

## Research Article

# Malarial Infection in HIV Infected Pregnant Women Attending a Rural Antenatal Clinic in Nigeria

R. S. Houmsou,<sup>1</sup> B. E. Wama,<sup>1</sup> S. O. Elkanah,<sup>1</sup> L. C. Garba,<sup>1</sup>  
T. D. Hile,<sup>2</sup> J. B. Bingbeng,<sup>3</sup> S. L. Kela,<sup>4</sup> and E. U. Amuta<sup>5</sup>

<sup>1</sup> Department of Biological Sciences, Taraba State University, Jalingo, Taraba State, Nigeria

<sup>2</sup> Higher Institute of Health Sciences, Adventist Cosendai University, Nanga Eboko, Cameroon

<sup>3</sup> Department of Biology, College of Education, Zing, Taraba State, Nigeria

<sup>4</sup> Department of Biological Sciences, Federal University Kashere, Gombe State, Nigeria

<sup>5</sup> Department of Biological Sciences, University of Agriculture, Makurdi, Benué State, Nigeria

Correspondence should be addressed to R. S. Houmsou; [rs.houmsou@gmail.com](mailto:rs.houmsou@gmail.com)

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Malaria still remains a challenging infection affecting the lives of several HIV infected pregnant women in sub-Saharan Africa. This study was undertaken to determine malarial infection in HIV infected pregnant women in relation to sociodemographic and obstetrical factors. The study also assessed relationship between malarial infection and haemoglobin level, CD<sub>4</sub><sup>+</sup> counts, and ART regimen, as well as predisposing risk factors that influenced occurrence of malarial infection in the women. Thick and thin blood smears were prepared and stained with Giemsa. Haemoglobin level was determined using a hematology analyzer, while the flow cytometry was used to measure CD<sub>4</sub><sup>+</sup> counts. Sociodemographic and obstetrical parameters were obtained through the administration of questionnaires. Of the 159 HIV infected pregnant women examined, 33.3% (59/159) had malarial infection. Malarial infection was significantly higher in pregnant women who were divorced, 40.24% (33/82) ( $\chi^2 = 5.72$ ;  $P = 0.05$ ), were at their first trimester (4–12 weeks), 54.8% (17/31) ( $\chi^2 = 14.85$ ;  $P = 0.01$ ), had CD<sub>4</sub><sup>+</sup> = [201–500 cells/ $\mu$ L], 42.42% (42/99) ( $\chi^2 = 10.13$ ;  $P = 0.00$ ), and those that had severe anaemia (<8 dg/L), 100.00% ( $\chi^2 = 45.75$ ;  $P = 0.00$ ). However, risk factors that influenced the occurrence of malarial infection in the pregnant women were occupation (farming) (AOR = 0.226;  $P = 0.03$ ), marital status (divorced) (AOR = 2.80;  $P = 0.02$ ), gestation (first trimester) (AOR = 0.33;  $P = 0.00$ ), haemoglobin level (Hb < 8 dg/L) (AOR = 0.02;  $P = 0.00$ ), and CD<sub>4</sub><sup>+</sup> counts (low CD<sub>4</sub><sup>+</sup>) (OR = 0.40;  $P = 0.05$ ). The study reported endemicity of malaria in HIV infected pregnant women living in rural areas of Benue State, Nigeria. Malarial infection was higher in women that were divorced, and at their first trimester, had low CD<sub>4</sub><sup>+</sup> count, and had severe anaemia. Farming, divorce, gestation, severe anaemia, and low CD<sub>4</sub><sup>+</sup> counts were predisposing risk factors that influenced malaria occurrence in the HIV infected pregnant women. It is advocated that HIV infected pregnant women should be properly and thoroughly educated on malaria preventive measures in rural areas so as to avoid unpleasant effect of malaria during their pregnancies.

## 1. Introduction

Malaria still remains a challenging infection affecting the lives of several HIV infected pregnant women in sub-Saharan Africa (SSA). At least 25 million pregnant women in malaria stable transmission areas of SSA are exposed to *Plasmodium falciparum* malaria each year [1]. However, HIV infection ranges from 10% to 40% and accounts for 10%–27% of malaria in pregnancy [2–4]. HIV infection has been known

to augment the risks of placental and peripheral malaria, high density parasitaemia, and febrile malaria illness among pregnant women [3–6]. These women are also at an increased risk of having premature delivery, severe anaemia, delivery of low birth weight babies, and maternal death as a result of frequent and severe malarial infections [4].

In Nigeria, malaria and HIV are serious life threatening problems besides noncommunicable diseases that are on the rise among populations. It is estimated that 3.3 million

individuals live with HIV/AIDS and over 100 million malaria cases are being reported yearly [7, 8]. From these numbers, 215,000 and 300,000 individuals, respectively, die every year from both diseases with pregnant women seriously affected despite limited data from previous studies.

In Benue State, Nigeria, pregnant women and their unborn babies are exposed to the risk of getting infected with malaria and HIV as both diseases overlap in the area. Malaria transmission is found to be stable [9–12] and HIV has been consistently on the rise from 10.5% in 2005 to 12.7% in 2010 [13] making the State to have the highest prevalence in the country.

Previous epidemiological and biomedical studies conducted on pregnant women tended to be characterized by a single disease approach. However, HIV-infected pregnant women who are already faced with poverty, discrimination, and other forms of violence in rural areas remain an understudied group. They are at heightened risk of malaria, HIV/AIDS, and various infectious diseases. They are not also provided with adequate health care services mostly due to poor health infrastructures with limited manpower and underequipped laboratory facilities. At present, there is a dearth of comprehensive epidemiological studies on malarial infection in HIV-infected pregnant women living in rural Nigeria. However, none has been reported in Benue State, Nigeria; thus we carried out this study to determine malarial infection in relation to sociodemographic and obstetrical factors of HIV-infected pregnant women attending antenatal clinic of a rural hospital in Benue State, Nigeria. The study also assessed malarial infection in relation to  $CD_4^+$  counts, haemoglobin level, and ART regimen, as well as risk factors predisposing HIV-infected pregnant women to malaria.

## 2. Materials and Methods

**2.1. Study Site.** The study was conducted at the antenatal clinic of the General Hospital Vandeikya, Vandeikya LGA, Benue State, central Nigeria. The area is located within the guinea zone savanna of central Nigeria where malaria is perennial but increases during the rainy season (April–October) when mosquito breeding is high [10]. The hospital provides antenatal services and has a well-established HIV care and treatment unit supported by the Centre for Integrated Health Programs (CIHP) a nongovernmental organization. The centre has a flow cytometer where HIV-infected patients get  $CD_4^+$  count measurements. All the pregnant women at the 2nd and 3rd trimesters were administered Sulfadoxine-Pyrimethamine (SP) but not cotrimoxazole as an intermittent preventive treatment.

**2.2. Study Population, Inclusion and Exclusion Criteria, and Data Collection.** This was a cross-sectional study conducted between June and October 2013. Prior to the commencement of the study, ethical approval was given by the Ministry of Health, Benue State. Management of the General Hospital Vandeikya, Vandeikya LGA, granted us permission to conduct the study. All pregnant women were briefed on the significance of the study and they consented before enrolment.

The study enrolled HIV-infected pregnant women attending routine antenatal services of the hospital. Pregnant women with at least 16 years of age and with gestation of at least 4 weeks were enrolled for the study. Women less than 20 years of age required additional consent from their guardians. Pregnant women with immediate life-threatening medical and obstetrical conditions were excluded.

Following enrolment, a structured questionnaire was administered to each pregnant woman to collect sociodemographic and obstetrical (gestation and gravidity) data. Thereafter, blood samples were collected for HIV and malaria screening, respectively.

### 2.3. Laboratory Tests

**2.3.1. HIV Screening and  $CD_4^+$  Counts.** About 4 mL of blood sample was collected into a vacutainer tube from each enrolled pregnant woman. Each tube was appropriately labeled and immediately sent for HIV screening and malaria parasitaemia, respectively. Pregnant women were screened for HIV using the Alere Determine HIV-1/2 rapid immunoassay test strip. Positive samples were confirmed using the Trinity Unigold HIV-1/2 kit (Trinity Biotech PLC, Ireland). The PARTEC cyflow counter version 2.4 (Flow cytometry) was used to count  $CD_4^+$  lymphocytes subsets of the HIV-infected pregnant women.

**2.3.2. Malaria Microscopy.** Thick and thin blood smears were prepared and stained with Giemsa for light microscopy [14]. Two independent laboratory technicians examined the stained slides for malaria parasites using oil immersion at X100 objective lens. All the positive slides reported *Plasmodium falciparum* parasite species.

**2.3.3. Determination of Haemoglobin (Hb) Concentration.** Anaemia was defined as haemoglobin concentration  $< 11$  g/dL [15]. Blood samples from the enrolled pregnant women were mixed for 15 minutes in tubes on a haematology mixer; each tube was then transferred to an abacus junior haematology analyzer to determine haemoglobin concentration. Anaemia was categorized as severe ( $Hb < 8$  g/dL), mild ( $Hb = 8.1$ – $10.9$  g/dL), and normal ( $Hb > 11$  g/dL).

**2.4. Statistical Analysis.** Collected data were entered into Excel 2007 worksheet and imported into SPSS version 19.0 for Windows. The chi-square test was used to compare malaria occurrence between sociodemographic variables, while the logistic regression was used to find association between risk factors that could influence malarial occurrence in HIV infected pregnant women. The adjusted odd ratios with 95% confidence interval were used to measure the strength of associations. All tests were 2-tailed and  $P$  values less than 0.05 were considered statistically significant.

## 3. Results

Table 1 describes malarial infection in relation to sociodemographic and obstetrical parameters of HIV-infected pregnant women attending a rural antenatal clinic in Nigeria. Malarial

TABLE 1: Univariate and multivariate analysis of malarial infection in relation to sociodemographic and obstetrical factors of HIV infected pregnant women attending a rural antenatal clinic in Nigeria.

Parameters	Malaria in HIV-infected pregnant women					
	Examined	Positive (%)	$\chi^2$	<i>P</i>	AOR (95% CI)	<i>P</i>
Prevalence	159	53 (33.33)				
Sociodemographic						
Age			3.51	0.47	1.02 (0.60–1.74)	0.92
[16–20]	26	6 (23.07)				
[21–25]	43	12 (27.90)				
[26–30]	59	24 (40.67)				
[31–35]	24	8 (33.33)				
[36–40]	7	3 (42.85)				
Education			0.50	0.91	1.04 (0.51–2.14)	0.00
No formal education	24	9 (37.50)				
Primary	84	27 (32.14)				
Secondary	51	17 (33.33)				
Occupation			3.08	0.21	0.26 (0.08–0.87)	0.02
Trading	57	14 (24.56)				
Farming	102	39 (38.23)				
Marital status			5.72	0.05	2.80 (1.18–6.64)	0.02
Single	53	11 (20.75)				
Married	24	9 (37.50)				
Divorced	82	33 (40.24)				
Obstetrical						
Gravidity			1.63	0.44	0.98 (0.48–2.03)	0.97
Primigravidae	23	5 (21.73)				
Multigravidae	136	48 (35.29)				
Gestation (weeks)			14.85	0.01	0.32 (0.17–0.62)	0.00
[4–12]	31	17 (54.83)				
[13–24]	79	29 (36.70)				
[25–36]	49	7 (14.28)				

Key: AOR = adjusted odd ratio.

infection was 33.33% (53/159) among the HIV-infected pregnant women examined. Age-related infection varied between 23.07% and 42.85% with no significant difference ( $\chi^2 = 3.51$ ;  $P = 0.47$ ). With regard to education and occupation of the women, malarial infection varied between 33.33% and 37.50% ( $\chi^2 = 0.50$ ;  $P = 0.91$ ) and between 24.56% and 38.23% ( $\chi^2 = 3.08$ ;  $P = 0.21$ ), respectively; while in relation to marital status, divorcees were more significantly infected, 40.24% (33/82), than the singles, 20.75% (11/53), and married, 37.50% (9/24) ( $\chi^2 = 5.72$ ;  $P = 0.05$ ). In relation to obstetrical parameters, malarial infection did not significantly vary with gravidity though multigravidae had higher infection, 35.29% (48/136), than the primigravidae, 21.73% (5/23) ( $\chi^2 = 1.63$ ,  $P = 0.44$ ). However, malarial infection was significantly higher in HIV-infected pregnant women at their first trimester (4–12 weeks) 54.83% (17/31) ( $\chi^2 = 14.85$ ,  $P = 0.01$ ). The sociodemographic and obstetrical risk factors predisposing HIV pregnant women to malarial infection in Vandeikeya are occupation (farming) (AOR: 0.26, CI (0.08–0.87),  $P = 0.02$ ), marital status (Divorce) (AOR: 2.80, CI (1.18–6.64),  $P = 0.02$ ), and gestational age (1st trimester) (AOR = 0.32, CI (0.17–0.62),  $P = 0.00$ ).

Table 2 shows malarial infection in relation to  $CD_4^+$  counts, haemoglobin level, and antiretroviral treatment regimen of the HIV-infected pregnant women. A significant malarial infection was observed in pregnant women with  $CD_4^+$  counts [201–500] cells/ $\mu$ L, (42.42%, 42/99) ( $\chi^2 = 10.13$ ;  $P = 0.00$ ) and haemoglobin level (Hb) < 8 g/dL, (100%, 19/19) ( $\chi^2 = 45.75$ ;  $P = 0.00$ ). Women on Pre-ART treatment had higher malarial infection (37.50%, 30/80) than those on ART (29.11%, 23/79) with no significant difference ( $\chi^2 = 1.25$ ,  $P = 0.26$ ). Low  $CD_4^+$  counts (201–500 cells/ $\mu$ L) (AOR = 0.40, CI (0.16–1.00),  $P = 0.05$ ) and haemoglobin level (Hb < 8 g/dL) (AOR = 0.02, CI (0.00–0.17),  $P = 0.00$ ) were observed to be malaria predisposing risk factors in the HIV-infected pregnant women.

#### 4. Discussion

Malaria and HIV are dreadful infections that affect pregnant women in areas where both infections overlap. In pregnancy, HIV has been hypothesized to impede the development of antimalaria antibodies, therefore predisposing pregnant women at increased risk of developing malaria; on the other

TABLE 2: Univariate and multivariate analysis of malarial infection in relation to CD<sub>4</sub><sup>+</sup> counts, haemoglobin level, and ART regimen of HIV infected pregnant women attending rural antenatal clinic in Nigeria.

Parameters	Malaria in HIV-infected pregnant women					
	Examined	Positive (%)	$\chi^2$	<i>P</i>	AOR (95%, CI)	<i>P</i>
CD <sub>4</sub> <sup>+</sup> count (cell/ $\mu$ L)			10.13	0.00	0.40 (0.16–1.00)	0.05
[201–500]	99	42 (42.42)				
[501–800]	50	10 (20.00)				
[801–1100]	10	1 (10.00)				
Haemoglobin (dg/L)			45.75	0.00	0.02 (0.00–0.16)	0.00
<8 (severe anaemia)	19	19 (100.00)				
8.1–10.9 (mild anaemia)	140	34 (24.28)				
>11 (normal anaemia)	0	0 (0.00)				
ART regimen			1.25	0.26	0.90 (0.35–2.31)	0.83
Pre-ART	80	30 (37.50)				
On ART	79	23 (29.11)				

Key: AOR = adjusted odd ratio.

hand, malaria could increase HIV mother to child transmission. The present study reports endemicity of malaria among HIV pregnant women in Vandeikya though infection level (33.33%) was below 50.00% as one would have expected the disease to be higher among rural HIV-infected pregnant women living in stable transmission area. The reduced prevalence among these women is the effort of the HIV unit established by the Centre for the Integrated Health Programs (a nongovernmental organization) into the General Hospital to care and treat HIV-infected patients. Pregnant women attending antenatal clinic of the hospital are thoroughly screened for both malaria and HIV and followed up for HIV until delivery and afterwards. Though being vulnerable to malaria because of their weak immune status and rural area of residence, this malaria prevalence is encouraging and found to be lower than 47.70% observed among HIV-infected pregnant women in Lagos, South West, Nigeria [16]. The malarial infection level found in this study is higher than 5.50% and 8.00% reported in Malawian and Rwandan HIV-infected pregnant women [17, 18].

Malarial infection did not significantly vary between age groups, education, and occupation of the women. This clearly shows that regardless of their sociodemographic characteristics women living in rural areas are all exposed to infections mostly because they have the same behavioural habits and living conditions. The increased malarial infection among the divorcees might be likely the exposure of these women to mosquito bites through their various occupations as most of them were either farmers or traders. However, the attitudes of not sleeping under bed nets might have greatly influenced malaria rise among these divorcees. This attitude was also observed among pregnant women in neighbouring Gboko Local Government Area who believed that bed nets are used to protect dead bodies from flies [9].

The higher malarial infection in the multigravidae might be likely due to the low level of antibodies against variant surface antigens (VSA) on infected erythrocytes binding chondroitin surface A (CSA) thought to be protecting against placental malaria in HIV seropositive multigravidae pregnant women [19, 20]. The observed high malarial infection

in these multigravidae is equivocal because in many stable malaria transmission areas malarial infection has been reported to be decreasing in HIV-negative multigravidae due to their immune build-up after several pregnancies and infections. Our finding corroborates the fact that HIV infection increases the risk of developing malaria in pregnant women irrespective of their gravidities.

Our study revealed that first trimester of pregnancy (4–12 weeks) is associated and found to be a risk factor predisposing the HIV-infected women to malaria as they were three times more exposed to malaria than women in other gestational ages. This shows that women at that stage of pregnancy were coming for their first antenatal clinic and were yet to be administered sulfadoxine-pyrimethamine (SP) used as the intermittent preventive treatment (IPT) advocated to start only with women at their second and third trimesters. However, pregnant women with low CD<sub>4</sub><sup>+</sup> counts (201–500 cells/ $\mu$ L) were significantly and four times more infected than those with higher CD<sub>4</sub><sup>+</sup> counts. Pregnant women in this category had most of their CD<sub>4</sub><sup>+</sup> lymphocytes depleted by the virus thereby reducing the production of malaria antibodies. Conversely, the low malarial infection observed in those having higher CD<sub>4</sub><sup>+</sup> count cells could be the reconstitution of their immune system due to the intake of highly active antiretroviral therapy (HAART) or they were newly infected women that still have strong immune system.

Malaria has been associated with severe and moderate anaemia in our HIV cohort. We cannot be conclusive about this because the causes of anaemia in HIV infected individuals are multifactorial. We did not take into consideration other HIV opportunistic infections that cause anaemia such as hookworm infection, schistosomiasis, and diarrheal diseases which are more common in HIV patients. However, the rural residence of these women might have also played a big role, because most of them might not have adequate information about nutritional diet and other factors that could cause anaemia.

Pregnant women on ART were less infected with malarial infection. This corroborates previous studies that reported reduced malarial infection in Nigerian and Ugandan HIV

patients on ART [21, 22]. However, Skinner-Adams et al. [23] hypothesized the inhibition of malaria parasite growth by certain active compounds like the protease inhibitors found in the HAART drugs.

Our study had various limitations. The cross-sectional nature of the study allowed us to collect only one blood sample for malaria detection. The financial and laboratory constraints did not allow us to carry out real time polymerase chain reaction technique (RT-PCR) which would have reported submicroscopic malaria infections than the light microscopy. The short duration of the study (5 months) was a limitation to assess the malaria effects on the birth weight of new born babies of HIV-infected pregnant women.

In conclusion, our study is the first epidemiological study reporting endemicity of malaria in HIV-infected pregnant women living in a malaria rural stable transmission zone of Benue State, central Nigeria. We found that sociodemographic (occupation, marital status), obstetrical (gestation), and haematological (anaemia and low CD<sub>4</sub><sup>+</sup> counts) factors have been reported to predispose HIV-infected pregnant women to malaria risk. It is therefore recommended that:

- (i) HIV infected pregnant women at their first trimester should be administered sulfadoxine-pyrimethamine (SP) as their counterparts at second and third trimesters or they should be given daily cotrimoxazole which is found to be effective for malaria prophylaxis in HIV patients.
- (ii) Iron deficiency, the primary cause of anaemia, should be screened properly and pregnant women should be treated promptly so as to curb anaemia known as complication from both malaria and HIV in pregnancy.
- (iii) HIV infected pregnant women should be properly and thoroughly educated on malaria preventive measures in rural areas so as to avoid unpleasant effect of malaria during their pregnancies.
- (iv) Health nongovernmental organizations should have a memorandum of understanding (MoU) with both public and private hospitals which are already underfunded and neglected by the stakeholders in rural areas. These NGOs can help plan effective screening and control programmes of both malaria and HIV infections and subsequent HIV care follow-up in pregnant women.

### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

### Authors' Contribution

R. S. Houmsou, S. L. Kela, and E. U. Amuta designed the study. R. S. Houmsou carried out statistical analysis and drafted the paper. B. E. Wama, T. D. Hile, and J. B. Bingbeng collected data and carried out laboratory analyses. L. C. Garba and S. O. Elkanah were involved in literature searches and

data collection. S. L. Kela and E. U. Amuta critically revised the paper. All authors read and approved the paper.

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