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RESEARCH ARTICLE

A Mother-to-Child Transmission Study in Nigeria: The Impact of Maternal HIV Infection and HAART on Plasma Immunoglobulins, Cytokine Profiles and Infant Outcome

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Abstract

Prevention of mother-to-child transmission (PMTCT) of HIV with highly active antiretroviral therapy (HARRT) allows the HIV^+ pregnant mothers to have vaginal delivery and breastfeed. Here we investigated the maternal plasma immunoglobulin, cytokine secretion and the outcome of the exposed infants among the HIV^+ HAART treated pregnant women in Nigeria. In this study, different plasma immunoglobulins and cytokines were measured in the HIV^+ HAART treated pregnant mothers. Pooled culture supernatants of B and T lymphocytes showed lower levels of IFN-γ, IL-10 and IL-4. There were lower IFN-γ and IL-10 secretions at 1st trimester; however, IL-10 continued to be lower throughout 2nd and 3rd trimesters. TNF-α secretion significantly decreased as pregnancy progressed to term. There were high plasma IgG and low IgM in the HIV^+ HAART treated pregnant women. Plasma IgG was high during 1st and 3rd trimesters. After one year of follow up, all the exposed children were seronegative for HIV-1 and HIV-2. Vaginal delivery and breastfeeding among HIV^+ HAART treated mothers have shown to be safe. The use of HAART by the infected mothers and the use of septrin and niverapin by the exposed infants prevented mother to-child transmission of HIV.

Keywords Human immunodeficiency virus (HIV) · Prevention from mother-to-child transmission (PMTCT) · Highly active antiretroviral therapy (HAART) · Lymphocyte stimulation · Mitogen · Cytokine · Immunoglobulins

Introduction

HIV weakens the immune strength of the pregnant mother through increase in HIV viremia, decrease in CD4+ cell counts, decrease in neutrophil phagocytosis, reduction of lymphocyte transformation, enhancement of Th1/Th2 shift in cytokine production and decrease in immunoglobulin A, G and M (Clerici *et al.* 2000; Müller *et al.* 2002; Pacheco *et al.* 2006; Ifeanyichukwu *et al.* 2010; Onyenekwe *et al.* 2010). The maternal immune response is regulated by a complex system of cytokines which act to promote proper growth and development of fetus while maintain the pregnancy till term (Burns *et al.* 2005; Gregory *et al.* 2006; Saraiva and O'Garra 2010; Kunzmann *et al.* 2013). In normal pregnancy, there is a decrease in Th-1 cytokine and an increase in Th-2 cytokine to allow host tolerance to an allograft (Burns *et al.* 2005; Gregory *et al.* 2006; Saraiva and O'Garra, 2010). The role of immunoglobulins in the maintenance of pregnancy cannot be overemphasized.

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Further support for an important role of anti-inflammatory cytokines in preventing reproductive failure was provided by a study in which intravenous immunoglobulin therapy in women with recurrent spontaneous abortions increased IL-4 and IL-10 levels and decreased the ratio of IFNγ+/IL-4+ T cells (Yamada *et al.* 2003).

Studies have shown that over a quarter of all cases of mother-to-child transmission (MTCT) of HIV in the world happen in Nigeria (UNAIDS 2017; UNAIDS 2018). This study is uniquely designed to study the safety of PMTCT (Prevention of MTCT) procedure for HIV^+ pregnant mothers in Nigeria otherwise known as Option B+ or "treat all" (Fasawe *et al.* 2013; WHO 2015) and the infants' outcome as against their counterparts in developed countries. This is a treatment approach recommended for low-resource/high-HIV burden settings (WHO 2010) and was adopted in Nigeria. Nigerian HIV^+ pregnant mothers are given HAART, allowed vaginal delivery and breastfeeding of their infants; the studies were done in developed countries with different PMTCT approach (HIV^+ pregnant mothers are given antiretroviral, cesarean section delivery and avoidance of breastfeeding). In this study, we aimed at assessing the possible effects of maternal HIV infection and HAART on the immunity of the pregnant mothers and the safety of the PMTCT procedure adopted by Nigerian government. This study also assessed the outcome of the exposed infants as regards their HIV-1 and HIV-2 status after one year.

Materials and Methods

Pregnant Mothers and Their Infants

Pregnant women were randomly selected from a case-controlled study. The pregnant mothers were in two categories; HIV^+ HAART treated pregnant mothers and HIV^- pregnant mothers. A total of 122 HIV^+ HAART treated pregnant mothers were seen at the PMTCT clinic of Mother of Christ Specialist, Hospital, Ogui Enugu, Nigeria. All HIV^+ pregnant mothers were placed on HAART irrespective of their CD4-T cell count through pregnancy, labour, delivery, breastfeeding and thereafter as the PMTCT guideline requires. HIV^+ HAART treated pregnant mothers were further grouped into 3 gestational stages: 1st trimester (n = 16), 2nd trimester (n = 52) and 3rd trimester (n = 54) respectively. A total of 72 HIV^- pregnant women were seen at the antenatal clinic section of Mother of Christ Specialist Hospital, Ogui Enugu, Nigeria as control. They were further divided into 1st trimester (n = 12), 2nd trimester (n = 35) and 3rd trimester (n = 25). Control and test subjects include all HIV^-/HIV^+ pregnant mothers without infection, hypertension, diabetes, smoking, alcohol and hard drug abuse. All pregnant women (test and control) were allowed normal vaginal delivery and breastfeeding. They breastfed their babies for 8 months to 1 year; the first 6 months was exclusive breastfeeding while the next 6 months was complementary (a combination of breast milk, water and food). The exposed infants received nevirapine syrup (1 mL for infant < 2.5 kg, 1.5 mL for infant > 2.5 kg) daily from birth till 6 weeks of age and then septrin until weaned. Detection of viral antigen using nucleic acid technique, polymerase chain reaction (PCR) method was done on the baby at 6 weeks, 6 months, 12 months of age and at some weeks after the baby is weaned. After one year of follow up, all the exposed infants tested negative to HIV using polymerase chain reaction (PCR) and termed seroreverters.

Sample Collection and Separation

About 10 mL maternal blood was collected into heparin container from both HIV^+ pregnant mothers and the control pregnant mothers. Within few hours (1–4 h) of collection, part of the whole blood was used to immediately separate peripheral blood mononuclear cells (PBMCs) and lymphocyte (B and T cell) stimulation was done using mitogens. The culture supernatant from PBMCs (lymphocyte) stimulation was used for the analysis of cytokines. The remaining whole blood was separated, and the plasma used for the analysis of Immunoglobulin (A, G, M).

Lymphocyte (B and T cell) Proliferation

Maternal whole blood was mixed with equal volume of phosphate buffered saline (PBS) also known as Hank's balanced salt solution (H9394-Sigma-Aldrich) to aid clear separation of different blood components. PBMCs were isolated from whole blood-PBS mixture by density gradient centrifugation using Ficol-Hypaque 1077 (Sigma-Aldrich, Germany). The isolated PBMCs were washed with PBS and diluted to 1×10^6 in RPMI 1640 culture medium (R7388-Sigma Aldrich, Germany). The RPMI was supplemented/enriched with penicillin–streptomycin (10 mL/L), amphotericin B (2.5 mg/L) and 10% v/v FBS (fetal bovine serum). The PBMC culture mixture was finally stimulated with pokeweed (PW, L9379; Sigma-Aldrich, Germany), phytohemagglutinin (PHA, P8754; Sigma-Aldrich, Germany) and concanavalin A (Con A, C5275; Sigma-Aldrich, Germany) for 72 h/3 days at 37 °C with 5% CO_2 in 96-well cell culture plate.

Cytokine Assay

Cytokine assays were performed in culture supernatants of lymphocyte (B and T cell) proliferation from HIV^+

pregnant mothers and HIV^- control pregnant mothers. Method of cytokine quantification employed the principle of enzyme-linked immunosorbent assay (ELISA). Cytokines in the class of Th1 (IL-2, TNF-α, IFN-γ) and Th2 (IL-4, IL-10) were assayed using customized Milliplex MAP Kit for human cytokine/chemokine magnetic bead panel. The selected cytokines were based on their roles in pregnancy.

Immunoglobulin Assay

Immunoglobulins (IgA, IgG, IgM) were quantified in plasma from HIV^+ pregnant mothers and HIV^- pregnant mothers. Estimation of Human IgA, IgG and IgM was done using the principle of ELISA.

Statistical Analysis

Cytokine results were expressed as median and range values. Mann–Whitney test was used to calculate the difference between medians of unpaired data. Immunoglobulin results were expressed as mean ± SD values. Column analysis, one-way analysis of variance (ANOVA) were used for the comparison of the means of the unpaired data. Statistical package used was GraphPad Prism 5. Significant value was set at $P < 0.05$.

Results

Characteristics and Treatments of Patients

The characteristics of the pregnant women in this study are summarized in Table 1. Each HIV^+ mother on HAART was either given a combination of combivir + nevirapine (lamovidine + zidovudine + nevirapine) or a combination of truvada + efavirenz (emtricitabine + tenofovir + efavirenz). All HIV^+ pregnant women went through vaginal delivery as the PMTCT protocol allowed but for three that had caesarean delivery. None of the pregnant women involved in the study smoked or used illegal substances. Pregnant women with other infections (diabetes, pre-eclampsia and high blood pressure) beside HIV infection were not included in the study as these factors might cause adverse pregnancy outcome. All the pregnant women in this study were given anti-malaria drug as a routine preventive therapy because malaria parasite is endemic in Nigeria. Regarding parity, more child-deaths were recorded among the HIV^+ pregnant mothers. There was a low turn-out of pregnant women during 1st trimester and a high turnout at 3rd trimester in both control and HIV^+ mothers. Most pregnant mothers attended antenatal during the last 3 months prior to their delivery. This was mainly due to a lack of finance, illiteracy and ignorance on the necessity of early antennal care. On the duration of HIV infection, it was observed that the HIV^+ pregnant mothers had been infected for a period of 1–16 years but they enrolled for HAART within a period of 1–8 years, suggesting a delay in the enrolment of HIV infected mothers with a HAART facility (PMTCT Clinic). Reasons for this delay ranged from unwillingness to let anyone know about their status, shame and stigmatization inherent in African society, illiteracy to poverty (Table 1).

Evaluation of Vertical Transmission

All the exposed infants whose mothers were HIV^+ HAART treated underwent vaginal delivery but for three whose mothers went through caesarean delivery. Exposed infants were breastfed while receiving nevirapine syrup (1 mL for infant < 2.5 kg, 1.5 mL for infant > 2.5 kg) daily from 0 to 6 weeks and septrin syrup from 7th week until weaned. Detection of viral antigen using PCR was done on the exposed babies. A follow-up on the outcome of the DNA-PCR testing showed that after one year, all the exposed infants whose mothers adhered to HAART

Table 1 Characteristics of the pregnant mothers (median and range).

Characteristics	HIV negative mothers (n = 72)			HIV positive mothers (n = 122)		
	T_1 (n = 12)	T_2 (n = 35)	T_3 (n = 25)	T_1 (n = 16)	T_2 (n = 52)	T_3 (n = 54)
Age at delivery (years)	27.5 (23–42)	29 (19–36)	29 (21–42)	27.5 (24–34)	32 (22–38)	30 (23–38)
Duration of infection	–	–	–	3 (1–6)	3 (1–16)	3 (1–10)
Duration of HAART treatment (years)	–	–	–	3 (1–6)	3 (1–7)	2 (1–8)
Months of gestational	3 (2–3)	5 (4–6)	8 (6–9)	3 (1–3)	5 (4–6)	8 (7–9)
Parity (alive–dead)	17	31-1	30-1	20-2	75-5	52-6
Blood pressure (mean, mm/Hg)	109/66	107/69	103/63	103/80	107/73	111/72
BMI index (kg/m^2)	27.3	27.6	28.7	28.3	27.2	33.9

HIV, human immunodeficiency virus; BMI, body mass index; T, trimester; HAART, highly active antiretroviral therapy.

procedure tested negative to HIV and are termed seroreverters. None of the exposed children born to HIV^+ HAART treated mothers was vertically infected with HIV-1 and HIV-2.

Cytokine Profiles

Cytokine secretions in the HIV^+ HAART treated and control pregnant women after T and B lymphocyte stimulation irrespective of trimester categorization are shown in Table 2. The B and T cells of the PBMC from both HIV^+ pregnant mothers and the control group were stimulated with mitogens (Con A, PW and PHA) to assess the cytokine secretion ability (immune strength) of their B and T lymphocytes. The estimation of secreted cytokines (IL-2, TNF-α, IFN-γ, IL-4, IL-10) were performed using customized Milliplex MAP Kit for human cytokine/chemokine magnetic bead panel. The cytokine quantification was done using ELISA. Results in Table 2 show that IFN-γ, IL-4 and IL-10 levels were significantly lower in the HIV^+ HAART treated pregnant women when compared with the HIV^- pregnant women. Low IL-4 and IL-10 secretions seen in the HIV^+ HAART treated pregnant women might be attributed to HIV infection.

To assess whether gestational stages affects cytokine secretion, cytokine levels of the HIV^+ HAART treated and control pregnant women were categorized into 1st, 2nd and 3rd trimesters respectively as shown in Tables 3, 4 and 5. Results showed that in the 1st trimester, IFN-γ stimulated by pokeweed had significantly lower value (1.0 pg/mL) when compared with the control group (59.3 pg/mL). Again, IFN-γ and IL-10 stimulated by phytohemagglutinin had significantly lower values than the control group (Table 3). In the second and third trimesters, the HIV^+ HAART treated pregnant women showed significantly low levels of IL-10 stimulations by pokeweed compared to their healthy control group ($P = 0.03$, $P = 0.02$). However, TNF-α, IL-2 and IL-4 secretions were similar in both the HIV^+ pregnant women on HAART and the control group throughout 1st, 2nd and 3rd trimesters respectively (Tables 3, 4, 5).

Table 6 compares cytokine secretions in HIV^+ pregnant women on HAART as the pregnancy progresses through 1st, 2nd and 3rd trimesters respectively. This intra comparison was intended to reveal the pattern of cytokine secretion within the HIV^+ HAART treated pregnant women as gestational age progresses to term. Results showed a significantly ($P = 0.02$; $P = 0.03$) lower secretions of TNF-α by phytohemagglutinin at the 2nd and 3rd trimesters compared to the 1st trimester (Table 6). The persistently low IL-10 secretion seen throughout 1st, 2nd and 3rd trimester in Tables 3, 4 and 5 could be attributed to HIV infection. Low IL-10 secretion is the body's immune strategy to allow the clearance of the virus. Decrease in TNF-α secretion as pregnancy progressed to term could be attributed to the regulatory function of HAART in preventing excessive inflammation (Table 6).

Table 2 Cytokine levels (in pg/mL, median and range) after stimulation with PW, Con A and PHA in the supernatant from mothers' peripheral mononuclear cell cultures.

		HIV uninfected control (n = 72)	HIV infected HAART treated (n = 122)	*P* value Control *vs* treated
IL-2	Con	3.5 (0–13,822)	0.0 (0–17,056)	0.32
	PW	3.3 (0–1718)	0.0 (0–9060)	0.28
	PHA	0.2 (0–5064)	0.0 (0–105.5)	0.58
TNF-α	Con	257.4 (11–5707)	33.6 (0–13,895)	0.11
	PW	169.8 (5.9–13,633)	59.6 (2.3–17,097)	0.30
	PHA	149.6 (5.8–24,325)	131.1 (0–12,933)	0.54
IFN-γ	Con	21.0 (0–4009)	1.1 (0–14,636)	0.04*
	PW	26.7 (15,985)	5.3 (0–17,673)	0.01*
	PHA	10.4 (0–17,673)	3.7 (0–8586)	0.01*
IL-4	Con	3.7 (0–18,379)	0.0 (0–11,070)	0.03*
	PW	0.0 (0–18,379)	0.0 (0–16,213)	0.24
	PHA	0.0 (0–16,213)	0.0 (0–16,213)	0.37
IL-10	Con	14.4 (0–19,577)	0.0 (0–12,960)	0.002*
	PW	12.8 (0–19,577)	0.0 (0–8486)	0.001*
	PHA	8.2 (0–13,945)	2.0 (0–1208)	0.02*

IL, interleukin; IFN-γ, interon gamma; TNF-α, tissue necrosis factor alpha; Con A, concanavalin A; PW, pokeweed; PHA, phytohymaglutinin A; *vs*, versus; HIV, human immunodeficiency virus; HAART, highly active antiretroviral therapy.

*Significant value set at ($P < 0.05$).

Table 3 Cytokine levels (in pg/mL, median and range) after stimulation with PW, Con A and PHA in the supernatant from mothers' peripheral mononuclear cell cultures at 1st trimester.

Types of stimulation	Subjects				
	IL-2	TNF-α	IFN-γ	IL-4	IL-10
Con A stimulation					
Pregnant women on HAART (n = 122)	0.0 (0–0.55)	18.5 (4–6619)	0.2 (0–4.7)	0.0 (0–0)	0.0 (0–0)
Control (n = 72)	2.4 (0–60.9)	108.7 (11–2035)	21.1 (0–2920)	4.2 (0–10,756)	86.3 (1–17,840)
P value	0.09	0.28	0.11	–	–
PW stimulation					
Pregnant women on HAART	0.0 (0–282)	39.8 (2–17,097)	1.0 (0–422)	0.0 (0–737)	0.0 (0–0)
Control	3.7 (0–616)	155.6 (6–3203)	59.3 (3–10,150)	0.0 (0–742)	72.1 (0–1445)
P value	0.15	0.59	0.01*	0.33	–
PHA stimulation					
Pregnant women on HAART	0.0 (0–15.8)	867.7 (0–10,842)	0.0 (0–162)	0.0 (0–0)	0.0 (0–1.9)
Control	0.0 (0–13.4)	906.3 (8.4–4225)	499.4 (4–6017)	0.0 (0–9876)	33.7 (0–13,945)
P value	0.83	0.79	0.03*	–	0.04*

IL, interleukin; IFN-γ, interon gamma; TNF-α, tissue necrosis factor alpha; Con A, concanavalin A; PW, pokeweed; PHA, phytohymaglutinin A; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus.

*Significant value set at ($P < 0.05$).

Table 4 Cytokine levels (in pg/mL, median and range) after stimulation with PW, Con A and PHA in the supernatant from mothers' peripheral mononuclear cell cultures at 2nd trimester.

Types of stimulation	Subjects				
	IL-2	TNF-α	IFN-γ	IL-4	IL-10
Con A stimulation					
Pregnant women on HAART (n = 122)	0.9 (0–17,056)	58.2 (0–13,895)	7.3 (0–5270)	0.0 (0–6502)	1.1 (0–12,960)
Control (n = 72)	4.6 (0–13,822)	250.5 (17–2219)	30.0 (0–4009)	1.4 (0–18,379)	19.7 (0–19,577)
P value	0.93	0.14	0.31	0.23	0.32
PW stimulation					
Pregnant women on HAART	3.1 (0–9060)	104.6 (3–11,331)	5.5 (0–17,673)	0.0 (0–4190)	0.5 (0–8067)
Control	1.1 (0–85.4)	103.5 (7–13,633)	70.6 (0–15,985)	2.5 (0–18,379)	32.5 (0–3690)
P value	0.81	0.57	0.19	0.45	0.03*
PHA stimulation					
Pregnant women on HAART	0.0 (0–105.5)	203.3 (4–12,933)	3.8 (0–8408)	0.3 (0–97)	2.5 (0–937)
Control	0.9 (0–34.8)	203.4 (6–6475)	6.8 (0–17,673)	0.0 (0–16,213)	3.7 (0–581)
P value	0.75	0.79	0.32	0.72	0.45

IL, interleukin; IFN-γ, interon gamma; TNF-α, tissue necrosis factor alpha; Con A, concanavalin A; PW, pokeweed; PHA, phytohymaglutinin A; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus.

*Significant value set at ($P < 0.05$).

Immunoglobulin Profiles

To assess the B cell compartment as part of the immune response in the HIV^+ HAART treated pregnant women and their healthy controls, plasma IgA, IgG and IgM were measured.

Pooled plasma IgA (mg/dL) level in the HIV^+ HARRT treated and HIV^- pregnant mothers showed no significant ($P > 0.05$) difference (Fig. 1A). Non significance in plasma IgA level suggests non-existence of mucosal infection among the HIV^+ HAART treated mothers.

Pooled data indicated significantly lower ($P < 0.05$) total plasma IgM level in the HIV^+ HAART treated

Table 5 Cytokine levels (in pg/mL, median and range) after stimulation with PW, Con A and PHA in the supernatant from mothers' peripheral mononuclear cell cultures at 3rd trimester.

Types of stimulation	Subjects				
	IL-2	TNF-α	IFN-γ	IL-4	IL-10
Con A stimulation					
Pregnant women on HAART (n = 122)	0.5 (0–8740)	33.6 (0–13,495)	3.7 (0–4636)	0.3 (0–11,070)	0.0 (0–4794)
Control (n = 72)	3.8 (0–315)	386.0 (14.7–5707)	22.6 (0–2141)	5.3 (0–13,000)	6.6 (0–6352)
P value	0.64	0.68	0.28	0.39	0.11
PW stimulation					
Pregnant women on HAART	0.0 (0–8438)	38.6 (9.1–2965)	20.9 (0–17,673)	0.0 (0–16,213)	1.1 (0–8486)
Control	3.5 (0–1718)	202.2 (5.8–2088)	26.6 (2.7–7806)	1.9 (0–413)	10.4 (0–19,577)
P value	0.42	0.95	0.60	0.57	0.02*
PHA stimulation					
Pregnant women on HAART	2.4 (0–57.1)	18.7 (0–1995)	3.6 (0–8586)	0.0 (0–16,213)	2.9 (0–1208)
Control	5.7 (0–5064)	40.5 (5.8–24,325)	6.9 (0–14,243)	2.7 (0–11,785)	24.2 (0–376)
P value	0.10	0.43	0.59	0.44	0.27

IL, interleukin; IFN-γ, interon gamma; TNF-α,tissue necrosis factor alpha; Con A, concanavalin A; PW, pokeweed; PHA, phytohymaglutinin A; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus.

*Significant value set at ($P < 0.05$).

pregnant mothers compared to the HIV^- control group (Fig. 1B). Low level of total plasma IgM suggests HIV infection going into chronic stage and at such IgM declines as soon as IgG rises to combat the infection.

Pooled total plasma IgG level in the HIV^+ HARRT treated pregnant mothers was significantly ($P < 0.001$) higher compared to their control group (Fig. 1C). The high IgG level seen in the HIV^+ pregnant mothers is suggestive of chronic HIV infection and secondary infection.

Plasma total IgA (mg/dL) level in the HIV^+ HAART treated and HIV uninfected control at 1st, 2nd and 3rd trimester showed no significant difference (Fig. 2A, $P > 0.05$).

Total plasma IgM (mg/dL) levels in the HIV^+ HAART treated and the HIV^- pregnant women at 1st, 2nd and 3rd trimester gestations also showed no significant difference (Fig. 2B, $P > 0.05$).

Data showed that plasma IgG levels at the 1st and 3rd trimesters in the HIV^+ HAART treated pregnant women were significantly higher ($P = 0.0005$; $P < 0.0001$) than that in the control group (Fig. 2C). Intra comparison within the HIV^+ HAART treated pregnant women and the HIV^- control pregnant women to evaluate the pattern of changes in IgG production as pregnancy progressed to term also showed that plasma IgG was significantly higher ($P < 0.001$) at 1st and 3rd trimesters compared to 2nd trimester in the HIV^+ HAART treated mothers; and significantly lower ($P < 0.05$) at 3rd trimester compared to 1st trimester in the HIV^- pregnant women (Fig. 2C). In summary, plasma total IgG was found to be significantly lower during second trimester in the HIV^+ HAART treated pregnant women and significantly lower during 3rd trimester in the control pregnant women; this is suggestive of intraplacental transfer of IgG from mother to fetus.

Discussion

In this study, we investigated the impact of maternal HIV infection and HAART on the immunity of the pregnant mothers and the safety of the PMTCT procedure in Nigeria. To determine the subject's immune response, lymphocyte stimulation assay was done. This is because of its specificity to assess lymphocyte function in cytokine stimulation. Therefore, PBMCs isolation and lymphocyte (T cell and B cell) stimulation using mitogens allow for a description of T cell responses without the influence of other whole blood components. In this study, lymphocyte proliferation assay was done using mitogens; Con A, PW and PHA. Three mitogens were used because, it is assumed that some individuals react differently in response to lymphocyte stimulation by mitogens. These mitogens provide strong stimuli that are not antigen specific, and usually do not discriminate as well as antigens in reflecting different levels of immunodeficiency. Some normal individuals may not respond to a given antigen but almost everyone's lymphocytes can be stimulated to proliferate nonspecifically by stimulating them *in vitro* with mitogens.

Findings from this study showed low secretions of IFN-γ, IL-4 and IL-10 in HIV^+ HAART treated pregnant

Table 6 Cytokine levels (in pg/mL, median and range) after stimulation with PW, Con A and PHA in the supernatant from mothers' peripheral mononuclear cell cultures at 1st, 2nd and 3rd trimester.

Trimester	IL-2	TNF-α	IFN-γ	IL-4	IL-10
Concanavalin A stimulation					
1st	0.0 (0–0.55)	18.5 (4–6619)	0.2 (0–4.7)	0.0 (0–0)	0.0 (0–0)
2nd	0.9 (0–17,056)	58.2 (0–13,895)	7.3 (0–5270)	0.0 (0–6502)	1.1 (0–12,960)
3rd	0.5 (0–8740)	33.6 (0–13,495)	3.7 (0–4636)	0.3 (0–11,070)	0.0 (0–47,944)
P value					
1st vs 2nd	0.13	0.49	0.11	–	–
1st vs 3rd	0.16	0.62	0.36	–	–
2nd vs 3rd	0.54	0.76	0.75	0.52	0.58
Pokeweed stimulation					
1st	0.0 (0–282)	39.8 (2–17,097)	1.0 (0–422)	0.0 (0–737)	0.0 (0–0)
2nd	3.1 (0–9060)	104.6 (3–11,331)	5.5 (0–17,673)	0.0 (0–4190)	0.5 (0–8067)
3rd	0.0 (0–8438)	38.6 (9.1–2965)	20.9 (0–17,673)	0.0 (0–16,213)	1.1 (0–8486)
P value					
1st vs 2nd	0.13	0.71	0.25	0.23	–
1st vs 3rd	0.32	0.92	0.07	0.27	–
2nd vs 3rd	0.77	0.98	0.68	0.98	0.85
Phytohemagglutinin stimulation					
1st	0.0 (0–15.8)	867.7 (0–10,842)	0.0 (0–162)	0.0 (0–0)	0.0 (0–1.9)
2nd	0.0 (0–105.5)	203.3 (4–12,933)	3.8 (0–8408)	0.3 (0–97)	2.5 (0–937)
3rd	2.4 (0–57.1)	18.7 (0–1995)	3.6 (0–8586)	0.0 (0–16,213)	2.9 (0–1208)
P value					
1st vs 2nd	0.78	0.42	0.27	–	0.12
1st vs 3rd	0.59	0.02*	0.11	–	0.16
2nd vs 3rd	0.65	0.03*	0.66	0.96	0.98

IL, interleukin; IFN-γ, interon gamma; TNF-α, tissue necrosis factor alpha; Con A, concanavalin A; PW, pokeweed; PHA, phytohymaglutinin A; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; vs, versus; 1st, 2nd, 3rd, trimesters.

*Significant value set at ($P < 0.05$).

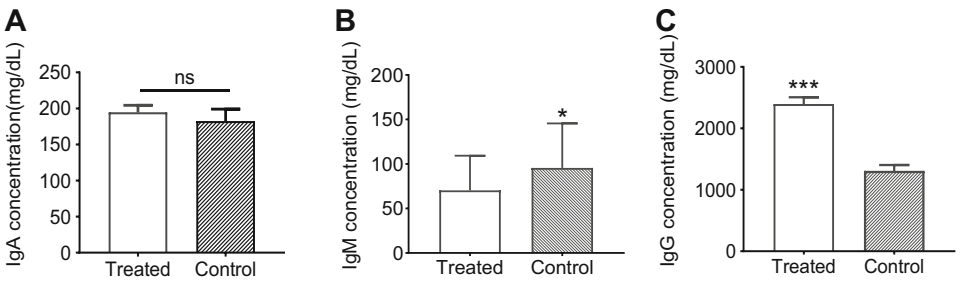

Fig. 1 Pooled total plasma IgA, IgM and IgG. **A** Plasma IgA (mg/dL) level in the HIV infected HARRT treated and the control pregnant mothers showed non-significant ($P > 0.05$) difference. **B** Data indicated significantly lower ($P < 0.0001$) total plasma IgM level in the HIV infected HAART treated pregnant mothers compared to the control group. **C** Total plasma IgG level in the HIV infected HARRT treated pregnant mothers was significantly ($P < 0.0001$) higher compared to their control group.

mothers irrespective of gestational stages. However, IL-10 secretion remained persistently low while TNF-α level decreased throughout gestation in HIV^+ HAART treated pregnant mothers. There was stability of B cell compartment. Importantly, after one year of follow-up, all the exposed infants were seronegative to HIV-1 and HIV-2. HAART showed to be effective in preventing mother to child transmission of HIV.

In this study, we found out that the HIV^+ HAART treated pregnant women had lower secretions of IFN-γ, IL-4 and IL-10. Low secretion of IFN-γ could be as a result regulatory effect of HAART on the pro-inflammatory cytokines. The low secretion of IFN-γ on the infected

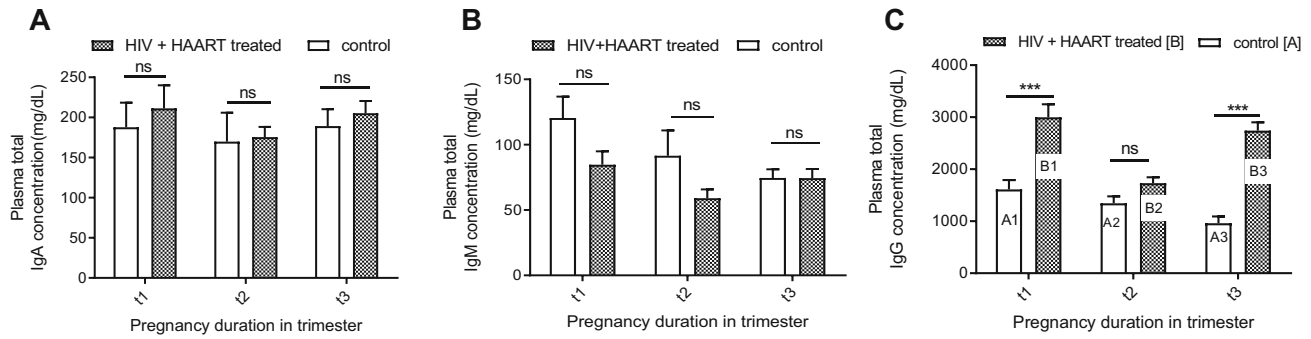

Fig. 2 Total plasma IgA, IgM and IgG at 1st, 2nd and 3rd Trimester. **A** There was non-significant—ns ($P > 0.05$) difference in plasma IgA through 1st, 2nd and 3rd trimesters in the HIV$^+$ HAART treated and control subjects. **B** Plasma IgM in the HIV$^+$ HAART treated pregnant women and control subjects showed non-significant (ns) difference ($P > 0.05$) throughout 1st, 2nd and 3rd trimester. **C** Plasma IgG levels at 1st and 3rd trimesters in the HIV infected HAART treated pregnant women were significantly higher ($P = 0.0005$; $P < 0.0001$) than that in the control group.

mothers is preferred as it curtails excess inflammation and its possible adverse effects. Data showed that the HIV$^+$ HAART treated pregnant mothers had lower IFN-γ and IL-10 during 1st trimester while IL-10 remained persistently low during the 2nd and 3rd trimesters. The persistently low IL-10 secretion seen throughout gestational ages can be attributed to HIV infection. It is the body's immune strategy to allow the clearance of the virus while HAART prevents overreaction of the inflammatory cytokines. TNF-α decreased as gestational age progressed to term in both test and control group. The decrease in the level of TNF-α as pregnancy progressed to term again is attributed to the effectiveness of HAART in preventing excess inflammatory reactions by TNF-α. HAART exerts regulatory effect on TNF-α in a bid to protect the fetus from possible complications of increased pro-inflammatory cytokines like abortion, ruptured membrane, infection with the virus (Fiore *et al.* 2006; Borges-Almeida *et al.* 2011).

There was significantly higher IgG, but lower IgM levels in HIV$^+$ HAART treated pregnant mothers. IgG is produced as part of secondary immune response and its increased production is synonymous to chronic HIV infection (Lo *et al.* 2013; Ifeanyichukwu *et al.* 2016) and possible opportunistic infections. Again, data showed that only IgG was found to be low at 2nd and 3rd trimester in both the test and control groups; this decrease is attributed to intraplacental transfer of IgG from the mother to the fetus for the purpose of passive immunity for the first few months of life before the fetus is able to produce its own immune cells.

After one year of monitoring and follow up, all the exposed infants tested negative to HIV-1 and HIV-2 by PCR. The seronegativity to HIV can be attributed to the exposed infants's mother adhering to the PMTCT procedure. Prior to the 2010 guidelines on HIV and infant feeding (WHO 2010), avoidance or early cessation of breastfeeding seemed logical or appropriate. However, the repercussions for the halt and survival of the infants were serious, with studies showing higher mortality rates due to diarrhea, malnutrition and other diseases in non-breastfed children. The 2010 recommendations are based on evidence of positive outcomes for HIV-free survival through provision of ARVs to breastfed HIV-exposed infants (UNICEF 2005).

The study of Borges-Almeida *et al.* (2011) showed seronegativity of exposed infants born to HIV$^+$ HAART treated mothers. However, their methodology showed that the HIV$^+$ HAART treated mothers underwent cesarean section and were not allowed to breastfeed their infants. In the past, Nigeria had tried adopting the method of PMTCT applied in the study of Borges-Almeida *et al.* (2011) by not allowing the mothers to breastfeed their babies to prevent mother to child transmission. The study was not favorable as many of the infants died from malnourishment and several opportunistic infections (UNICEF 2005; Sibeko *et al.* 2009; WHO 2010; Kindra *et al.* 2012). Our study is remarkably different since in our study the HIV$^+$ HAART treated mothers underwent vaginal delivery and breastfeeding of their infant, yet none of the exposed infants tested positive to HIV after one year of follow-up. It should be noted that the exposed infants received prophylaxis (nevirapine, septrin) from birth till months after they were weaned, which likely contributed to their seronegativity to HIV.

Some studies (Fiore *et al.* 2006, Borges-Almeida *et al.* 2011) have attributed increased TNF-α to antiretroviral treatment which resulted in maternal Th2 to Th1 shift; also, the high maternal production of TNFα at the end of pregnancy was more common in drug abusing mothers. On the contrary, our study revealed a decline in TNF-α secretion as the pregnancy progress to term. This can be as a result of the combination of HAART used, and none of the HIV$^+$ used in this study was a drug user.

High IL-10 has been reported in normal pregnancy (Mosser and Zhang 2008; Iyer and Cheng 2012) as it curtails the adverse effect of pro-inflammatory cytokines. The low IL-10 seems ideal because the pregnant women were infected with HIV and so needed regulated clearance of the virus and opportunistic infection that might adversely affect the fetus. However, observation on the outcome of the exposed infants has shown that in as much as IL-10 secretion was lower in the HIV^+ HAART treated mothers, HAART tightly controlled the activities of TH1 cytokines evidence in decreased TNF-α secretion as pregnancy progressed to term and in zero vertical transmission among the exposed infants.

This study demonstrates the effectiveness of HAART in Nigeria in preventing mother-to-child transmission of HIV as all the exposed infants were seronegative for HIV. The use of HAART by the HIV^+ mothers and the use of septrin and niverapin by the exposed infants prevented mother-to-child transmission of HIV. Vaginal delivery and breast-feeding of the exposed infants by their HIV^+ HAART treated mothers have shown to be safe.

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Authors' Contributions COE-A and CCO did conception and design of the work. COE-A interviewed the patients and collected samples. COE-A and OU did sample shipment. COE-A and LR analyzed samples. COE-A and PM did follow-up on the exposed infants. COE-A, LR and CCO interpreted the patient data. COE-A, LR and CCO were a major contributor in writing the manuscript. COE-A, LR checked and finalized the manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Animal and Human Rights Statement Additional informed consent was obtained from all patients for which identifying information is included in this article. Ethical clearance was obtained from the Ethical Committee of the Department of Medical Laboratory Science, Faculty of Health Science, Nnamdi Azikiwe University, Nnewi Nigeria. Ethical approval letter to see the subjects was obtained from ethical committee of Mother of Christ Specialist, Hospital, Ogui Enugu, Nigeria. The aim of the research was explained to all the HIV^+ HAART treated pregnant women and the control pregnant women seen at the PMTCT clinic and antenatal clinics of Mother of Christ Specialist, Hospital, Ogui Enugu, Nigeria. All the subjects were allowed to participate willingly and withdrawal at any time was allowed. All subjects filled a questionnaire and signed a consent form before blood samples were collected. Procedures followed were in accordance with the ethical standards of the Helsinki Declaration (1964, amended most recently in 2008), which was created by the World Medical Association. Ethical clearance letters are attached.

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