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ORIGINAL RESEARCH ARTICLE

The Incidence of Asymptomatic and Symptomatic Malaria Infection in HIV Positive and Negative Individuals in Osun State, Nigeria

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ABSTRACT

Malaria infection remains a public health problem in the sub-Saharan African region, including Nigeria. Clinical symptomatic malaria cases are treated when diagnosed. However, asymptomatic malaria cases are most often undetected and, therefore, untreated, resulting in a significant source of gametocytes that serve as carriers of malaria transmission. This study was conducted to determine the prevalence of malaria cases in both asymptomatic and symptomatic individuals who are HIV positive and negative in Osun State, Nigeria, as a call for effective diagnosis and treatment awareness. One hundred and twenty-two participants were enrolled in the study. Primary clinical biodata was examined to determine the fitness of the participants. Blood samples were collected for malaria parasite (*Plasmodium falciparum*) analysis using microscopy and polymerase chain reaction. Gametocytes were detected using nested PCR targeting gametocyte-specific marker, Pf377g. Malaria was detected in 31 (25.4%) HIV-positive individuals and 91 (74.6%) HIV-negative individuals. The prevalence of symptomatic malaria was 8.2% in HIV-positive individuals. The prevalence of symptomatic malaria was 31.2% in HIV-negative patients. However, it was 43.4% in the same group of people (HIV-negative patients) that are asymptomatic. CD4 cell count ($p<0.0001$) and parasite density ($p=0.005$) were significantly lower in symptomatic malaria individuals than their asymptomatic malaria counterparts. The higher prevalence of asymptomatic malaria individuals recorded in this study with the presence of gametocytes, a significant carrier of the disease, may contribute to the spread of the infection. Therefore, to reduce the fatality of malaria infection and limit its transmission, accurate and improved diagnostic strategies are essential for the treatment and management of asymptomatic malaria carriers.

ARTICLE HISTORY

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KEYWORDS

Nested PCR, *Plasmodium falciparum*, HIV, Symptomatic, Asymptomatic infection

INTRODUCTION

Malaria, caused by *Plasmodium falciparum*, remains a significant public health challenge in sub-Saharan Africa. While symptomatic infections are well-documented, asymptomatic carriers—who harbor gametocytes that facilitate parasite transmission—pose a major hurdle in malaria elimination efforts (Sumari *et al.,* 2017; Adesoye *et al.*, 2024). The ability to acquire partial immunity against severe malaria depends on age and repeated exposure in high-endemic areas (Qu *et al.,* 2021; Farid *et al.,* 2017). However, individuals with compromised immune systems, such as those living with HIV, face an increased risk of both symptomatic and asymptomatic malaria due to impaired malaria-specific immunity, particularly the depletion of CD4+ T cells (Roberds *et al.,* 2021).

The HIV-infected individual acquires an array of opportunistic infections compared with the HIV-uninfected individual due to impairment of malaria-specific immunity inflicted by HIV (Roberds *et al.,* 2021). Studies have shown that a decline in CD4+ counts increases the prevalence of both malaria symptomatic and asymptomatic infection (Sumari *et al.,* 2017). The CD4 T-lymphocyte is known as a primary immune system implicated in the formation of antimalarial immunity

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(Okonkwo *et al.,* 2014). It controls malaria infection by producing immune cells and facilitates B cells to generate antimalarial antibodies (Kengue-Ouafo *et al.,* 2019; Adesoye *et al.,* 2024).

Despite efforts to control malaria, undiagnosed asymptomatic cases continue to sustain transmission. Standard diagnostic methods such as microscopy and rapid diagnostic tests (RDTs) often fail to detect low-level parasitemia, whereas polymerase chain reaction (PCR) techniques offer higher sensitivity for identifying asymptomatic carriers (WHO, 2018; Matangila *et al.,* 2014; Coalson *et al.,* 2016).

There is limited data on the prevalence of symptomatic and asymptomatic *P. falciparum* infections in HIV-positive and HIV-negative individuals, particularly regarding gametocyte carriage. This study aims to bridge this knowledge gap by determining the prevalence of *P. falciparum* infections—both symptomatic and asymptomatic—among HIV-infected and uninfected individuals in Osun State, Nigeria. The findings will contribute to improved malaria control strategies, particularly for high-risk populations.

METHODOLOGY

It was a cross-sectional study carried out from March to September 2020 in Osun State, located in the tropical zone of South Western Nigeria. The state has a land area of 8,802 square kilometers and a population of 3,423,535 (Ojurongbe *et al*., 2015). The annual rainfall is between 800 mm and 1500mm, with a yearly mean temperature of 21.1℃ to 31.1℃ (Egbewale *et al.,* 2018).

The study involved confirmed HIV-infected patients duly registered into the antiretroviral treatment (ART) program at Institute of Human Virology of Nigeria (IHVN) clinic, Ladoke Akintola University of Technology Teaching Hospital Osogbo, General Hospital Ede and Obafemi Awolowo University Teaching Hospital Complex Wesley Guild, Ilesa, and HIV negative individuals who visited General Out-Patient Department (GOPD) of these facilities, presenting with uncomplicated *falciparum* malaria with the presence of fever (≥37.5^{o}C) following WHO criteria, while the assessment of asymptomatic enrollment presenting with no history of fever in the past 48hrs sample was collected; thus asymptomatic infection was defined as no measured of fever at the time of the study (Tiono *et al.,* 2023). Biodata capturing the socio-demographic and clinical history of the respondents was taken. Weight was taken using a weighing scale, and the axillary temperature was determined using a digital clinical thermometer (Teh *et al.,* 2018). Written informed consent was obtained from patients before recruitment into this study.

Sample size determination

The sample size was determined using Charan & Biswas, 2018. as follows:

$$n = \frac{Z^2P(1-P)}{d^2}$$

where:

n = required sample size

Z = Z-score for the desired confidence level (1.96 for 95% confidence)

P = estimated prevalence =0.50 (since there is no prior data is available)

d = margin of error (precision), =0.089 (≈8.9% margin of error)

$$n = \frac{1.96^2 \times 0.5(1-0.50)}{0.089^2}$$

$$n = \frac{0.9604}{0.007921}$$

$n \approx 121.2$

Since the sample size should be a whole number, we round up to 122.

So, the required sample size is 122.

Specimen Collection and Identification of *Plasmodium* Species

5mls of blood samples were aseptically collected by venipuncture into an ethylene tetra-acetic acid (EDTA) container and mixed. Thick and thin blood films were prepared and stained with Giemsa stain and examined under the microscope using 100X objective with immersion oil according to the guidelines of the WHO, 2015. Parasite density was recorded as the number of parasites per microliter of blood, adopting a relative WBC count of 8000 cells/µl (WHO, 2016). *Plasmodium falciparum* asexual parasitemia was classified according to the following criteria: low (800 parasites/µl), moderate (801 to 8,000 parasites/ µl), and high (> 8,001 parasites/ µl) Edgar *et al.* (2022).

The smears were stained with 10% Giemsa solution for 10 minutes. Rinsed with buffered water (pH 7.2) and allowed to air-dry before examining under a microscope.

The stained slides are examined under 1000× magnification using an oil immersion lens.

The thick smear was used for parasite quantification, while the thin smear was used for species identification.

The WHO recommends two standard methods:

Asexual-stage parasites (trophozoites, schizonts) were counted against 200 white blood cells (WBCs) in the thick smear. In the case of fewer than 10 parasites per 200 WBCs, the count was extended to 500 WBCs for better accuracy.

Parasite density was calculated using the formula:

$$\text{Parasites per µL of blood} = \frac{\text{PC} \times \text{TWBC count per µl}}{\text{WBCs Counted}}$$

Where:
PC = Parasites counted
TWBC = Total WBC

HIV Diagnosis

HIV status was determined using a Rapid Test kit (Abbott Laboratories, Co., Ltd. Minato-Ku, Tokyo, Japan) to confirm the HIV status of participants, followed by UNI GOLD or STAT PAK concurrently according to the serial algorithm of the Federal government of Nigeria (FMOH, 2016).

CD_4^+ count, Packed Cell Volume (PCV), and Hemoglobin Concentration (Hb)

The CD_4^+ T cell count was estimated using two-color single-platform flow cytometry (Partec, GmBH, Germany) (Mwau *et al.,* 2022). The packed cell volume (PCV) and hemoglobin concentration (Hb) were estimated with a Sysmex XT—21N Hematology Analyzer Automated machine (Sysmex Corporation, Japan 2012 Model), strictly adhering to the manufacturer's instructions (McKenzie & Williams. 2019).

Molecular Analysis

Parasite Genomic DNA Extraction

The protocol for extracting parasite genomic DNA from the whole blood was done by crude extraction method as described by the Institute of Medical Research and Training, University College Hospital, Ibadan (Malaria unit). 1000µl of elution buffer was added to elute the DNA and stored at -20^{0}C until further analysis.

The primers used to amplify a segment of the stevor gene, which belongs to the multigene family involved in *P. falciparum* antigenic variation was:

Table 1: Primary and secondary PCR primers used for this study

Primer Name	Primer sequence (5' – 3')
P5	5´-GGG AAT TCT TTA TTT GAT GAA GAT G-3´
P18	5´-TTT CA(C/T) CAC CAA ACA TTT CTT-3´
P19	5´-AAT CCA CAT TAT CAC AAT GA-3´
P20	5´-CCG ATT TTA ACA TAA TAT GA-3´
P17	5´-ACA TTA TCA TAA TGA (C/T) CC AGA ACT-3´
P24	5´-GTT TGC AAT TAT TCT TTT TCT AGC-3´
MSP-1	FP 5´-TGA AGA TGG GGG TCA CTC CA-3´
	RP 5´-TGT GAG TTG CGG ATT GAG CA-3´
MSP-2	FP 5´-ATG AAG GTA ATT AAA ACA TTG TCT ATT ATA-3´
	RP 5´-ATA TGG CAAAAG ATAAAA CAA GT-3´
GLURP	FP 5´-AGA TGA TGT GCC TTC CCC TA-3´
	RP 5´-TGT GAG TTG CGG ATT GAG CA-3´
*Pf*377g	FP 5´-AAG ATG ACG AAG GGG ATG AAG TA-3´
	RP 5´-AGTCCATAT GTT GGTTAT GATG-3´

Key: FP =Forward Primer
RP = Reverse Primer
MSP-1 = Merozoite Surface Protein-1
MSP-2 = Merozite Surface Protein-2
GLURP = Glutamate Rich Protein
*PF*377g = *Plasmodium falciparum* 377 gametocyte

Species-Specific PCR for *P. falciparum* StevorGene

The Stevor gene family was used here for parasite detection because it is exclusive to *P. falciparum* and absent in other malaria-causing species like *P. vivax*, *P. ovale*, and *P. malariae*. This makes it a highly specific target, reducing false positives due to cross-reactivity. PCR assay includes a primary and nested reaction to enhance specificity. As Ojurongbe et al. (2014) described, primary amplification was performed with primers. Genus-specific primers (P5, P18, P19, and P20) were used for the first amplification cycle. *P. falciparum* species were detected using a species-specific primer (P17 and P24) in the second cycle (nested) reaction. The PCR assays were performed using the Gene Amp PCR system 9700 Thermal cycler (Biotron, Gottingen, Germany). Thermal profile was 93^{o}C for 3 minutes, 30 seconds of 25cycles at 93^{o}C, 50 sec at 50^{o}C, 30 seconds at 72^{o}C, and 3minutes at 72^{o}C. 2µl of the primary PCR product was analyzed in the nested PCR reaction for 25 cycles. The final concentration of each reagent was 5.0µl x 10 reaction buffer, 200µM dNTP, 1.25 units Taq polymerase, and 0.4 pM primers (Jena Bioscience, Deutschland). PCR products (1100bp and 205pb, respectively) were visualized by gel electrophoresis and ethidium bromide staining.

Detection of Pf377g.

The PCR analysis for *Pfg377* genes in the patient samples was carried out using the PCR conditions with high-fidelity Taq DNA polymerase described previously

(ThermoFisherTaq) (Schneider *et al.,* 2015; Kumar *et al.,* 2019). The amplified products were analyzed by electrophoresis on a 2% molecular grade agarose gel and visualized by UV transilluminator after gel SYBR-safe staining. The size of the PCR products was estimated using image lab software version (3.0) (Biorad), with size computed automatically by the software based on the 100 base pair DNA ladder calibrator (Real Biotech Corporation).

Statistical Analysis.

Data were analyzed using GraphPad Prism 5 (GraphPad Software Inc. USA) to generate means, standard deviation, median, and frequency distributions. Analysis of variance was utilized to investigate the relationship between the variables. The association between categorical variables was tested using the chi-square test ($\chi 2$). Statistical significance was defined as a p-value < 0.05. All variables of interest were normally distributed after using Kolmgrov-Smirnov. 95% Confidence Interval (CI) on total malaria prevalence was determined using logistic regression

RESULTS

The total *Plasmodium falciparum*-positive population in this study was 122; out of the 122 positive malaria cases, 31 participants were HIV positive (co-infected with malaria), while ninety-one were HIV negative (mono-infection) (p=0.004). The proportion of females having malaria is significantly high (82) compared with their male counterparts (40) (p=0.008). There were 33 individuals with a temperature ≥ 37.5ºC and 89 with a temperature ≤ 37.5ºC. This was statistically significant (p=0.0001). The mean age for asymptomatic cases was 28.48± 15.38 years, and for symptomatic participants, it was 29.05 ± 13.50 years (p=0.224). The mean weight was significantly higher (57.15 ± 0.99) in asymptomatic individuals than their symptomatic counterparts (46.35±1.25) (p=0.0001). Seventy-four percent of the study participants were asymptomatic for malaria, while forty-eight were symptomatic. Of the one hundred and twenty-two microscopically positive participants for the malaria parasite, 20 asymptomatic and symptomatic malaria cases were subjected to PCR amplification of the Stevor gene for *Plasmodium falciparum* detection. Twenty-five (20.5%) were gametophyte positive as revealed by the PCR (Table 2).

Figure 1 shows the gel picture of the group-specific antigenic variances of *P. falciparum* spanned to polymerase MSP-1 gene on 1% agar rose gel stained with ethidium bromide. The well was labeled from 1 to 8 to accommodate the samples and the negative control. Five samples in lanes 2, 3, 5, 6, and 8 showed the expected base pair band for the MSP-1 gene. A gel picture of the polymerase MSP-2 gene is shown in Figure 2 with the expected molecular weight of the DNA. Samples in lanes 3 and 5 showed the expected base pair bands for the protein; Figure 3 shows the gel image of GLURP protein with the expected molecular weight of the DNA, and the sample on well 3 showed the expected base pair bands of the gene. The sample in lane 4 gave the expected base pair band of the gene. Also, Figure 4 shows the gel image of the Pf377g gene of the parasite on 1% agar rose gel. Four samples in lines 5, 6, 7, and 8 showed the expected base pair band for the gametocyte gene of *P. falciparum*.

The Packed cell volume was found to be significantly (p < 0.0001) lower in symptomatic malaria participants compared with asymptomatic malaria counterparts regardless of HIV status (Table 3). Hemoglobin concentration was considerably lower (p= 0.001) in the symptomatic malaria individuals compared with asymptomatic malaria counterparts (Table 3). The parasite density was observed to be significantly (p < 0.005) higher in symptomatic malaria-positive participants than those with asymptomatic malaria (Table 2). The CD4 T cell count was further observed to be significantly (p < 0.0001) lower in symptomatic malaria participants than those having asymptomatic malaria among the study participants (Table 3).

Table 2: Baseline Characteristics of the Study Population (N=122)

	Asymp Mal (%)	Symp Mal (%)	Total (%)	CT	P-value
HIV status					
HIV Positive	21(17.2)	10(8.2)	31(25.4)	17.7- 33.1	0.004*
HIV Negative	53(43.4)	38(31.2)	91(74.6)	66.9- 82.3	
Gender					
Male	22 (18.0)	18(14.8)	40(32.8)	27.3- 38.3	0.008*
Female	52 (42.6)	30(24.6)	82(67.2)	59.3- 75.1	
Temperature					
≥ 37.5ºC	9(7.4)	24(19.7)	33(27.1)	22.3- 31.9	
≤ 37.5ºC	65(53.3)	24(19.7)	89(72.9)	65.1- 80.7	0.0001*
Mean age (years)	28.48 ± 15.38	29.05 ± 13.50		-	
Mean body weight (Kg)	57.15 ± 0.99	46.35 ± 1.25		-	0.0001*
Microscopy positive	74 (60.7)	48 (39.3)	122(100)	-	
***P.falciparum* positive by stevor gene**	20 (16.4)	20(16.4)	40(32.8)	27.3- 38.3	0.574
Gametocyte PCR positive	18(14.8)	7(5.7)	25(20.5)	16.7- 24.3	0.314

***Chi-square test** **KEY: CT: 95 % Total Confidence Interval, CI**

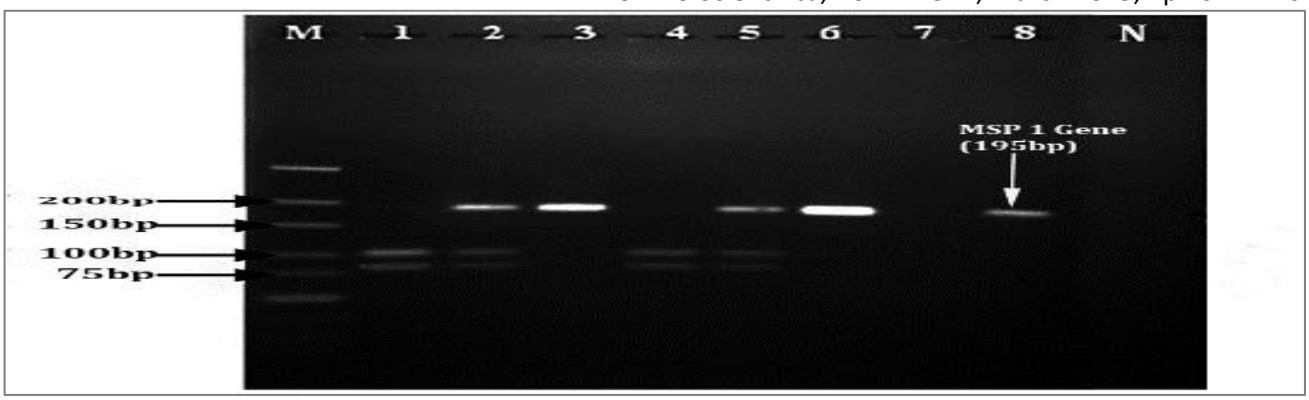

Figure 1: Electropherogram of merozoite surface protein 1(MSP-1)
Key note: *Amplicon on 1% Agarose gel, (M) showing the graduated ladder in bands of 1000bp (DNA marker), (N) showing the negative control, and well 8 showing a positive band*

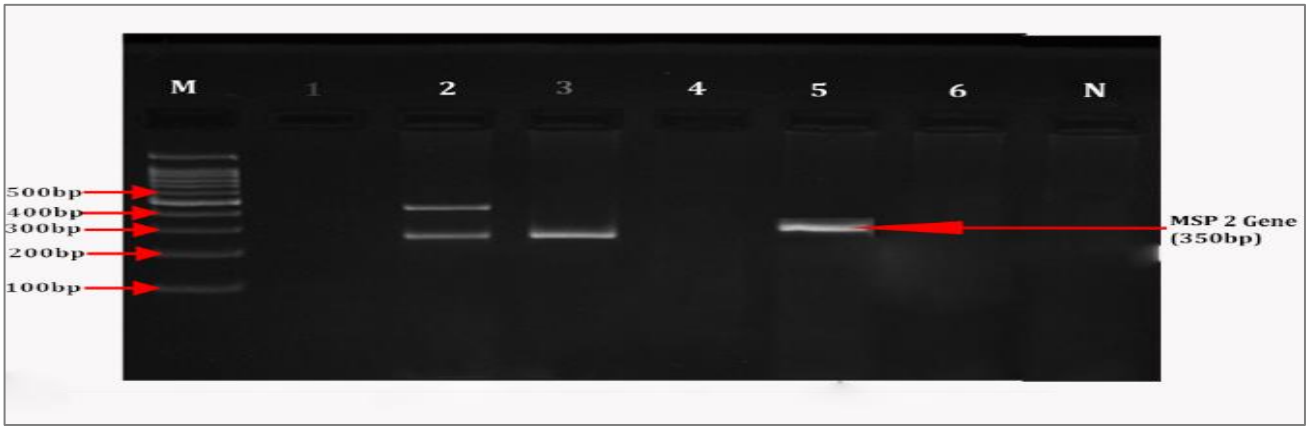

Figure 2: Electropherogram of merozoite surface protein 2 (MSP-2)
Key note: *Amplicon on 1% Agarose gel, (M) showing the calibrated ladder in bands of 1000bp (DNA marker), (N) showing the negative control, and well 5 showing the positive band.*

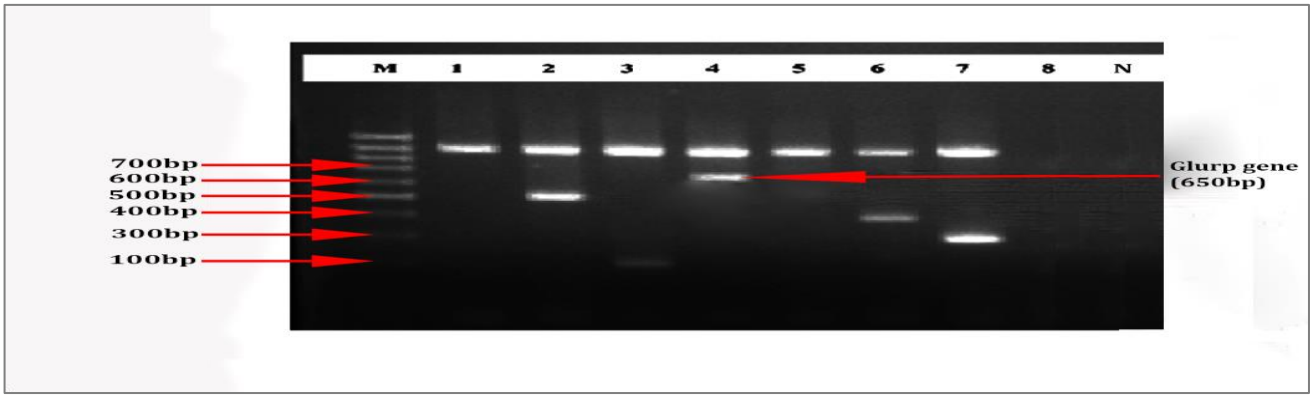

Figure 3: Electropherogram of glutamate-rich protein (GLURP)
Key note: Amplicon on 1% Agarose gel, (M) showing the calibrated ladder in bands of 1000bp (DNA marker), (N) showing the negative control, and well 4 shows a positive band.

DISCUSSION

This study evaluated the prevalence of asymptomatic and symptomatic malaria among HIV-infected and uninfected individuals in Osun State, Nigeria. The total asexual *falciparum* prevalence using the PCR stevor gene was high in both symptomatic and asymptomatic participants. At the same time, the sexual stage of the parasite was substantially higher among asymptomatic malaria-positive individuals (60.7%) than among symptomatic (39.3%) participants. This result is similar to the finding by Sumari *et al.* (2017) with a higher gametocyte report.

This outcome indicates that to obtain an accurate diagnosis and exact *falciparum* prevalence in malaria surveillance within the population, there is a need to utilize molecular investigating tools. The molecular technique deployed in this study exhibited higher sensitivity; other studies have previously proved this (Mwau *et al.,* 2022; Kumar *et al.,* 2019). The infective stage (gametocytes) detected was higher in malaria asymptomatic HIV-positive and negative participants in this study. Despite the lack of symptoms, the gametocyte was identified; although it is not specific for the burden of the disease, it should not be left untreated because they are known

reservoirs of falciparum and act as indices for continuous transmission in the population. This concurs with the finding stating that the asymptomatic infected individuals are gametocyte carriers, identified as reservoirs of infection, as reported by Schneider *et al.* (2015) and Sumari *et al.* (2017). Although the real mechanism behind the spread of gametocyte transport is still unknown, this could be associated with the damage of T-cell-dependent antibody responses influencing the delay in developing transmission-blocking immunity. The *falciparum* positive by microscopy was higher (60.7%) in asymptomatic than symptomatic, proving that even though molecular tools are sensitive, microscopy remains a gold standard, highly useful in quantifying parasitemia level and speciation. However, microscopy is limited in its accuracy and precision; one of the limitations is that it requires an experience malaria microscopist.

Figure 4: Electropherogram of *Plasmodium falciparum377gametocyte* (*Pf377g*)
Key note: Amplicon on 1% Agarose gel, (M) showing the graduated ladder in bands of 1000bp (DNA marker) (N) showing the negative control and well 5, 6, 7, and 8 positive bands.

Table 3: Hematological and Immunological parameters in comparison to malaria status.

Variables	AM/HIV Pos Mean ± SD	SM/HIV Pos Mean ± SD	AM/HIV Neg Mean ± SD	SM/HIV Neg Mean ± *SD*	*F value*	*p-value*
PCV	34.32±0.21	29.26±0.30	40.84 ± 0.26	33.36 ± 0.29	18.40	<0.001
HC. (g/dl)	10.50 ± 0.20	9.20 ± 1.39	11.54 ± 1.07	9.95 ± 0.18	4.76	0.001
PD (P/ µl)	949.96±448.53	6478.74±625.18	1470.46±1218.37	5742.64±500.13	4.49	<0.005
CD_4 C (cell/µl)	375.98±9.24*	203.21±6.30*	595.40±9.24	315.95±14.98	5.61	<0.001

*One-way ANOVA test;
KEY: AM: Asymptomatic Malaria; SM: Symptomatic Malaria; PD: Parasite Density; HC: Hemoglobin conc; P/ µl: Parasite/µl of blood; CD_4 C: CD_4 Count

There is a strong direct relationship between body temperature and symptomatic malaria. The high temperature could be due to disturbances in the schizogonic cycles due to the heterogenicity of the malaria parasite. This stimulates the immune system to secrete TNF-α, IL-1, and other body cytokines, causing resistance that leads to an over-regulation of endothelial cells that generate paroxysmal febrile (Laban *et al.,* 2015). There is a significant relationship between malaria infection and the mean body weight, as observed in this study. It could be due to poor nutritional lifestyle and loss of appetite, which allows malaria attack due to weakened immune response and malnutrition (Oyeniran *et al.*, 2014; Sumbele *et al.*, 2016; Sumari *et al.,* 2016).

The result obtained in this study showed a significant decrease in PCV and Haemoglobin concentration (as low as <40% in males and < 30% in females, so also <12g/dl), indicating anaemia among malaria symptomatic and asymptomatic HIV-infected participants compared with the HIV negative malaria counterparts. The mean PCV and Hb values were significantly lower in asymptomatic malaria participants than in the symptomatic group. Malaria-triggered anemia can result from erythrocyte lysis, which accelerates the clearance of parasites and non-parasites (Shikur *et al.,* 2016), thus lowering PCV and hemoglobin concentrations. This is in line with the findings of Bawah *et al.* (2018), who reported higher rates of malaria-inducing anemia among children in Ho municipality, and is also supported by the studies conducted in Ghana (Sakzabre *et al.,* 2020) in Nigeria (Bawah *et al.,* 2018) and Western Kenya (Robert et al. 2023), linking anemia to malaria parasitism.

Malaria-associated anemia (MAA) is a significant complication of *Plasmodium* infections, particularly in endemic regions. It results from a complex interplay of red blood cell (RBC) destruction, impaired erythropoiesis, and immune-mediated mechanisms (White *et al.,* 2018). The direct destruction of RBCs occurs due to parasite-induced hemolysis when infected erythrocytes rupture during the asexual stage of *Plasmodium* development. Additionally, uninfected RBCs are prematurely cleared by the spleen, contributing to anemia (Haldar & Mohandas, 2019). Beyond RBC destruction, malaria suppresses bone marrow function, leading to ineffective erythropoiesis and a decreased production of new RBCs, exacerbating anemia (Nti & Perkins 2020). The inflammatory response

triggered by *Plasmodium* infection plays a crucial role, as cytokines such as TNF-α, IL-6, and IFN-γ disrupt iron metabolism and erythropoietin activity, further worsening anemia (Kalyesubula & Nankabirwa 2020). Severe malaria-associated anemia is particularly common in children and pregnant women due to their relatively weaker immune responses and higher parasite loads (White, 2018). The condition is often linked to high morbidity and mortality, increasing the risk of secondary infections, organ dysfunction, and cognitive impairment in children (Nankabirwa *et al.,* 2014). Effective management involves antimalarial treatment, blood transfusion in severe cases, and iron or folate supplementation, though the latter is controversial due to its potential impact on parasite growth (Kariuki & Williams, 2020). Addressing malaria-associated anemia requires integrated malaria control strategies, including vector control, improved access to healthcare, and nutritional interventions, particularly in high-transmission areas.

High parasitemia in symptomatic malaria has been implicated in to decrease in hemoglobin value, thereby resulting in anemia (Shikur *et al.,* 2016)

Symptomatic malaria has been reported to be associated with a regularly reduced hemoglobin value, leading to anemia (Osaro *et al.,* 2019). Previous studies in Nigeria (Tiono *et al.* 2023), Cameroon (Tchinda *et al.,* 2012), and a study in Ethiopia (Sahle *et al.,* 2017) reported low significant levels of hemoglobin that can increase malaria parasitemia developing into complicated malaria. Furthermore, this could also be due to the antiretrovirals taken by HIV-infected individuals, which have been documented to cause anemia in conjunction with the destruction of red blood cells by *Plasmodium* malaria. Anemia of *Plasmodium* malaria may occur during erythrocyte lysis, organ attack, and ingestion, elimination of infected and uninfected erythrocytes, parasite load, and defective development of erythrocytes due to non-functionality of the bone marrow (Rattanapunya *et al.,* 2015).

The lowest CD4 (203 cells/µl) was observed among symptomatic HIV-infected participants, which is consistent with a similar study by Ojurongbe *et al.* (2015) and Tay with his colleagues in Osogbo, Nigeria (Tay *et al.,* 2015). This may be due to the associated decrease in CD4 among those infected with HIV. However, several studies have separated malaria from a low CD4 count observed among HIV-infected individuals (Sahle *et al.*, 2017). Although, it is not quite the same as when malaria was higher in CD4 below 200 cells/µL (Rattanapunya *et al.,* 2015). HIV-malaria co-infection presents a significant public health challenge, particularly in regions where both infections are endemic. The interaction between HIV-induced immunosuppression and malaria infection leads to more severe disease outcomes, increased parasitemia, and higher mortality rates (Alemu *et al.,* 2013). HIV weakens the adaptive and innate immune responses, particularly by depleting CD4+ T cells, which are crucial for controlling *Plasmodium* infections (Omalu *et al.,* 2019). Reduced CD4+ T cell levels impair the ability to mount effective T-helper 1 (Th1) responses, leading to decreased interferon-gamma (IFN-γ) production, which is essential for malaria immunity (Whitworth *et al.,* 2010). HIV-induced monocyte and macrophage dysfunction also compromise the body's ability to clear Plasmodium-infected red blood cells, resulting in higher parasite burdens and severe malaria presentations (Schneidewind *et al.,* 2017). HIV infection is also associated with hyperactivation of B cells, leading to dysfunctional antibody responses, which weaken long-term immunity against malaria (Mphahlele *et al.,* 2016). Furthermore, malaria itself contributes to HIV disease progression by increasing immune activation and systemic inflammation, leading to higher HIV viral loads and accelerated CD4+ T cell depletion (Skinner-Adams *et al.,* 2012). The immunosuppressive effects of co-infection are particularly severe in pregnant women and children, increasing the risk of maternal anemia, low birth weight, and vertical transmission of HIV (Omalu *et al.,* 2019). Effective management requires integrated approaches, including antiretroviral therapy (ART), intermittent preventive treatment for malaria, and vector control measures to mitigate the dual burden of both diseases (Naing *et al.,* 2020).

The mean value of malaria parasite density (MMPD) of 4,078 and 5,742 parasites/µl were significantly higher in symptomatic malaria cases in both HIV-positive and negative individuals, respectively, than their asymptomatic malaria counterparts. There is a direct relationship between CD4 cell count and malaria intensity. This is expected due to a possible impairment in the body's defense mechanism, such as cellular responses regulated by CD4 and thus affecting cellular and humoral immune responses against malaria antigen, which then support the spread of the malaria parasite into the host system (Mohandas & An, 2012; Etusim *et al.,* 2013; Sahle *et al.,* 2017).

CONCLUSION

The study observed higher *falciparum* gametocyte prevalence among the asymptomatic HIV-infected and uninfected individuals than symptomatic malaria counterparts. In addition, the study established asymptomatic individuals as gametocyte carriers, indicating that they are suitable reservoirs of infections. Therefore, prompt diagnosis using highly sensitive malaria diagnostic tools such as PCR to detect low parasitemia and gametocytes is expedient and recommended. Therefore, to reduce the fatality of malaria infection and limit its transmission, accurate and improved diagnostic strategies are essential for treating and managing asymptomatic malaria carriers.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

ETHICAL APPROVAL

The Ethical approval for the study was issued by the ethical committee of the Ladoke Akintola University of Technology Teaching Hospital (LTH/EC/201/11/243) Osogbo and Osun State Ministry of Health Ethical Committee Osogbo (*OSHREC/PRS/569T/41*).

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