Comparative Analysis of Asymptomatic Malaria Parasitaemia in Human Immunodeficiency Virus Positive and Negative Pregnant Women in Sagamu, Ogun State, South West Nigeria

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Abstract
BACKGROUND: Malaria parasitaemia among pregnant women is associated with complications to the mother and the unborn fetus. Human Immunodeficiency Virus (HIV) infection is known to increase the prevalence of malaria parasitaemia. This study aims to determine and compare the prevalence of asymptomatic malaria parasitaemia and the degree of anaemia in matched HIV-positive and HIV-negative pregnant women.

METHODS: This was a cross-sectional study of asymptomatic malaria parasitaemia among HIV-positive pregnant 300 women attending the booking clinic. HIV-negative women (300) served as control. The prevalence, density of malaria parasite and degree of anaemia were compared in the two groups. CD4+ count was done for the HIV-positive participants.

RESULTS: The prevalence of asymptomatic malaria parasitaemia among HIV-positive pregnant women was found to be 77.0% compared with 31.0% among HIV-negative pregnant women. The prevalence of asymptomatic malaria parasitaemia was found to be higher among younger pregnant women, women with low parity, and among HIV-positive with lower CD4+ count. The prevalence of anaemia was found to be 70% among the HIV-positive pregnant women compared to the 20% found among the HIV-negative pregnant women (p = 0.000). The degree of anaemia increased as the density of malaria parasitaemia increases and as the CD4+ cell count decreased (p = 0.000).

CONCLUSION: There is higher prevalence of asymptomatic malaria parasitaemia and anaemia among HIV positive pregnant women compared to their negative counterparts. There is a need for enforcement of preventive strategy and supplementations to reduce the burden of malaria parasitaemia in pregnancy.

Key Words: Asymptomatic malaria parasitaemia, HIV, Anaemia.

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I. Introduction

Malaria remains a major health concern worldwide mostly in the tropical region; mainly due to the significant contribution to the economic burden of in these areas where it is endemic [Uzochukwu et al., 2010; Panti et al., 2012]. The major specie in this region is P. falciparum and also the most virulent [Uneke et al., 2007; WHO, 2015].

World Health Organization estimates that each year, 300-500 million people develop malaria and 1.5 -3 million people, mostly children, die of malaria [Noppadon et al., 2009; Hochman and Kim, 2009]. It constitutes about 10% of overall disease burden worldwide [Hochman and Kim, 2009]. Most affected are countries in Tropical Africa, where more than 90% of disease burden of malaria exist and where rapid evolution of drug resistance is increasingly complicating malaria treatment [WHO, 2011].

Asymptomatic malaria parasitaemia constitutes a menace in malaria endemic areas and has contributed directly or indirectly to maternal morbidity and mortality in developing countries especially in Nigeria [Hochman and Kim, 2009]. It is defined as the presence of malaria parasites in the blood in the absence of malaria-related symptoms and it is common in malaria-endemic regions with stable malaria where a high degree of infestation has engendered a substantial level of immunity within the population [Lindblade et al, 2013; Gudo et al., 2013; Noppadon et al 2009]. A vast majority of infestations with plasmodium falciparum in pregnancy in areas with stable malaria transmission remain asymptomatic, undetected and untreated [Shulman, 1993; Desowitz and Alpers, 1992].

Malaria infestation is a major cause of fever and anaemia in pregnant women resident in endemic areas of Africa and this is basically due to reduced immunity to malaria during pregnancy [Klufo, 1992]. It has been
demonstrated that severe anaemia is twice as common in pregnant women with peripheral parasitaemia compared with those without parasitaemia [Shulman et al., 1996].

Asymptomatic malaria parasitaemia is present in both pregnant and non-pregnant women. The progression to symptomatic malaria infestation is generally enhanced in pregnancy due to the increased immunosuppression and depression of cell mediated immune response to plasmodium antigens Rasheed et al., 1993; Riley et al., 1989]. The prevalence of malaria in HIV-positive pregnant women is twofold or more for any parity when compared with HIV-negative counterparts [Verhoeff et al., 1999].

Asymptomatic malaria parasitaemia constitutes a major risk factor for developing symptomatic malaria infestation in pregnancy and may be associated with adverse effect on fetal and maternal health. Considering the myriad of adverse maternal and fetal effects associated with untreated asymptomatic malaria parasitaemia, early detection via screening and appropriate treatment would be justified.

HIV infection impairs a pregnant woman’s ability to control malaria infestation and also increases the risk of placental malaria, high density malaria parasitaemia and febrile illness [Onwujeckwe et al., 2008; Okpere, 2010]. It is also known that the effects of asymptomatic malaria parasitaemia such as anaemia and low birth weight are potentiated by HIV infection and anaemia may be one mechanism by which asymptomatic malaria parasitaemia causes adverse birth outcome [Muhangi et al., 2007].

The protective effect of parity on the complications associated with malaria among pregnant women cuts across all parities with the effect apparent early in gestation. HIV infection impairs immunity and an increase in susceptibility to malaria in the higher parity groups has been found [vanGeertrudeen and D’Alessandro, 2007].

The high prevalence of HIV and malaria in this region (sub-Saharan Africa) makes co-infection very common [Abu-Raddad et al., 2006]. This has a negative implication since both infections have negative impact on the health of pregnant women and their newborns. While infection with either malaria or HIV/AIDS can cause illness and death, infection with one can make infection with the other worse and/or more difficult to treat [Alemu et al., 2013]. This study compares the prevalence of asymptomatic malaria parasitaemia in HIV-positive and HIV-negative pregnant women in Sagamu.

II. Methods

Study Design: This was a cross-sectional study. HIV-positive pregnant women who have been counseled, tested and confirmed HIV-positive were the cases while clients that tested HIV-negative served as the control arm.

Study setting: This study was carried out in the antenatal clinic of OlabisiOnabanjo University Teaching Hospital (OOUTH) Sagamu, Ogun State, Southwestern Nigeria. OOUTH is the only Teaching Hospital in the state and serves as a referral centre for private, primary and secondary healthcare facilities in Sagamu and its environs. Obstetric services are provided for both high and low risk pregnant women by Nurses/Midwives, Resident Doctors and Consultant Obstetricians. OOUTH also carries out HIV counseling and testing (HCT), programme for the prevention of mother-to-child transmission (PMTCT) of HIV, and treatment of adult and paediatric HIV cases.

Study Population: This is composed of pregnant women attending their booking antenatal clinic at the OOUTH.

Sample Size Determination: The sample size for the subjects of the study was taken as 300 using a prior prevalence of 23.8% and also making up for attrition. The minimum sample size was estimated using the statistical formula of Fisher for determining sample size when calculating the prevalence of a factor in a study [Araoye, 2004].

Inclusion criteria: Pregnant women attending their first antenatal (booking) clinic at OOUTH, who agreed to have HIV screening test after counseling and who gave informed consent to participate were included in the study.

Exclusion criteria: Women with signs and symptoms suggestive of malaria infection such as fever, headache, malaise, vomiting and history of use of antimalarial medication within the last four weeks before the study.

The screening was done using two different rapid test kits (determine & stat-Pak). If a discordant result was obtained, confirmation of result is done with another rapid test kit (UniGold) as “tie-breaker”. Clients with both tests positive were considered HIV-positive.
The consenting HIV-positive clients served as subject of the study and were recruited consecutively until the required sample size was attained. HIV-negative pregnant women suitably matched for age, parity and gestational age served as controls. They were also recruited consecutively at the booking clinic until the required sample size was reached.

**Ethical consideration:** The study was carried out after obtaining clearance from the Health Research Ethics Committee of OOUTH. Written informed consent was obtained from the eligible participants and the laboratory tests were done at no cost to the participants.

Data collected consisted of three sections. The first section was used to record the demographic characteristics of the participants which included the Age, Occupation of patient, Gravidity, Parity, Gestational age, Educational level, tribe and religion of the patient. The second section was used to record the following laboratory findings: Haemoglobin level, HIV status, CD4⁺ count and malaria parasitaemia using the blood film microscopy. Data was collected over a period of 7 months.

Data analysis was done using the statistical software package for social sciences (SPSS) version 17. Results are presented as simple percentages and tables. Means were compared using “t” test while proportions were compared using Chi-square test. A P-value of <0.05 was considered significant.

**Limitations of the study:** Difficulty in getting consent to participate in the study and the inability to do viral load estimation of the HIV-positive participants.

### III. Results

The mean age and mean gestational age for both subjects and controls were similar. There is no statistically significant difference in the mean gestational age for subjects (25.4 ± 6.8) and control (25.60 ± 6.0). This is because both subjects and controls were well matched. The prevalence of malaria parasitaemia among HIV positive pregnant women was 77.0% (231/300) compared with 31.0% (93/300) among HIV negative pregnant women. This was statistically significant (x² = 1.278, P value = 0.000).

**Table 1** shows relationship between prevalence of asymptomatic malaria parasitaemia and maternal age and parity. Parasitaemia was more common in the age group 20-29 years and for each group, the prevalence was more among the HIV-positive pregnant women. Also, parasitaemia was more prevalent in the low parity group. Both age and parity were statistically significant. Except for the Para1 group, the prevalence of parasitaemia decreases as parity increases.

**Table 2 and figure 1** showed the prevalence of parasitaemia in the different gestational age groups. There is an apparent increase in the prevalence of parasitaemia as the gestational age increases among the HIV positive pregnant group which is not so among the HIV negative pregnant group. The prevalence of anaemia was 270 (45.0%) among the participants. Anaemia was more prevalent among HIV-positive pregnant women 210 (35.0% of total study group) compared with HIV negative women 60 (10.0%). This difference was statistically significant (x² = 1.515, P value = 0.000).

There is an inverse relationship between the level of haemoglobin and the prevalence of malaria parasitaemia i.e. more participants with high density of malaria parasite had low haemoglobin level. This is statistically significant with a chi-square value of 4.328 and a P value of 0.000 (Table 3).

It was observed that as the CD4 cell count decreases more of the subjects developed asymptomatic malaria parasitaemia (Table 4). This is statistically significant (x² = 1.601, P value = 0.000).

**Table 5** revealed that as the CD4 cell count increases, lesser number of participants with HIV presented with anaemia. At CD4 cell count of 200–499/mm³, 61.0% of the participants have anaemia while only 9.0% presented with anaemia at CD4 cell count ≥500/mm³. This is significant with X² of 2.479 and a p-value of 0.000.

### IV. Discussion

Asymptomatic malaria parasitaemia continues to be a major health problem mostly in areas of malaria endemicity like the Sub-Saharan Africa where the ravaging effect of HIV infection is more and aggravates the problem [WHO, 2011; Alemu, 2013; WHO, 2000]. It constitutes a major health risk to both the mother and the unborn child especially if the mother is HIV-positive [Panti et al., 2012; Sule-Odu et al., 2002; Nyirjesy et al., 1993]. The co-infection has more negative impact on pregnancy outcome [Onwujeke et al., 2008; Okpere et al., 2010; Muhangi et al., 2007; Alemu, 2013]. The prevalence of asymptomatic malaria parasitaemia was found to be significantly higher among HIV positive pregnant women (77.0%) compared to HIV negative pregnant women (31.0%). This is similar to findings from the works of other researchers [Verhoeff, 1999; VanGeertruyden and D’Alessandr, 2007; Abu-Raddad et al., 2006; Dibua, 2013; WHO, 2004]. This is basically due to the fact that there is a synergistic pathological interaction between HIV and malaria [Abu-Raddad et al., 2006]. The immune deficiency caused by HIV infection reduces the immune response to malaria and therefore increase in prevalence of malaria parasitaemia [Alemu et al., 2013]. More so, studies have revealed that people who are infected with HIV are more susceptible to malaria infection [VanGeertruyden and D’Alessandr, 2007].
This study demonstrated increased prevalence of asymptomatic malaria parasitaemia among young pregnant women (women under 30 years, especially those under 20 years). This is similar to the findings of other workers [Akinboro et al., 2010]. The fact that the prevalence of asymptomatic parasitemia (AMP) was more among the younger age group could be explained on the premise that they have low antibodies developed against the placenta sequestered parasites. These antibodies prevent the binding of parasites to the chondroitin sulphate-A receptors in placenta. These antibodies only develop in subsequent pregnancies [Ogbodo et al., 2009].

Malaria parasitaemia decreases with increase in parity, this preferential susceptibility of people with low parity could be as a result of the fact that immune-suppression associated with pregnancy occurs more in the first than subsequent pregnancies [Riley et al., 1989; Ogbodo et al., 2009]. This finding agrees with the works of other researchers [Panti et al., 2012; Akinboro et al., 2010; Ogbodo et al., 2009; Agan et al., 2010].

The prevalence of AMP has been found to decreases as gestational age increases for pregnant women that are not HIV positive. This is because of the acquired immunity against plasmodium as pregnancy advances [Nyamgee et al., 2014]. In this study, there is increased prevalence of malaria parasitaemia as gestational age of the HIV positive pregnant women increases and this was statistically significant. This could be because the protective effect of increase immunity against malaria normally acquired as the gestational age increases is blunted out by the HIV infection [Okpere et al., 2014].

The incidence of anaemia in the general population was higher among the HIV positive parturients (35%) with asymptomatic malaria parasitaemia compared with the HIV negative counterparts (10%). This was in agreement with the works of other researchers [Muhangi et al., 2007; Alemu et al., 2013]. Anaemia is known to be one of the mechanisms through which the adverse effects of HIV and malaria parasite in pregnancy occur. The number of pregnant women presenting with moderate to severe parasitaemia also increases. These findings are in tandem with earlier findings from the works of other researchers [Okpere et al., 2010; Nwouwu et al., 2009]. This is as a result of the fact that as the degree of maternal parasitaemia rises anaemia becomes worsen due to excessive removal of parasitized and non-parasitized erythrocytes, immune destruction of parasitized red cells, impaired erythropoiesis as a result of bone narrow dysfunction and hypersplenism [Adesina et al., 2009].

This study also confirms the inverse relationship that exists between the CD4+ count on one hand and the degree of parasitaemia and anaemia in HIV positive pregnant women on the other hand (tables 4 and 5). As the CD4+ count becomes lower, parasitaemia and anaemia increases. It is known that CD4+ T- lymphocytes decline temporarily during clinical malaria episodes in HIV-infected and HIV-uninfected patients and that repeated malaria infections are associated with a more rapid decline in CD4+ T lymphocytes over time, suggesting that malaria may lead to faster disease progression from HIV to AIDS[6]. Malaria provides an ideal microenvironment for the spread of the HIV virus among the CD4+ cells making it easier for its destruction and subsequent depletion and further increasing the viral load [Alemu et al., 2013].

The work of Hochman and Kim, 2009, on the impact of HIV and malaria co-infection revealed that HIV causes reduction in the CD4+ T lymphocytes, reduction in the maternal antibody to the variant surface antigens (VSAs) on malaria-infected erythrocytes which plays important role in pregnancy-related immunity to malaria and reduction in the plasma opsonizing activity. All these lead to reduction in the ability to combat malaria and thereby increasing parasitaemia. The question for further research will be: does this decrease in CD4+ count seen during acute malaria infection have long term consequences on HIV progression to AIDS?

V. Conclusion And Recommendation

The study has shown that there is increased prevalence of asymptomatic malaria parasitaemia among HIV positive pregnant women compared with their negative counterparts. The degree and prevalence of anaemia was found to be higher among HIV positive pregnant women compared with their negative counterparts. From this study, risk factors like young maternal age, low parity, higher gestational age (in HIV-positive pregnant women), non-use of ITN and low socio economic level are associated with increased prevalence of asymptomatic malaria parasitaemia.

It is recommended that the strategy of “screen and treat” for malaria at antenatal booking be adopted, especially for HIV positive pregnant women among whom the prevalence of AMP is higher so as to prevent the adverse feto-maternal effects of malaria parasitaemia and placental parasitization in pregnancy. Provision of ARV drugs for use by HIV positive pregnant women will help to reduce viral load, increase CD4+ count and ultimately prevent the potentiation of the adverse effects of AMP and the increased risk of Mother-To-Child Transmission (MTCT) of HIV.

Further research activities are recommended so as to know more about the interaction between malaria and HIV and their impact on MTCT of HIV, placental parasitization and management and control of these diseases.

COMPETING INTEREST: there was no conflict of interest in conducting and publishing of this work.
AUTHORS’ CONTRIBUTIONS: Nathaniel G.V. initiated the study, supervised data collection, carried out the first analysis and wrote the final version of this work. Odusoja O.L. supervised the design and helped interpret the results. Jagun O.E. helped with conducting the statistical analysis and composition of the manuscript.

References

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**Tables**

**Table 1:** Relationship between prevalence of asymptomatic malaria parasitaemia and maternal age and parity

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Negative parasitaemia</th>
<th>Positive Parasitaemia</th>
<th>Total N=600</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV Positive</td>
<td>HIV Negative</td>
<td>HIV Positive</td>
<td>HIV Negative</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>3 (1.1%)</td>
<td>9 (3.3%)</td>
<td>15 (4.6%)</td>
<td>15 (4.6%)</td>
</tr>
<tr>
<td>20-29</td>
<td>27 (9.0%)</td>
<td>10 (3.3%)</td>
<td>105 (32.4%)</td>
<td>39 (12.0%)</td>
</tr>
<tr>
<td>30-39</td>
<td>33 (12.0%)</td>
<td>99 (35.9%)</td>
<td>93 (28.7%)</td>
<td>33 (10.2%)</td>
</tr>
<tr>
<td>&gt;40</td>
<td>6 (2.2%)</td>
<td>12 (4.3%)</td>
<td>18 (5.6%)</td>
<td>6 (1.9%)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>21 (7.6%)</td>
<td>51 (18.5%)</td>
<td>66 (20.4%)</td>
<td>45 (13.9%)</td>
</tr>
<tr>
<td>1</td>
<td>12 (4.3%)</td>
<td>48 (17.4%)</td>
<td>39 (12.0%)</td>
<td>9 (2.8%)</td>
</tr>
<tr>
<td>2</td>
<td>15 (5.4%)</td>
<td>45 (16.3%)</td>
<td>72 (22.2%)</td>
<td>15 (4.6%)</td>
</tr>
<tr>
<td>3-4</td>
<td>18 (6.5%)</td>
<td>60 (21.7%)</td>
<td>45 (13.9%)</td>
<td>21 (6.5%)</td>
</tr>
<tr>
<td>≥5</td>
<td>3 (1.1%)</td>
<td>3 (1.1%)</td>
<td>9 (2.8%)</td>
<td>3 (0.9%)</td>
</tr>
<tr>
<td>Gravidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15 (5.4%)</td>
<td>42 (15.2%)</td>
<td>39 (12.0%)</td>
<td>36 (11.1%)</td>
</tr>
<tr>
<td>2</td>
<td>15 (5.4%)</td>
<td>42 (15.7%)</td>
<td>33 (10.2%)</td>
<td>15 (4.6%)</td>
</tr>
<tr>
<td>3-4</td>
<td>18 (6.5%)</td>
<td>84 (30.4%)</td>
<td>111 (34.3%)</td>
<td>27 (8.3%)</td>
</tr>
<tr>
<td>≥5</td>
<td>21 (7.6%)</td>
<td>39 (14.1%)</td>
<td>148 (48.4%)</td>
<td>15 (4.6%)</td>
</tr>
</tbody>
</table>

**Table 2:** Prevalence of parasitaemia in the different gestational age groups.

<table>
<thead>
<tr>
<th>Gestational Age</th>
<th>Negative Parasitaemia</th>
<th>Positive Parasitaemia</th>
<th>Total N=600</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV Positive</td>
<td>HIV Negative</td>
<td>HIV Positive</td>
</tr>
<tr>
<td>&lt;14 weeks</td>
<td>3 (0.5%)</td>
<td>3 (0.5%)</td>
<td>15 (2.5%)</td>
</tr>
<tr>
<td>14-27 weeks</td>
<td>47 (7.8%)</td>
<td>118 (19.7%)</td>
<td>108 (18.0%)</td>
</tr>
<tr>
<td>≥28 weeks</td>
<td>31 (5.2%)</td>
<td>74 (12.3%)</td>
<td>130 (21.7%)</td>
</tr>
</tbody>
</table>

**Table 3:** Relationship between Anaemia and parasitaemia.

<table>
<thead>
<tr>
<th>Parasitaemia</th>
<th>Mild parasitaemia</th>
<th>Moderate parasitaemia</th>
<th>Severe parasitaemia</th>
<th>Total (n=270)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;7g/dl</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>3(1.1%)</td>
<td>3(1.1%)</td>
</tr>
<tr>
<td>7.8-9.9g/dl</td>
<td>0(0%)</td>
<td>33(12.2%)</td>
<td>34(12.6%)</td>
<td>67 (24.8%)</td>
</tr>
<tr>
<td>9-11g/dl</td>
<td>21(7.8%)</td>
<td>167(61.9%)</td>
<td>12(4.4%)</td>
<td>200 (74.1%)</td>
</tr>
</tbody>
</table>

P value =0.000   X² = 4.328
Table 4: Frequency of Asymptomatic malaria parasitaemia according to CD4 cell count strata in HIV positive pregnant women

<table>
<thead>
<tr>
<th>CD4 cell count</th>
<th>Positive malaria parasitaemia</th>
<th>Negative parasitaemia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200/mm³</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>200–499/mm³</td>
<td>195(65.0%)</td>
<td>30(10.0%)</td>
<td>225(75%)</td>
</tr>
<tr>
<td>≥500/mm³</td>
<td>36(12.0%)</td>
<td>39(13.0%)</td>
<td>75(25%)</td>
</tr>
<tr>
<td>Total</td>
<td>231(77.0%)</td>
<td>69(23.0%)</td>
<td>300(100%)</td>
</tr>
</tbody>
</table>

$X^2 = 1.601 \quad P \text{ value} = 0.000$

Table 5: Relationship between CD4 count and degree of anaemia in HIV positive pregnant women

<table>
<thead>
<tr>
<th>CD4 cell count</th>
<th>No anaemia (%)</th>
<th>Mild anaemia (%)</th>
<th>Moderate anaemia (%)</th>
<th>Severe anaemia (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200/mm³</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>200–499/mm³</td>
<td>33(11%)</td>
<td>113(37.7%)</td>
<td>67(22.3%)</td>
<td>3(1.0%)</td>
<td>216(72.0%)</td>
</tr>
<tr>
<td>≥500/mm³</td>
<td>57(19%)</td>
<td>27(9.0%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>84(28.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>90(30%)</td>
<td>140(46.7%)</td>
<td>67(22.3%)</td>
<td>3(1.0%)</td>
<td>270(100%)</td>
</tr>
</tbody>
</table>

$X^2 = 2.479 \quad P \text{ value} = 0.000$

Figure 1: Prevalence of parasitaemia in the different gestational age groups.