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Effect of Malaria on Cellular Immunity of Pregnant Women Coinfected with Malaria and HIV in Sokoto State, North-Western Nigeria

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Abstract: Malaria during pregnancy remains a serious public health problem especially in cases when there is a coinfection with Human Immunodeficiency virus (HIV). This case control study investigated the effects of malaria parasitaemia/HIV infection on packed cell volume, CD4+ T cell count and CD8 + T cell count of HIV pregnant women attending antenatal clinics (ANC) in three tertiary hospitals in Sokoto state, Nigeria. One hundred and three (103) HIV infected pregnant women participated in the study and were placed in two groups after performing malaria parasite identification and count. 45 HIV infected pregnant women served as the control subjects while 58 pregnant women coinfected with malaria and HIV served as the test subjects. All subjects were on antiretrovirals but didn't know the line of drugs they were taking from the questionnaire administered. Packed cell Volume, CD4+ T cells and CD8+ T cells count were carried out by Microhaematocrit Method, BD FACS counter, and Enzyme Linked ImmunoSorbent Assay, respectively. There was no statistical significant between malaria and packed cell volume between the test and control group (p = 0.597) however the test subjects and control subjects had mean±sd of (31.62±3.12) and (31.39±2.69) respectively. There was a statistical association between Malaria and CD4 (p=0.00). Pregnant women with coinfection had significantly low CD4 as compared with the control counterparts, (425.45 ± 276.16) cells/mm^3 and (707.49 ± 212.94) cells/mm^3. There was no statistical association between CD8 count of both the test and control groups (p= 0.21). MP/HIV coinfections exist in our study area especially in pregnant women. Without quick clinical interventions, cases of severe anemia will suffice, and low CD4+ T-cell count.

Keywords: Gestational Malaria, Cellular Immunity, HIV/Malaria Coinfection, Nigeria

1. Introduction

Malaria a tropical disease is caused by the protozoa of the genus Plasmodium species and transmitted by the female anopheles mosquito [1]. Severe malaria and HIV co-infection is a disastrous syndemism especially in the face of antimalarial resistance and pregnancy. Malaria and HIV are two important global health infectious diseases. Studies show that malaria is the fourth leading cause of death of children < 5 years and pregnant women in developing Countries [2]. Malaria cases tend to increase each year because of poor healthcare delivery systems, emergence of drug and insecticide resistance and climate changes [3]. Malaria and human immunodeficiency virus (HIV) infection accounted for over 3 million deaths in 2007 and millions more are adversely affected each year.

Studies have shown that the co-infection exists in many parts of the world. Geographically, Malaria and HIV/AIDS

co-infections overlap, primarily in sub-Saharan Africa, Southeast Asia and South America. In sub-Saharan Africa, there is an estimated 40 million people are living with HIV and more than 350 million episodes of malaria occur yearly [4]. There is also evidence of a negative interaction between these two infections. HIV increases the risk of malaria infection and the development of clinical malaria. Conversely, malaria increases HIV replication [5]. Malaria and HIV infections are also the most deleterious conditions in sub-Saharan African pregnant women, in terms of the morbidity and mortality they cause in mothers and their newborns [6 - 12].

HIV/AIDS can increase the adverse effects of malaria in pregnancy, including anemia, placental malaria infection and low birth weight [13]. Recent assessments propose that in malaria-endemic sub-Saharan Africa, each year approximately 25 million women become pregnant and are at increased risk of infection with Plasmodium falciparum, particularly in their first two pregnancies. This results in maternal anemia and reduced neonatal birth weight due to preterm delivery and intrauterine growth retardation (IUGR) [14, 15]. The vast majority of these infections are low-grade, frequently sub-patent [16, 17] and in most women are asymptomatic and therefore undetected and untreated [18].

A significant effect of HIV-related immunosuppression on the presentation and severity of malaria is expected, because CD4+ T cells, in conjunction with B cells and antigen-presenting cells, are integral to the immune response to malaria. Malaria infection is associated with heightened CD4+ cell activation and up-regulation of proinflammatory cytokines, providing an ideal microenvironment for the spread of HIV in CD4+ cells and thus for rapid HIV replication [5]. Understanding the human immune response to malaria and HIV leads us to expect that either infection might influence the clinical course of the other. The immune deficiency caused by HIV infection should, in theory, reduce the immune response to malaria parasitaemia and therefore increase the frequency and severity of clinical attacks of malaria. So HIV infection affects the clinical presentation, severity and response to treatment of malaria cases [5].

A study conducted in Nigeria 19 indicated a higher prevalence of malaria in HIV infected patients and also revealed that patients co-infected with malaria and HIV were more likely to be anaemic. It is now evident that T cells play a major role in the acquisition and maintenance of protective immune response to malaria infection. Initial studies in both animal models and human points to a major role of the CD4+ T cells alone are able to confer protection against malaria but recent studies have shown that CD8+ T cells also contribute to immunity against malaria infection. Mice with severe combined immunodeficiency (SCID) and reconstituted with T cells from immune donors suppress parasite growth, suggesting a protective role of T cells against malaria parasites. B cell-deficient mice are also able to suppress parasitemia at the same rate as normal mice [20].

CD8 T-cells play a crucial role in controlling HIV replication during the early phase of infection. HIV-specific CD8 T-cells are targeted at the dominant viral variant and their emergence is associated with a rapid fall in viral load before the development of an antibody response. A majority of the CD8 T-cells generated during primary infection die within a few weeks, leaving a reservoir of HIV-specific CD8 memory T-cells that will persist, regardless of the presence of antigen or CD4 helper T-cells. Researchers have found that viral load is better controlled in people whose HIV-specific CD8 T-cells mature fully into 'effector memory' T-cells [21].

Strong CD4 T-helper cell and CD8 T-cell responses correlate with long-term non-progression. A therapeutic vaccine that would restore HIV-specific CD4 T-cell and CD8 T-cell responses is one approach that has been looked at to help immune control of HIV. Very rarely, an efficient CD8 T-cell response can occur before HIV has started to replicate in CD4 T-cells or macrophages. This can prevent HIV infection before the production of HIV antibodies. This may occur more frequently in newborn babies than in adults. It is widely accepted that CD4 T cells play critical roles during blood stage malaria but the role of CD8 T cells remains controversial. Their action is probably limited to the liver and the silent stage of malaria infection in hepatocytes has so far been neglected in research. Mice depleted of CD4 cells had significantly higher parasitemisia on day 7 as well as significantly higher peak parasitemia [22].

Depletion of CD8 lymphocytes was found to have no effect on the early course of infection or on the level of peak parasitemia. However mice depleted of CD8 cells experienced two recurrent bouts of parasitemia during the later stage of the infection and required more than 5 weeks to eliminate the parasites. The results confirmed the importance of CD4 T cells in acquired immunity and demonstrate a role for CD8 cells in the resolution of infection. Another study showed that CD8+ exhaustion drives chronic malaria [23]. In patients whose CD4 counts are below 200cells/μL the delay in parasitemia clearance is longer. Depletion of CD4+ T cells from such mice lead to a loss of the mice ability to suppress parasitemia. This indicates that CD4+ T cells can act independently of B cells in the resolution of the parasites.

In humans, direct studies of the responding T cells during malarial infection are difficult, as these cells may leave the peripheral circulation and sequester in the spleen or other tissues [24, 25]. CD4+ T cells play a central role in regulating the immune responses to the asexual blood stages of P. falciparum via cytokine production and B-cell helper [26]. It has been shown that CD4+ T cells from individual naturally exposed to malaria, respond to blood stage P. falciparum by proliferation, production of IFN-y and/or IL-4 secretion in vitro. Such production of IL-4 was neither associated with proliferation nor with IFN-y production, but was well correlated to serum antibodies to the peptides used to activate the T cells [26]. This is in line to the finding that malaria-specific CD4+ T cells can provide help for B cells to produce P. *falciparum*-specific antibodies [27, 28]. A correlation between resistance to fever and high parasitaemia and in vitro T cell responses to P. *falciparum* blood stage

antigens has been reported [29].

In contrast, other studies failed to demonstrate such correlations [30, 30]. The role of CD4+ T cells has been questioned as there is no evidence that the advent of AIDS has exacerbated malaria [32]. Available evidence indicates an important role of MHC class 1-restricted CD8+ T cells in the pre-erythrocytic immunity [33, 34] and contribution to protection against severe malaria [35 36]. Recent studies conducted in Nigeria [36, 37] detailed in the absence of appropriate and prompt clinical interventions, SMP/HIV coinfections may lead to severe anemia and CD4 lymphopenia. Also a similar study conducted in 2015 [38], showed a reduction in CD4 below WHO recommended in pregnant women with co-infection of HIV and Malaria. This study sought to determine the effect of malaria on packed cell volume of red blood cells, CD4+ T cells and CD8+ T cells count in HIV pregnant women.

2. Materials and Methods

2.1. Study Site and Design

This is a case control study carried with of 103 HIV infected pregnant women attending ante-natal clinic visit at three tertiary hospitals in Sokoto State, Nigeria, viz; Specialist Hospital, Maryam Abacha Hospital and Women and children Welfare clinic in Sokoto State.

2.2. Study Area

The study was conducted in three hospitals in Sokoto State. Sokoto is the capital of Sokoto State and lies on latitude 13.0059°N and longitude 5.2476°E, and occupies an area of 25,973 square kilometers. Sokoto State is bordered by the Republic of Niger to the North, it also shares boundaries with kebbi state to the west and south, and Zamfara to the south and east. The climate of Sokoto has a mean annual maximum temperature of 28.3°C. Sokoto State comprises of 23 local governments. The population of the state as at the March 2006 census was 3.70 million

2.3. Ethical Consideration and Informed Consent

The ethical clearance was obtained from the ethical committee of Sokoto state ministry of health, Nigeria. Informed (written) informed consent was obtained from all study subjects.

2.4. Eligibility Criteria

The inclusion criteria include those who were positive for HIV and with febrile illnesses regardless of onset and duration of illness, those who were pregnant women and with no signs of fever, those that consented to voluntarily participate in the study while the exclusion criteria included those that did not consent to voluntarily participate, those who have started taking antimalarial drugs already, those diagnosed with leukaemia and Diabetes mellitus.

2.5. Analytical Laboratory Protocols

After obtaining informed consent, five millitres (5mL) of blood was collected blood by sterile venipuncture into two EDTA vacutainer tubes. The vacutainer tubes were immediately transported to General Murtala Mohammed Hospital for $CD4^+$ analysis. Within six hours of sample collection $CD4^+$ total enumeration was be performed on the whole blood using BD FACS cytometer CD4 technique. The remaining blood was used for thin and thick blood smear for malaria parasitemia examination and count; Packed Cell Volume estimation at Heamatology Department of Specialist Hospital, the EDTA blood sample was centrifuged at 3000 rpm for five minutes in the Laboratory in order to obtain the plasma which was subsequently used to run the CD8+ ELISA assay. The plasma samples remained stored at -20°C until analysis. CD8+ T cells were estimated using ELISA technique.

2.5.1. Principle of CD8 T Cell Count

The kit uses enzyme linked immunosorbent assay-double antibody sandwich principle to assay CD8 level in the sample. The Microelisa stripplate provided in this kit has been coated by Purified CD8 antibody to make solid-phase antibody, then add CD8 to wells, combine with CD8 antibody labeled by HRP, become antibody - antigen - enzyme-antibody complex. After washing to completely remove the uncombined enzyme, Chromogen Solution A and Chromogen Solution B were added, the color of the liquid solution changed to blue. The reaction was stopped and the color finally becomes yellow. The intensity of colored product was measured spectrophotometrically at a wavelength of 450 nm. The concentrations of CD8 T cell in the samples were determined by comparing the Optical Density of the samples to the standard curve provided by the kit manufacturer.

2.5.2. Malaria Parasite Detection and Identification

Using a clean grease-free microscope slide, a small drop of blood was placed to the centre of the slide, without delay, the blood was spread to make the thick smear. After drying, the slides was stained for 10–15 min with 10% Giemsa solution. When the thick film was completely dry, a drop of immersion oil was placed to an area of the film which appears mauve colored (usually around the edges). The Slides were examined for malaria parasites and malaria pigments (if any) using x 100 objective lens.

2.6. Data Collection and Statistical Analysis

Data generated from laboratory test results was analyzed using Statistical package for Social Sciences computer software version 21.0. A p-value of < 0.05 was considered significant in all statistical comparison.

3. Results

There was no statistical significant between malaria and packed cell volume between the test and control group (P;

0.597) notwithstanding the test subjects (31.62±3.12) and control subject (31.39±2.69) had low PCV. There was a statistical association between Malaria and CD4 (p=0.00). Pregnant women with coinfection had significantly low CD4 as compared with the control counterparts, (425.45 ± 276.16) cells/mm^3 and (707.49 ± 212.94) cells/mm^3. There was no statistical association between CD8 T cell count of both the test and control groups (p = 0.21) (Table 1).

Table 1. *Comparison of packed cell volume, CD4 T cell and CD8 T cell count of test and control subjects.*

Variables	Test n= 58	Control n=45	S.E (T)	S.E (C)	*p*-value
PCV (%)	31.62±3.12	31.31±2.69	0.41	0.40	0.597
CD4+ T cell count (cell/mm^3)	425.45±276.15	707.49±212.94	36.26	31.74	0.00
CD8+ T cell count (cells/mm^3)	47.44±31.02	58.53±52.44	4.07	7.82	0.21

Data are expressed as mean ± SD; Significance difference as determined by unpaired student's *t*-test.
S.E- Standard Error

4. Discussion

This present study also compared the packed cell volume level in malaria/HIV coinfected pregnant women with that of those infected with HIV alone. There was no statistical significant between the mean packed cell volume between the test and control group. Notwithstanding the test subjects and control subject had mean PCV± SD of (31.62±3.12) and (31.39±2.69) respectively. This appreciable PCV may be as a result of antiretroviral therapy which has been showed to improve PCV, Haemoglobin, WBC, and CD4 counts of the subjects [38].

In this study, despite the fact that HIV pregnant women do not know which line of ART they are presently placed on, those coinfected with MP/HIV had mean CD4+ count was <500 cells/ mm^3 while the mean CD4+ cell count of subjects without malaria but HIV infection was > 500 cells/ mm^3. This outcome is similar to a case-control study carried out by Nasir *et al.* [36]. With the absence of appropriate and prompt clinical interventions, SMP/HIV coinfections may lead to severe anemia and CD4 lymphopenia [36, 38].

A study by Ekwempu *et al* [39] to compare CD4 counts level in both HIV seropositive and seronegative women, study suggests that pregnancy may partially deplete CD4 cells because a significant difference was observed in mean (SD) CD4 cell count in HIV-seropositive and HIV seronegative pregnant women at various gestational ages, although this present sheds more light on the effect of malaria parasitaemia as a close factor in depletion of CD4 T cells.

The low CD4 count in the test subjects is connected with progressive immune deterioration caused by dual pathogens [40]. This is in conformity with previous report [41]. They consider the major reason of these to be due to the fact that patients present themselves very late for consultations. Fear of stigmatization and inadequate counseling on the part of healthcare workers favour late consultations and appropriate management of MP/HIV coinfected patients. The relatively low CD4 cells count in the test subjects could also be due to ART resistance

Futhermore it confirms the claim that the immune deficiency caused by HIV infection should, in theory, reduce the immune response to malaria parasitaemia and therefore increase the frequency and severity of clinical attacks of malaria. So HIV infection affects the clinical presentation, severity and response to treatment of malaria cases and malaria infection in-turn causes heightened CD4+ cell activation leading to its depletion and up-regulation of proinflammatory cytokines, providing an ideal microenvironment for the spread of HIV in CD4+ cells and thus for rapid HIV replication. There was no significant association in CD8 count between the control and test subjects of the research. The mean± standard deviation of the test subjects is 47.44±31.02 while that of the control group is 58.53±52.44.

The burden of malaria especially amongst HIV infected pregnant women, underscores the importance of effective prevention, treatment and control measures that will stem the tide of the high prevalence recorded in this study and many others. This will significantly reduce morbidity and mortality among pregnant women and improve the overall health of women of child-bearing age.

5. Conclusion

In the absence of prompt and appropriate clinical interventions, MP/HIV coinfection may likely lead to severe anemia and progression to AIDS. Malaria infected pregnant women especially those with HIV coinfections should be periodically and closely monitored for presence of antimalarial and ART resistance.

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