Clinical Microbiology and Infectious Diseases

Research Article ISSN: 2398-8096

Post-Natal Cytokine Levels of Neonates of Seropositive Mothers: An Indication of Vertical Transmission?

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Abstract

Studies have implicated increased plasma levels of TNF-α in clinical malaria and vertical transmission of HIV. The intricate imbalances in cytokines levels threaten successful pregnancies. In this study, pregnant women were polled-stratified for interview and subsequently enrolled in a longitudinal study for eighteen months in the endemic area of Saki. Spot Blood samples (2 mL) were collected for laboratory diagnosis of HIV and Malaria at delivery and post natal. The plasma concentration of cytokines: TNF-α, IL-2, IL-10 and IFN-γ was determined by cytokine ELISA techniques. Forty-five (30.2%) of pregnant women were sero-positive with 63.3% in babies. The co-infection rate was 22.8 % in pregnant women and 26.3% in babies. The levels of TNF-α was high (30.0×10^{-3} pg/μL) in non-infected babies at delivery whereas IL-2, IL-10 and IFN-γ peaked at (70 10^{-3} pg/μL, 40×10^{-3} pg/μL) and 16.0×10^{-3} pg/μL respectively in uninfected babies. At delivery, the levels of TNF-α, IL-2, IL-10 and IFN-γ was 18.3×10^{-3} pg/μL, 69.8×10^{-3} pg/μL, 41.0×10^{-3} pg/μL and rise to 28.3×10^{-3} pg/μL in co-infected babies. The level of IL-10 was highest in co-infected babies at the second month post-delivery. Also, there was a progressive increase in the level of IFN-γ at the third month among the un-infected, co-infected and seropositive babies. The parasite density in co-infected babies in the first three months and the plasma levels of IFN-γ and TNF-α could be an indication of vertical transmission of both HIV and P. falciparum.

Introduction

HIV infection poses a great threat to a successful pregnancy outcome, in that a precarious shift from type 1 to type 2 cytokines is necessary for a successful pregnancy; this is, however, reversed in HIV infection and indicates HIV progression [15,28]. Malaria induced the production of various cytokines, including interleukins and tumor necrosis factor, which have been shown to stimulate HIV-1 replication [18]. HIV infection appears to impair a pregnant woman's ability to control malaria parasitemia, resulting in more frequent and higher density parasitemia than in HIV-un-infected pregnant women [25]. The TNF-α and IFN-γ appear to be protective against the parasite, very high serum concentrations of pro-inflammatory cytokines are associated with great morbidity [25,13,26,8]. The current study examined the implication of plasma concentration of two pro-inflammatory cytokines (TNF- α and IFN- γ) on mother-to-child-transmission of HIV and malaria infection.

Materials and Methods

The mean temperature of Saki is 33°C while the sunshine hours per day range from 3.4 hours in August to 11 hours in February. Rice and roots crops are the main agricultural products. Saki is a Peri-urban border town of Nigeria and Cotonou. The target population was pregnant women who reported for routine ante-natal check-up. Pregnant mothers who consented to participate in the study after being adequately informed of the project objectives, protocol and benefits were then enrolled. All subjects were encouraged to deliver their babies at the selected hospitals of the project. Thin and thick blood smears were made from each of these samples for microscopy having been stained in 30% Giemsa (thick) and Leishman (thick). Standardized Parasitemia was performed by counting parasite asexual stages per 200 leukocytes on both thick and thin blood films. The number of parasites per microliter of blood was calculated by assuming an average white blood cell count of 8,000/μL. The preparation of both thick and thin blood films for confirmation of parasitemia followed the methods described by [8]. The degree of parasitemia was graded by modified technique of [31] into Mild Low (1-999/μL) and High above (>1000-9999/μL). They were systematically examined under immersion lens. A negative result was recorded after thorough examination of 100 fields without any parasite. Quality control was ensured, by using freshly reconstituted filter Giemsa stains for parasitemia.

Measurement of cytokines in the plasma by ELISA

The concentration of IL-10 Anti-inflammatory (IL-10) and Pro-inflammatory cytokines (IL-2, IFN-γ, TNF-α) of 179 samples were measured by using ELISA techniques. The cytokine detection kits purchased from (Mabtech, Stockholm, Sweden) were used for this assay. ELISA plates (Corning Incorporated, Nunc Maxicorb) were coated with the purified anti-cytokine capture monoclonal antibody to (1μg/ml) in a coating buffer (Sodium bicarbonate). Monoclonal antibodies used were TNF-α, I-D1K, 9D7 and IL-2-I for TNF-α, IFN-γ, IL-10 and IL-2 respectively. One hundred microliter (100 μL) of diluted antibody was added to each well, sealed in order to prevent

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Key words: *ELISA, polled-interviewed, vertical transmission, P. falciparum, seropositive, post-natal*

Received: September 02, 2017; **Accepted:** September 25, 2017; **Published:** September 28, 2017

evaporation and incubated at 4°C overnight. The plates were washed with phosphate buffered saline-Tween 20 (PBS/Tween) and blocked with 200 µL of blocking (0.05% skimmed milk) appropriately diluted in PBS/Tween and incubated at room temperature 2 hours and for dilution of standards and plasma samples. Specific binding and blocking buffer (1% milk) were applied to the plates immediately before the addition of the samples and standards and incubated at 4°C overnight. The plates were washed 4 times in duplicates (1:1) with PBS/Tween into the pre-coated plates. Bound cytokines were assayed using Biotinylated anti-cytokine detection monoclonal antibody which was diluted to 2µg/mL in blocking buffer (PBS/Tween) followed by 100µL of diluted antibody was added to each well and incubated at room temperature for 2 hours. The enzyme conjugate (Streptavidin-ALP) was diluted to its pre-titrated optimal concentration in blocking buffer/Tween by using a 1:1000 dilution factor according to the manufacturer's instruction. 100µL of diluted enzyme conjugate was added per well and incubated at room temperature for 45 minutes. The substrate (Para Nitro Phenyl Phosphate) was prepared by dissolving 20µg in 20 ml of de-ionized water thereafter 150µL was dispensed into each well. The plates were incubated at room temperature in the dark for colour development for 80 minutes. The optical density for each well was determined with a Biotrak II microplate reader at 405nm. The optimal absorbance values were obtained by plotting a standard curve. The average ODs (Optical Density) were calculated and its corresponding concentrations were plotted on graph as the standard graph.

Statistical Analysis

Subjects' infection status were adopted from similar literatures [1,32] by using students t-test and ANOVA by using SPSS version 15 at p= 0.05 as the level of significance.

Results

Low level of TNF-α was observed from 30.0 x 10^{-3} pg/µL in un-infected to 17.0 x 10^{-3} pg/µL, 20.0 x 10^{-3} pg/µL and 0.8 x 10^{-3} pg/µL in co-infected HIV only and those infected with *P. falciparum* a six month only. The mean plasma concentrations of cytokines in babies during the follow-up (Figure 1) and post-delivery is shown in Figure 2-4**.** TNF-α level were significantly higher from 15.0 × 10^{-3} pg/µL in un-infected to 26.0 × 10^{-3} pg/µL in co-infected and 30.0 × 10^{-3} pg/µL in HIV infected. However, the plasma concentration of TNF-α decreased progressively (30.0 × 10^{-3} pg/µL) in un-infected to (10.0 × 10^{-3} pg/µL) in malaria infected babies. The levels of TNF-α reduced in uninfected and HIV infected babies in second and third month post-delivery.

From figure 1, the levels of TNF-α is high (30.0 × 10^{-3} pg/µL) in non-infected babies at delivery whereas IL-2, IL-10 and IFN-γ peaked at (70 × 10^{-3} pg/µL, 40× 10^{-3} pg/µL) and 16.0 × 10^{-3} pg/µL respectively in uninfected babies. At delivery, the levels of TNF-α, IL-2, IL-10 and IFN-γ was 18.3 × 10^{-3} pg/µL, 69.8 × 10^{-3} pg/µL, 41.0 × 10^{-3} pg/µL and rise to 28.3 × 10^{-3} pg/µL in co-infected babies. Figure 5 showed that TNF-α decreased progressively in uninfected and HIV infected post-delivery babies. IL-2 levels increased in the second month in no-infection categories of babies. In co-infected babies, there was a progressive decrease in the cytokine levels as indicated in figure 2. The level of IL-10 was highest in co-infected babies at the second month post-delivery (Figure 3). Figure 2 showed progressive increase in the level of IFN-γ at the third month among the un-infected, co-infected and seropositive babies.

Discussion

However, the parasite density in co-infected babies (in the first three months post-delivery) could be suggested as vertical transmission

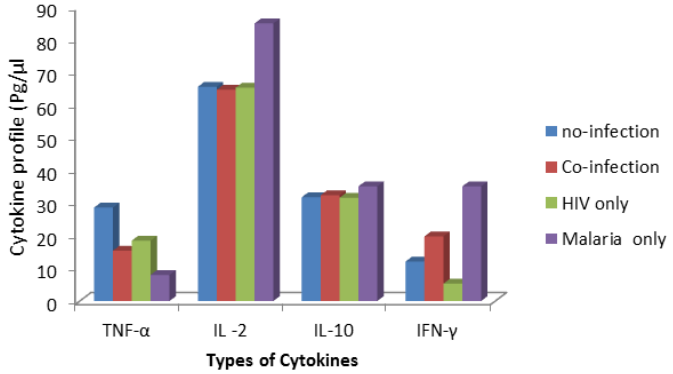

Figure 1. Mean plasma cytokine levels at delivery and categories of infection in babies born to the HIV infected mothers.

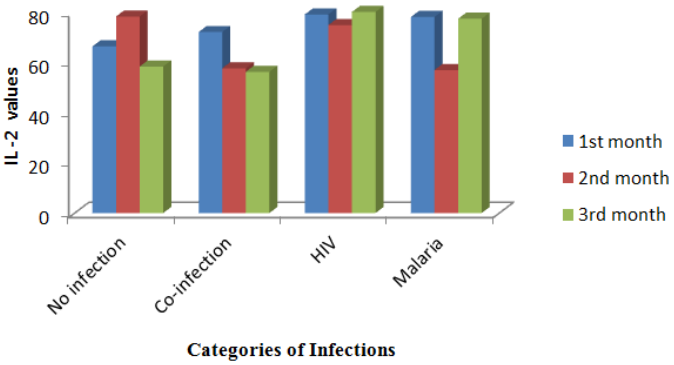

Figure 2. IL-2 levels and classes of infection in babies three month post-delivery.

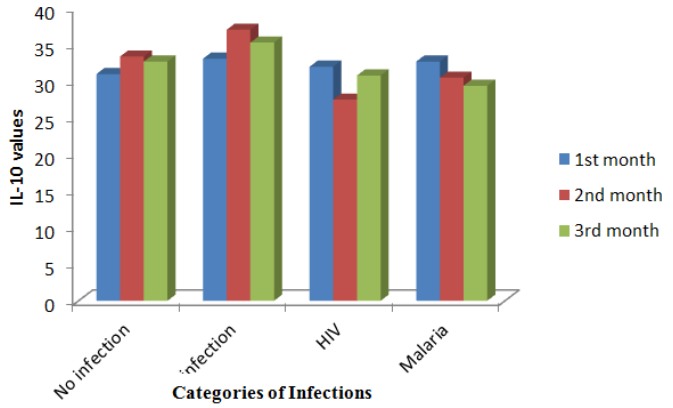

Figure 3. IL-10 levels and classes of infection in babies three month post-delivery.

of both HIV and *P. falciparum* supporting the fact that both infections remain major public health issues in Nigeria [7,12,14]. The decrease in the parasite density from the fourth months could be as a result of transient potency of the innate immunity to clear parasitemia within the first six months of life. There was no mortality recorded in this study was a plus for the effectiveness of antimalarial and HAART was unlike the outcome of a prospective study in Malawi, where the maternal mortality rate within six weeks of pregnancy was 370 per 100,000 women [27]. Perhaps, reasons for this differences in these studies may lie in the fact that both antimalarial and HAART are effective in reducing mother-to-child-transmission of HIV [21,33,35]. Low circulating levels of IFN-γ have been previously associated with severe malaria and mortality in children especially infants with cerebral

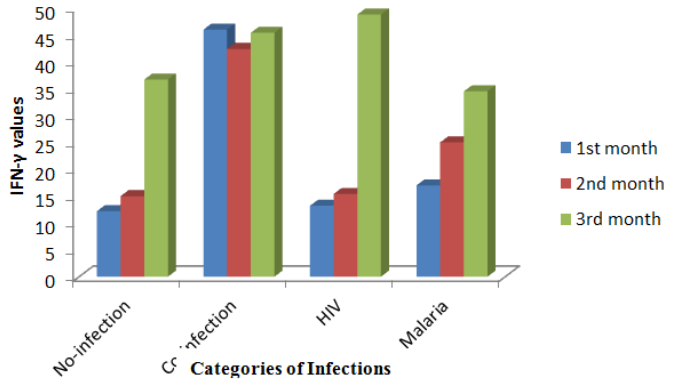

Figure 4. IFN-γ levels and classes of infection in babies three month post-delivery.

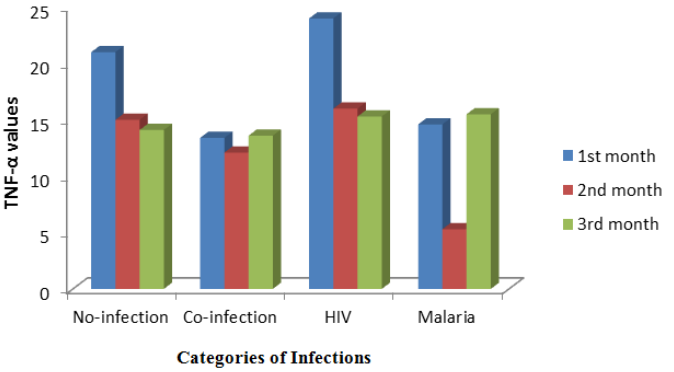

Figure 5. TNF-α level in the infected groups' status during Post-delivery follow-up in babies.

malaria. The concentration of IFN-γ followed the same trend that was observed in the levels of IL-2 in this study. IFN-γ was expected to have played a crucial role in the clearance of intracellular parasites [24], and associated with high malaria severity in young African children [10,30]. Its reduction in all infectious cases (especially in co-infected and HIV infected only) in this study could be regarded as being pathological biomarker for impaired resistance against infection which corroborated the result obtained in similar study by Angulo et al. [2] and Ned et al. [29].

It was established that in newborns of HIV infected mothers, HIV and *falciparum* co-infection was a strong predictor of adverse perinatal outcomes (anemia), and indicative of impaired immunity thereby increasing the susceptibility of newborn to infections [22]. The general reduction in TNF-α level that was observed in this study was a confirmation of that assertion. As suggested earlier, the similar low levels of observed in newborns of HIV infected could be an indication of vertical transmission of impaired immunity from their HIV infected mothers. This might have led to increased IFN-γ concentration among co-infected babies and malaria only infected. The high level of IFN-γ in non-infected mothers compared to the levels of IL-10 was supposed to be deleterious to successful pregnancy and a comparative indication of severe perinatal outcome and high parasite density which would be potentially harmful for the pregnancy [10,11,30].

The pattern observed in the level of IFN-γ in this study contradicts studies [16,29,34,36]; the concentration of IFN-γ was low upon infection. Low circulating levels of IFN-γ have been previously associated with severe malaria and mortality in children especially infants with cerebral malaria. The concentration of IFN-γ followed the same trend that was observed in the levels of IL-2 in this study. IFN-γ was expected to have played a crucial role in the clearance of intracellular parasites [24], and associated with high in malaria severity in young African children [10,30]. The general reduction in TNF-α level that was observed in this study was a confirmation of that assertion. This might have led to increased IFN-γ concentration among co-infected babies and malaria only infected. The high level of IFN-γ in non-infected mothers compared to the levels of IL-10 was supposed to be deleterious to successful pregnancy and a comparative indication of severe perinatal outcome and high parasite density which would be potentially harmful for the pregnancy [10,11,30]. The pro-inflammatory cytokine levels in this study tended to be low with either *P. falciparum* or HIV prevalence. This is in contrast with [2] who implicated pro-inflammatory cytokines of Th1 subsets: TNF-α and IFN-γ in clinical malaria. However, Ogbodo et al. [31] and Singh et al. [31] noted that pro-inflammatory cytokines in the placental environment served as a stimulant for HIV-1 expression and possible facilitator of mother-to-child-transmission of HIV. It is known that, cytokines which favour the increased incidence of *P. falciparum* infection tend to increase the proliferation or replication of HIV [34].One potential mechanism for this was evaluated *in vitro*: binding of recombinant *P. falciparum* adhesion to chondroitin sulfate A on human placental cells increased HIV replication in those cells, possibly via TNF-α stimulation [35]. Thus in consonance with Kfutwah et al. [18], Singh and John [34] and Verhoeff et al. [36], TNF-α was suggested as an inducer of viral replication and high levels of IFN-γ indicate increase in the chances of mother-to-child-transmission of HIV [34,35]. This may serve as an explanation for the observed TNF-α level in this study. The observed increase in TNF-α level among those infected with HIV only and the co-infected women when compared to their uninfected peers, is contrary to recent reports [35,36]. Ordinarily, increased concentrations of TNF-α as reported by Kfutwah et al. [18] was an indication of increased HIV expression and could facilitate MTCT of HIV. However, it is important to note that there was statistical direct relationship between TNF-α and HIV replication but an inverse relationship between IFN-γ and HIV infection. Also, in contrast with the study by Kfutwah et al. [18], there was a direct relationship between infection categories and plasma concentration of TNF-α.

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