**ORIGINAL RESEARCH ARTICLE**

# Influence of Maternal Characteristics during Pregnancy on the Infant Early Life Immune Responses in a High HIV Burdened Setting in Harare, Zimbabwe

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## Abstract

This study aimed at investigating the maternal characteristics that in turn influence the immunological status of infants in asymptomatic enteric pathogen carriers in mother baby pairs (MBPs) in a high HIV burdened population in Harare, Zimbabwe. BIOPLEX immunoassay was used to analyse serum samples from 39 MBPs for 27 cytokines and 6 immunoglobulins. The MBP were purposively selected based on HIV infection and *Entamoeba histolytica* carriage. Logistic regression was used to identify any link between maternal demographic and clinical data with infant cytokine and immunoglobulin levels. Maternal *E. histolytica* carriers were more likely to have infants with low levels of IL-12p70, FGF-basic, GM-CSF and TNF-α cytokines (OR: 0.14; 95% CI: 0.03-0.79) and high levels of IgA immunoglobulin (OR: 8.1; 95% CI: 1.45-45.06). HIV infected mothers were more likely to have infants with low levels of IgG2 (OR: 0.24; 95% CI: 0.06-1.00) and IgA (OR: 0.22; 95% CI: 0.05-0.90) immunoglobulins. Notably, it was highly likely to deliver infants with low IgG4 levels (OR: 0.24; 95% CI: 0.06-1.02) for maternal mean age above 30.38 years (Standard deviation 6.09) though not significant (p=0.05). Maternal *E. histolytica* asymptomatic carriage, and HIV-infection status result in low levels of pro-inflammatory cytokines IL-12p70, FGF-basic, GM-CSF and TNF-α and immunoglobulins IgG2, IgG4 and IgA on their infants. *(Afr J Reprod Health 2018; 22[3]:43-50).*

**Keywords:** Cytokines; Immunoglobulins; HIV infected mothers; enteric infections; *Entamoeba histolytica;* HIV-exposed infants.

## Résumé

Cette étude visait à étudier les caractéristiques maternelles qui, à leur tour, influent sur le statut immunologique des nourrissons chez les porteurs de pathogènes entériques asymptomatiques chez les pairs mères-nourrissons (PMN) dans une population fortement atteinte du VIH à Harare, au Zimbabwe. Le dosage immunologique BIOPLEX a été utilisé pour analyser des échantillons de sérum provenant de 39 PME pour 27 cytokines et 6 immunoglobulines. Les PMN ont été sélectionnées sur la base de l'infection du VIH et du transport d'Entamoeba histolytica. La régression logistique a été utilisée pour identifier tout lien entre les données démographiques et cliniques de la mère et les taux de cytokines et d'immunoglobulines chez le nourrisson. Les porteuses d'E. Histolytica étaient plus susceptibles d'avoir des nourrissons présentant de faibles taux de cytokines IL-12p70, FGF-basique, GM-CSF et TNF-α (OR: 0,14; IC 95%: 0,03-0,79) et des taux élevés d'immunoglobuline IgA (OR: 8,1; IC à 95%: 1,45-45,06). Les mères séropositives étaient plus susceptibles d'avoir des nourrissons avec de faibles taux d'immunoglobulines d'IgG2 (OR: 0,24; IC 95%: 0,06-1,00) et d'IgA (OR: 0,22; IC 95%: 0,05-0,90). Notamment, il était très probable que les nourrissons ayant un faible taux d'IgG4 (OR: 0,24; IC 95%: 0,06-1,02) délivrent un âge moyen maternel supérieur à 30,38 ans (écart type 6,09), mais non significatif (p = 0,05). MaternalE. Histolytica asymptomatique et le statut infectieux du VIH entraînent des taux bas de cytokines pro-inflammatoires IL-12p70, FGF-basique, GM-CSF et TNF-α et des immunoglobulines IgG2, IgG4 et IgA chez leurs nourrissons. *(Afr J Reprod Health 2018; 22[3]: 43-50).*

**Mots-clés**: Cytokines; immunoglobulines; mères séropositives; infections entériques; entamoeba histolytica; nourrissons exposés au VIH

## Introduction

Despite the success of prevention of HIV mother-to-child transmission (PMTCT) programs in Zimbabwe and world-wide, there have been noted an increased burden in mortality and morbidity of the HIV exposed uninfected (HEU) infants as compared to HIV unexposed ones (HUUs)[1,2]. Most common causes of morbidity and mortality in HEUs include diarrheal diseases, pneumonia and bacterial sepsis, but the actual cause is still insufficiently defined[3]. Maternal and antenatal environmental factors, such as viral, bacterial, and parasitic infections, impact on fetal and/or infant's innate and adaptive immunity development. HEU infants have a wide range of phenotypical and functional immunological abnormalities that may contribute to the high morbidity and mortality from infections[4,5]. Such factors surrounding the abnormal infant immune development are not well described.

HAART exposure in fetal and early neonatal life has also been linked to lower immunoglobulin G (IgG) titres[6]. Cytokine profile, when properly designed could complement the data from viral load and CD4+ T lymphocyte count in the analysis of the disease status, thus aiding in the decision making among possible therapeutic interventions aimed at persistently decreasing immune activation and chronic inflammation[7,8]. Thus, soluble markers in body fluids reflect immune status and therefore may be important in assessing HIV transmission, progression and even mortality. Decrease in IL-2, IFN-γ and concomitant increases of IL-4 and IL-10 have been associated with a decline in antigen-specific immune responses, resulting in opportunistic infections[9,10].

Logically, exposure of the infant to maternal environmental factors such as viral, bacterial, parasitic and other factors impact on fetal and/or infant's innate and adaptive immunity development. There is still paucity of information with regards the relationship between such maternal characteristics and the infant immune responses like cytokine and immunoglobulin levels. In this study it was hypothesized that maternal factors such as age, tribe, asymptomatic carriage of enteric pathogens, HIV status as well as immunological status influences the infant's immunological response which will probably in turn have a bearing on the infant's health.

Infants are highly susceptible to infectious diseases possibly due to immaturity of their immune system and susceptibility to tolerogenic signals. At some early stages of life, infant antigen presenting cells and CD4 T cells were found to 'display reduced ability to produce cytokines as well as cytokine receptors, which may result in decreased cytotoxic effector cell function and a decreased ability to provide adequate B-cell assistance'[11]. This, therefore, results in the high levels of susceptibility to infections in infants.

Individuals that are asymptomatically infected with *Entamoeba histolytica* could represent an important group enabling the study of immune responses that are critical to the outcome of an infection[12]. This study investigated the maternal demographic and clinical factors suspected to influence cytokine and immunoglobulin production in infants in a HIV burdened setting.

## Methods

### *Ethics approval and consent to participate*

The approval for this study was granted by Medical Research Council of Zimbabwe, Approval Code MRCZ/A/2043 as well as the Biomedical Research Ethics Committee (BREC) of University of KwaZulu-Natal, Durban, South Africa, Approval Code 409/15. Consent to take part in the study was granted by the study participants as evidenced by their response to the questionnaire and agreeing to provide both maternal and infant samples.

### *Study population and sampling*

This study was a sub-study based on stored plasma samples collected from the University of Zimbabwe College of Health Sciences Birth Cohort of HIV infected ($HIV^+$) and HIV uninfected ($HIV^-$) pregnant women during the period February 2016 to February 2017. The main study was approved by the Medical Research Council of Zimbabwe, approval code MRCZ/1948 and the Joint Research Ethics Committee, approval code JREC81/15. The study participants were from Dzivarasekwa, Glenview and Kuwadzana, three selected high-density suburbs in Harare, Zimbabwe, based on previous burden of pathogens of interest. The mothers recruited in this study were between 18 and 41 years with mean age of 30.38 (6.09) years.

### *Study procedure*

The pregnant women on their routine antenatal care visits were invited to participate in the study. After

**Table 1**: Summary table for serum sample collection points for infants in Harare, Zimbabwe

| Serum point of collection | Frequency | Percentage |
|---|---|---|
| 10 days | 4 | 10.26 |
| 30 days | 1 | 2.56 |
| 6 weeks | 15 | 38.46 |
| 10 weeks | 4 | 10.26 |
| 14 weeks | 15 | 38.46 |
| Total | 39 | 100 |

agreeing, an informed consent was administered and signed by participant and the interviewer before a questionnaire administration to capture demographic data such as HIV status, ART uptake and regimen.

The mothers were followed up to delivery where information on delivery mode, infant sex and weight as well as infant HIV statuses were taken, and infant blood collected for sera where possible.

Those mother-baby pairs (MBP) who were able to provide stool samples had cultures and ELISA done on the samples. The MBPs that had *E. histolytica* as well as other enteric pathogens detected were considered for cytokine and antibody profiling.

### *Sample collection*

Stored serum for 39 MBPs was purposively selected based on *Entamoeba histolytica* infections and other microbial infections as well as maternal HIV statuses results based on the outcome of the screening process of this study. Controls were based on lack of enteric pathogens and being HIV negative. Maternal prenatal samples were obtained on recruitment and for infant the sera were as close to delivery as possible. Most infant samples were collected at 14 weeks and 6 weeks as shown in *Table 1* .

### *Cytokine and chemokine profiling*

All the 39 MBPs were analysed using Bio-plex Pro Assay 27 plex kit, Bio-Rad, USA. A total 27 serum cytokines were multiplexed using this magnetic-bead based multiplex immunoassay according to the manufacturer's instructions. The plate washings were performed on a Bio-Plex Pro and Bio_Plex Pro 11, Revision B, Wash Station and incubation were performed at room temperature on a Bio-Rad Shaker Fax – 2200. Bio-Plex® 200 system (Bio-Rad, California, USA) which uses Bioplex Manager Software was used for Data acquisition which gave out data output as Median Florescence Intensity and concentration in $pgml^{-1}$. The 27 cytokines and chemokines investigated were IL-1β, IL-1r, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17a, Eotaxin, basic PDGF, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1(MCAF), MIP-1α, MIP-1β , PDGF-BB, RANTES, TNF- α and VEGF. In this study, only those cytokines which were analysed to have significance were further analysed to see the maternal influences on the infant immune responses linking to maternal characteristics.

### *Cytokines analysed*

Analysis was done on influence of maternal factors such as age, HIV status, *E. histolytica* carriage, tribe and area of residence on the infant immune response based on the levels of the 27 cytokines and 6 immunoglobulins tested.

### *Data analysis*

Data analysis was based on logistic regression to determine any associations between maternal characteristics and infant cytokine and immunoglobulin production. During analysis, MBP outliers per variable were dropped irrespective of whether the outlier is maternal or infant. STATA version 13 (StatCorp, Texas, USA) was employed for the data analysis.

## Results

### *Demographic data*

The maternal mean age years and standard deviation (SD) was 30.38(6.09) and were fairly distributed among the three residential suburbs. Twenty-four of the mothers were HIV negative and 15 were HIV positive. Of the 15 HIV positive, 14 were on antiretroviral therapy (ART) and the other one aged 24 year stated that the husband refused her to take medication. Out of the 14 on ART, 12 were on Telonam E, 1 on Telonam N and 1 did not indicate her ART regimen.

### *Sampling and sample collection*

A total of 39 MBP who had cytokines and antibody profiling done were considered for this study. About 38.5% of the mothers were HIV positive and 61.5% were HIV negative based on HIV rapid tests. Of the 39 mothers 23.1% had *E. histolytica* infection. Of the 39 babies, twelve babies (30.8%)

**Table 2:** Influence of maternal E. histolytica carriage on infant cytokine concentration levels in Harare, Zimbabwe

| Infant Cytokine | Cytokine class | Maternal *E. histolytica* carriage | | |
|---|---|---|---|---|
| | | Odds Ratio | P-value | 95% Confidence Interval |
| IL-1r | Anti-inflammatory | 0.5 | 0.337 | 0.12-2.08 |
| IL-2 | Anti-inflammatory | 0.83 | 0.798 | 0.21-3.38 |
| IL-4 | Anti-inflammatory | 0.83 | 0.798 | 0.21-3.38 |
| IL-5 | Anti-inflammatory | 0.27 | 0.08 | 0.06-1.17 |
| IL-10 | Anti-inflammatory | 0.34 | 0.176 | 0.07-1.61 |
| IL-13 | Anti-inflammatory | 0.5 | 0.337 | 0.12-2.08 |
| IL-6 | Pro-/Anti-inflammatory | 0.28 | 0.103 | 0.06-1.29 |
| IL-15 | Pro-/Anti-inflammatory | 0.57 | 0.445 | 0.14-2.40 |
| IL-1b | Pro-inflammatory | 0.5 | 0.337 | 0.12-2.08 |
| IL-7 | Pro-inflammatory | 0.7 | 0.636 | 0.16-3.1 |
| IL-8 | Pro-inflammatory | 0.34 | 0.176 | 0.07-1.61 |
| IL-9 | Pro-inflammatory | 0.83 | 0.798 | 0.21-3.38 |
| IL-12 (p70) | Pro-inflammatory | 0.14 | **0.026** | 0.03-0.79 |
| IL-17A | Pro-inflammatory | 0.83 | 0.798 | 0.21-3.38 |
| G-CSF | Pro-inflammatory | 0.28 | 0.103 | 0.06-1.29 |
| GM-CSF | Pro-inflammatory | 0.14 | **0.026** | 0.03-0.79 |
| IFN-γ | Pro-inflammatory | 0.28 | 0.103 | 0.06-1.29 |
| IP-10 | Pro-inflammatory | 0.5 | 0.337 | 0.12-2.08 |
| MCP-1 mcaf | Pro-inflammatory | 0.21 | 0.113 | 0.03-1.45 |
| MIP-1α | Pro-inflammatory/chemotactic | 0.49 | 0.335 | 0.12-2.08 |
| MIP-1β | Pro-inflammatory/chemotactic | 0.5 | 0.337 | 0.12-2.08 |
| TNF-α | Pro-inflammatory | 0.14 | **0.026** | 0.03-0.79 |
| Eotaxin | Chemotactic | 0.28 | 0.103 | 0.06-1.29 |
| RANTES | Chemotactic | 0.28 | 0.103 | 0.06-1.29 |
| FGF basic | Growth factor | 0.14 | **0.026** | 0.03-0.79 |
| PDGF-BB | Growth factor | 0.83 | 0.798 | 0.21-3.38 |
| VEGF | Growth Factor | 0.28 | 0.103 | 0.06-1.29 |

**Table 3**: Influence of maternal E. histolytica carriage, HIV infection and age on infant immunoglobulin concentration levels in Harare, Zimbabwe

| Infant Immunoglobulin | Maternal HIV Infection | | | Maternal Age | | | Maternal *E. histolytica* carriage | | |
|---|---|---|---|---|---|---|---|---|---|
| | Odds Ratio | P-value | 95% Confidence Interval | Odds Ratio | P-value | 95% Confidence Interval | Odds Ratio | P-value | 95% Confidence Interval |
| **IgG1** | 1.35 | 0.649 | 0.37-4.92 | 0.58 | 0.422 | 0.15-2.21 | 2.3 | 0.248 | 0.55-9.83 |
| **IgG2** | 0.24 | **0.049** | 0.06-1.00 | 1.27 | 0.729 | 0.33-4.97 | 2.2 | 0.288 | 0.52-9.27 |
| **IgG3** | 0.88 | 0.839 | 0.24-3.18 | 0.92 | 0.898 | 0.24-3.46 | 1.4 | 0.649 | 0.34-5.62 |
| **IgG4** | 0.4 | 0.183 | 0.10-1.55 | 0.24 | 0.053 | 0.06-1.02 | 2.2 | 0.288 | 0.52-9.27 |
| **IgA** | 0.22 | **0.035** | 0.05-0.90 | 1.1 | 0.898 | 0.29-4.12 | 8.1 | **0.017** | 1.45-45.06 |
| **IgM** | 0.32 | 0.102 | 0.08-1.25 | 1.2 | 0.790 | 0.31-4.59 | 2.1 | 0.288 | 0.52-9.27 |

were HIV exposed uninfected (HEUs), three (7.7%) were sero-convertors and 24 (61.5%) were HIV unexposed uninfected (HUUs). A total of 8 (20.5%) infants had *E. histolytica* infections of which 5 were HUUs and 3 were HEUs. No enteric pathogen was isolated in the three babies who sero-converted.

### *Relationships between maternal characteristics and infant cytokine and immunoglobulin levels*

None of the maternal demographic characteristics analysed seemed to have an influence on the infant cytokine production levels (p>0.05).

### *Influence of maternal E. histolytica carriage on infant cytokine and immunoglobulin levels*

Maternal *E. histolytica* carriage seemed to significantly (p=0.026) influence infant Interleukin (IL)-12p70, Fibroblast Growth Factor-basic (FGF-basic), Granulocyte Monocyte-Colony Stimulating Factor (GM-CSF) and Tumor Necrosis Factor (TNF)-α cytokines.

The odds of having high levels of these cytokines in infants were reduced by 86% if the mother was a carrier of *E. histolytica* during

pregnancy (OR: 0.14; 95% CI: 0.03-0.79). This is indicated in Table 2. Immunoglobulin (Ig)A also appear to be significantly influenced (p=0.017) by maternal *E. histolytica* carriage. An infant born to a mother with *E. histolytica* carriage has 8.1 odds of having high levels of IgA greater than an infant born to a mother without *E. histolytica* carriage (OR: 8.1; 95% CI: 1.45-45.06). This is shown in *Table 3*.

### *Influence of maternal HIV infection on infant cytokine and immunoglobulin production*

Maternal HIV infection did not seem to influence infant cytokine production levels (Results not shown). However maternal HIV infection seemed to influence infant IgG2 and IgA immunoglobulin levels. Infants born to HIV positive mothers were significantly associated (p=0.049) with lower odds of having high IgG2 levels, that is the odds of having high IgG2 immunoglobulin levels was reduced by 76% in infants born to HIV infected mothers compared to those born to HIV uninfected mothers (OR: 0.24; 95% CI: 0.06-1.00).

Similarly, an infant born to a HIV infected mother was significantly associated (p=0.035) with lower odds of having high IgA immunoglobulin levels compared to the one born to HIV uninfected mothers (OR: 0.22; 95% CI: 0.05-0.90) as shown in *Table 3*.

### *Influence of maternal age on infant cytokine and immunoglobulin production*

Maternal age seemed not to influence infant cytokine levels (results not shown) but appears to impact on the infant IgG4 immunoglobulin levels. Being born to a mother above mean age years and standard deviation (SD) of 30.38(6.09) reduces the odds of having high IgG4 compared to those born to mothers below mean age years and standard deviation (SD) of 30.38(6.09), though not significant but on the border line (p=0.053) as indicated in *Table 3*.

## Discussion

The need to improve maternal and child well-being is one of the millennium development goals which is critical in public health since it affects the health of the next generation. Concentrating on maternal and child health will assist in the prediction of future challenges with regards individuals, families, communities and national public health challenges including the health care systems. It has always been believed that the maternal immunity totally or somehow influences the infant immune response[13].

This study demonstrated how maternal demographic and clinical variables influence infant cytokine and immunoglobulin levels. The observed trends in this study between the relationships of maternal characteristics and their neonates which were not significant might indicate true possibility of these trends if sample size were increased. This study only used 39 mother-baby pairs (MBP) of which the characteristics under study were further reduced in number, making some analysis not statistically robust, hence the need to carry out the research at a larger scale. The results however provide some baseline insight.

This study revealed that maternal *E. histolytica* carriage is a strong confounder for infant IL-12p70, FGF-basic, GM-CSF, and TNF-α cytokines production levels and IgA immunoglobulin levels. There are conflicting evidences on the function of TNF-α during amoebic infections. Some studies have indicated TNF-α to have a protective effect against *E. histolytica* infection while others indicated an increased susceptibility of the host to *E. histolytica* infection in the presence of TNF-α. It was also observed that TNF-α blocking agents lead to healing of tissue inflammation in gastro-enteric diseases such as inflammatory bowel diseases (IBD)[14].

Also, Zhang *et al.*[15], have noted that TNF-α blocking agents '…reduced inflammation and intestinal damage in amoebic infection, while inhibition of IL-1 reduced cytokine production but had less marked effects on inflammation and disease'. In this regard, the reduced levels of the cytokine in infants born to *E. histolytica* carriers might mean that the parasite produces the blocking agents to TNF-α to avoid destruction by the host, and these blocking agents might be transferred to the infant in breast milk. This however needs further investigations.

Speculatively, the low TNF-α levels in infants born to mothers with *E. histolytica* carriage maybe also due to the immune modifications on the maternal TNF-α gene so that the TNF-α cytokine is produced in low concentration to avoid destruction. The defected cytokine genes might be genetically passed on to the infants since the cytokine genes can be inheritable[14,16].

GM-CSF is basically a hematopoietic and myelopoietic growth factor[17,18] which also promotes production of pro-inflammatory cytokines such as

'TNF-α, IL-6, IL-12p70, IL-23 and IL-1β as well as chemokines like CCL22, CCL24, CCL5 and CCL1 which promote leukocyte recruitment'[17]. Thus, it is expected that this cytokine should be elevated in infection. In our study, this cytokine was in low levels in infants born to *E. histolytica* carriers which might explain one of the strategies by *E. histolytica* to evade the host immune response. Possibly the parasite will modulate the host immune system leading to down regulation of GM-CSF which eventually results in less leukocytes to fight the parasite. Since the GM-CSF production will be down regulated in the mother, we expect the infant to also have low level of the cytokine. Since GM-CSF is in low concentration and has a role in the production of TNF-α and IL-12p70, we expect these two cytokines to also be in low concentrations.

FGF-basic has not been well studied in asymptomatic *E. histolytica* carrier pregnant mothers hence there is limited data in this regard. There is need for more research on the relationship between this cytokine with asymptomatically infected *E. histolytica* maternal carriers and their infants.

FGF-basic has been shown, among other factors, to promote healing of wounds and reduction of scars although it is still not clear how this occurs at molecular level[19,20] as such, this cytokine is expected to be in high concentration during acute infections and reduced in concentrations as the wound heals. *E. histolytica* pathogenesis involves development of a "flask shaped" ulcer[21] which the host must clear and in chronic infections, the amoeba will have learnt to live with the host hence there is possibly remarkable reduction in host damage hence the reduced concentration of FGF-basic, which will be transferrable to the infant.

The raised IgA immunoglobulin levels in infants born to *E. histolytica* carriers observed in this study correlates to Nakada-Tsukui and Nozaki, who have indicated an increase in IgA transportation 'across the intestinal epithelium and promotion of neutrophil infiltration'[22] in the presence of *E. histolytica* infection. The infants might therefore be exposed to this immunoglobulin during breastfeeding, and if infected due to exposure to the infected mother, will also start producing their own IgA immunoglobulins. It was shown that HIV infected mothers were more likely to have infants with low levels of IgG2 and IgA immunoglobulins from this study. This finding is in concordance with Abu-Raya *et al*.,[23] who, in their review, indicated the capability of HIV infection to alter the transfer of maternal immune factors to the exposed new-borns and young infants.

'This may relate to the immune activation in HIV-infected pregnant women, associated with the production of inflammatory cytokines at the maternofetal interface associated with inflammatory responses in the new-born'[23].

It has also been noted by earlier studies that the immunoglobulin IgG is the only antibody class capable of significantly crossing the placenta and this crossing is highly dependent on the '(i) maternal levels of total IgG and specific antibodies, (ii) gestational age, (iii) placental integrity, (iv) IgG subclass, and (v) nature of antigen, being more intense for thymus-dependent ones'[24]. As such, since HIV infection has a negative impact on the immune cells production, it is possible that it reduces the IgG2 production in the pregnant mothers which effectively will be transported in small concentrations to the fetus and to the new-born via breast milk.

We also observed that mothers giving birth at a mean age above 30.38 years (Standard deviation 6.09) were more likely to have infants with low IgG4 levels though this observation was not significant. Palmeira *et al*., found no influence of 'maternal age, weight, parity and type of delivery' on 'placental antibody transfer'[24]. Our study however was not looking at placental antibody transfer but on the influence of maternal characteristics on the infant immunoglobulin and cytokine levels. We therefore speculate that as one gets older, so do immune cells.

'As age advances, the immune system undergoes profound remodelling and decline, with major impact on health and survival'[25]. So, with age, it is possible that maternal IgG4 was now produced in low concentrations which were also transferred in low concentrations in utero or postpartum via breast milk.

The wide confidence intervals observed in this study are more likely due to the small sample size used for this study. Individual immune response differences may be influenced by cytokine polymorphisms.

In conclusion, it is critical to monitor maternal age, HIV status and *E. histolytica* carriage as these eventually modulate the infant immune development, which might possibly lead to development of auto-immune diseases.

## Study Limitations and Recommendations

The sample size was small and better insight could have been achieved if a larger sample size was used for analysis. Also, samples were collected at just one data point and could have given a better insight if multiple data collection points were used.

## Significance and Impact of Study

Our study reiterates the need to consider maternal characteristics such as age, HIV status, immunological status and enteric pathogen carriage in pregnancy as these might impact on their infants' immune responses. This will assist in disease treatment and prevention; management of pregnant women and might assist in developing effective immunotherapy for reducing autoimmune diseases in HIV exposed uninfected infants.

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## Conflict of Interests

All the authors declare that they do not have any competing interests.

## Author Contributions

AFN conceived the idea, designed the experiments, performed the assays, analyzed the data and developed the final written manuscript; TM, BSP, AI and TN supervised the work throughout, provided technical guidance to AFN. TJC and EPS provided technical guidance to AFN; KD and FG coordinated collection of samples and provided technical guidance to AFN. CM performed sample collection and processing prior to shipment, was involved in data entry, cleaning and analysis. GK was involved with sample collection, data entry and review of the manuscript. TB provided technical guidance on study design, data cleaning and analysis to AFN. All authors contributed to the writing of the manuscript.

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