIV un-infected mothers.

The limitations of the study are a relatively small sample size (though the minimum number required for the outcome was calculated and recruited), non-screening for placenta malaria and non-consideration of effect of previous vaccination on tetanus specific IgG values. Primigravidae were recruited in an attempt to limit the effect of previous vaccination, as multigravidae would have been vaccinated in previous pregnancies.

CONCLUSION

HIV infection did not significantly reduce maternal tetanus antibody production and tetanus antibody transfer among pregnant HIV infected primigravidae on ARV therapy in Ibadan. Adherence to antenatal protocols, guidelines and compliance with ARV therapy in HIV positive pregnant women must be emphasized and periodically evaluated in all health facilities.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1. WHO.WHO Media centre, HIV/AIDS factsheet NO360, July 2015. http://www.who.int/mediacentre/factsheets/fs360en, accessed 25/8/15.
2. National AIDS/STIs Control Programme, Federal Ministry of Health, Abuja, Nigeria. Integrated National Guidelines for HIV Prevention Treatment and Care (Chapter 1). Epidemiology of HIV in Nigeria. Federal Ministry of Health, 2015: 4.
3. **Agboghoroma CO,** Audu LI, Iregbu KC. Effectiveness of prevention of mother-to-child transmission of HIV program in Abuja, Nigeria. J HIV Hum Reprod. 2015; 3: 7-13.
4. **Olaniyi JA,** Arinola OG. Humoral immunoglobulin factors and nitric oxide levels in HIV patients with low CD4+ T-lymphocyte count. Intl. J of Health Research. 2011; 4(2): 67-70
5. **Arinola OG,** Salawu L., Ojurongbe O. Immunoglobulin classes (IgG,A and M) and acute phase proteins in pregnant women with urinary schistosomiasis. 2005; West African Journal of Medicine; 24(1): 44-48.
6. **Cumberland P,** Shulman CE, Chris Maple PA, Bulmer JN. Maternal HIV infection and placental malaria reduce transplacental antibody transfer and tetanus antibody levels in newborns in Kenya. J Infect Dis 2007; 196: 550-557.
7. **De Milito A.** B-lymphocyte Dysfunctions in HIV Infections. Curr. HIV. Res. 2004; 2: 11-21.
8. **Creazy R** and Resnik R.(eds.) Maternal-Fetal Medicine, 5th edn. Philadelphia, Saunders 2004.
9. **Brair ME,** Brabin BJ, Milligan P et al. Reduced transfer of tetanus antibodies with placental malaria. Lancet 1994; 343: 208-209.
10. **Romero R.** Novel aspects of neutrophil biology in human pregnancy. Am J Reprod Immunol. 2005; 53: 275.
11. **Mor G.** Inflammation and pregnancy: the role of toll-like receptors in trophoblast-immune interaction. Ann N Y Acad Sci. 2008; 1127: 121–128.

12. **Terpstra FG.** Longitudinal Study of Leukocyte Functions in homosexual men sero-converted for HIV: rapid and persistent loss of B-cell function after HIV infection. Eur J Immunol 1989; 19: 667-673.
13. **deMoraes-Pinto MI,** Verhoeff F, Chimsuku L. Placental Antibody Transfer: Influence of maternal HIV infection and placental malaria. Arch Dis Child Fetal Neonatal Ed 1998; 79: F202-205.
14. **deMoraes-Pinto MI,** Almeida AC, Kenj G. Placental transfer and maternally acquired neonatal IgG immunity in Human Immunodeficiency Virus Infection. J Infect Dis 1996; 173: 1077-1084.
15. **Han YW,** Ikegami A, Bissada NF, *et al.* Transmission of an uncultivated Bergeyella strain from the oral cavity to amniotic fluid in a case of preterm birth. J Clin Microbiol. 2006; 44: 1475–1483.
16. **Srinivas SK,** Ma Y, Sammel MD, *et al.* Placental inflammation and viral infection are implicated in second trimester pregnancy loss. Amer J Obstet Gynecol. 2006; 195: 797–802.
17. **Rahamon SK,** Arinola GO. Immunoglobulin Classes and Acute Phase Proteins in the Breast Milk and Plasma of Nigerian HIV-Infected Lactating Mothers. Eur J Gen Med 2012; 9: 241-246.
18. **Onyenekwe CC,** Meludu SC, Arinola OG, Salimonu LS. Tetanus toxoid antibody level in Asymptomatic *Plasmodium falciparum* malaria parasitaemic pregnant women. Afr J Biomed Res 2003; 6: 73-77.
19. **Onyenekwe CC,** Meludu SC, Arinola OG, Salimonu LS. Relationships between P. falciparum density, haptoglobin, transferrin and packed cell volume in apparently healthy pregnant women. Afr J Biomed Res. 2005; 8: 21-24.
20. **Mel'nikova VF,** Aksenov OA. Infectious placentitis and characterization of the placenta as an immune barrier. Arkh Patol. 1993; 55: 78–81.