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Original Research Article

Current prevalence of *falciparum* malarial infection among HIV patients on highly active antiretroviral therapy in university of Uyo teaching hospital, Uyo Nigeria

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ABSTRACT

Background: Malaria and HIV remain two leading causes of morbidity and mortality to patients in developing African countries. Both infectious diseases have been documented to account for an enormous morbidity and mortality in Sub-Saharan Africa. The geographical overlap in sub-Saharan Africa and South America has led to similarities in co-infection with *Plasmodium* and HIV, this has resulted in the quick progression and severity of both diseases particularly among the poor, and contributes to the poverty of sub-Saharan African nations by taking a toll on young people who contribute greatly to the workforce of the economy. The present study was conducted to determine the prevalence of malarial infection in HIV patients receiving high active antiretroviral therapy in university Uyo teaching hospital, Uyo Nigeria.
Methods: A predesigned structured questionnaire was administered to collect bio data and socio-demographic characteristics from the participants consisting 35 HIV infected adult patients and 32 non HIV infected adults as controls. All HIV patients were receiving HAART during this study. The HAART regimens used by HIV infected patients consist of zidovudine, lamivudine, efavirenze, and nevirapine.
Results: About 5 (14.2%) HIV patients on HAART had *falciparum* malaria. No *falciparum* malaria was detected in HIV negative participants. Of the five positive malaria cases detected in HIV patients, 8.5% were females and 5.7% were males.
Conclusions: There was no significant difference of malaria parasite infection by gender (P = 0.88), age group (P = 0.17), and CD4+ count (O.R:1.0, P = 0.81).

Keywords: Current Prevalence, *falciparum*, HIV Patients, HAART

INTRODUCTION

Malaria and HIV remain two leading causes of morbidity and mortality to patients in developing African countries.[1] Both infectious diseases have been documented to account for an enormous morbidity and mortality in Sub-Saharan Africa.[2] In 2015, there were about 214 million malaria cases that led to 438,000 deaths in Sub-Saharan Africa. Sub-Saharan Africa has the most HIV and AIDS epidemic in the world.[3] In 2013, an

estimated 24.7 million people were living with HIV, accounting for 71% of global total.[3] In the same year, there were an estimated 1.5 million new HIV infections and 1.1 million AIDS related deaths.[4] The geographical overlap in sub-Saharan Africa and South America has led to similarities in co-infection with *Plasmodium* and HIV, this has resulted in the quick progression and severity of both diseases particularly among the poor, and contributes to the poverty of sub-Saharan African nations by taking a toll on young people who contribute greatly to the workforce of the economy.[5] In malaria endemic areas such as Uyo, *Plasmodium falciparum* is the most predominant species and carries a substantial risk of death, causing the most morbidity and mortality.[6]

HIV infection can increase the risk and severity of malaria infection and parasite burden which may facilitate high rate of malaria transmission. HIV individuals living in malaria endemic areas, such as Uyo Nigeria, particularly, are considered semi-immune to malaria and can develop clinical malaria if they are untreated. This has become a significant public health threat as a result of increase risk factors associated with high malaria burden in HIV infected persons with low $CD4^+$ T-cell counts, low immune status, gender, and among others. Low $CD4^+$ T-cells at late stages of HIV infection result to decrease $CD8^+$ T-cell counts and function, thereby causing a severe change in the immune response against other agents of disease including *Plasmodium*.[6] *Parasitaemia* is more common among HIV infected patients, as low $CD4^+$ are linked with higher parasite densities.

Treatment failure of antimalarial drugs in HIV patients is another challenge, especially when there is inability of antimalarial drugs to produce the desired therapeutic effect. Treatment failure is caused by various factors which include drug resistance and drug to drug interaction.[7]

The advent of highly active antiretroviral therapy (HAART) has been narrated to reduce the morbidity and mortality caused by HIV infection. Despite the introduction of HAART, there are reports of malarial infection among HIV infected patients on HAART and artemisinin-based combination therapy (ACT).

In a previous study in Uganda, a prevalence of 5% asymptomatic malaria was reported among HIV infected patients receiving HAART, while in Benin City Nigeria, it was reported that HIV infected patients on HAART and ACT had a prevalence of 2.1% and 9.8% respectively.[8,9] Detailed accurate information is lacking on the co-infections of malaria and HIV infected patients receiving HAART in Uyo, Nigeria.

This study was aimed to evaluate the prevalence of malaria infection in HIV patients who were receiving HAART in University of Uyo Teaching Hospital, Uyo-Nigeria.

METHODS

Study area

The University of Uyo Teaching Hospital is a tertiary hospital located in Uyo, the capital of Akwa Ibom State, Nigeria. It offers general medical treatment and surgical operation to HIV infected and non infected patients in Uyo and other local government Areas in Akwa Ibom State. The Latitude of Uyo, Nigeria is 5.038963, and longitude is 7.909470 with GPS coordinates of 5º 2’ 0” N and 7º 55’ 0’’ E and elevation of 65m, 213 feet above sea level. The dry season spans from mid-October to March whereas the rainy season lasts between April and September.

Study population

A total of 67 participants were involved in the study. This consisted of 35 HIV infected adult patients and 32 non HIV infected adults as controls.

All HIV patients were receiving HAART during this study. The HAART regimens used by HIV infected patients consist of zidovudine, lamivudine, efavirenze, and nevirapine. HAART naive HIV patients and those with AIDS defining conditions were excluded from the study.

Study design

This was a case control hospital based study consisting of HIV infected adult patients, and non HIV infected adults attending the University of Uyo Teaching Hospital for treatment.

Administration of questionnaires

A predesigned structured questionnaire was administered to collect bio data and socio-demographic characteristics from the participants.

Collection of samples

A sterile multi-sample needle was used to draw 5ml of intravenous blood sample into an EDTA vacutainer glass tubes.

HIV test

A plastic pipette was used to add 1 drop of whole blood to the sample well of Uni-gold cassette kit (Trinity Biotech, Ireland). Three drops of diluents was added into the sample well. In 20 minutes, result was read.[10]

$CD4^+$ count test

$CD4^+$ T-lymphocyte cell count was analyzed using flow cytometry (University of Uyo, Nigeria).[11]

Microscopic analysis

A Plastic pipette was used to add 3 drops of blood sample on a labelled clean glass slide. Another clean glass slide was used to make a thick and thin blood film sample at a spot on the slide glass. After 10 minutes, the dried thin films were stained with 1-2 drops of methanol to affix the blood samples and then 10% of Giemsa stain, leaving the filmed glass slide to dry for 10 minutes. Distilled water was then poured to wash, drain and air dry for microscopy examination.[12]

DNA molecule extraction

Four hundred (400µl) microlitre pipettes was used to add Genomic lysis buffer to 100µl of blood sample, mixed completely by a vortex mixer for 4-6 seconds, thereafter allowed to stand for 5-10 minutes at room temperature. The mixture was then transferred to a Zymo-spin™ column in a collection tube. Two hundred (200µl) microlitres of DNA pre-wash buffer was added to the spin column. This was centrifuged at 10,000x for one minute. The spin column was transferred to a clean micro-centrifuge tube and less than five hundred (<500µl). Elution Buffer was added to the spin column and incubated for 2-5 minutes at room temperature.[13]

Molecular screening for falciparum malarial infection

After DNA molecule extraction, further molecular screening of samples for *P. falciparum* was done to confirm the microscopy test using Polymerase Chain Reaction (PCR). A Nested PCR approach was used for the molecular screening of extracted DNA samples for *Plasmodium falciparum* detection.

Primary stage of nested PCR

Sixty-seven (67) PCR tubes were labelled. These were placed in an Ice rack. A calibrated pipette of 1.0µl was used to draw up a master mix and transferred to a 1.5µl Eppendorf tube. This was followed by adding 0.4µl primers-(Forward IMDR1/F: GTCAAACGTGCATTTTTATTAATGACCATTTA and reverse R IMDRI/R: AAAGATGGTAACCTCTCAGTATCAAAGAGAG), $Mgcl_2$ 0.5µl, 5mM DNA template and a high molecular grade water of 3.29µl to dilute the other reagent in their correct concentrations. The primary nested PCR final volume (20µl) was taken from PCR cocktail and then transferred into the labelled PCR tubes for amplification by a Bio system PCR machine.

Secondary stage of nested PCR

All 67 PCR tubes were labelled like that of the Primary stage of Nested PCR and placed in an Ice rack. A calibrated pipette of 12.5 was used to draw up a master mix and transferred to a 1.5µl Eppendorf tube. This was followed by adding 0.2µl primers (Forward IMDR1/F: GTCAAACGTGCATTTTTATTAATGACCATTTA and reverse R IMDRI/R: AAAGATGGTAACCTCTCAGTATCAAAGAGAG), 0.5µl $MgCl_2$, 0.5µl DNA template, and an 11.1µl. High molecular grade water to dilute the other reagent in their correct concentrations. The primary and secondary nested PCR final volume (25µl) was taken from PCR cocktail and then transferred into the labelled PCR tubes for amplification by a Bio system PCR machine. The secondary PCR process was carried out by using the amplicons from the primary nested PCR to carry out the secondary nested PCR process to ascertain the desired *Plasmodium falciparum* gene. Agar gel Electrophoresis was then carried out to separate the DNA gene molecule (*Plasmodium falciparum* infective gene) of interest.

Data analysis

Proportions between two variables were compared by evaluating P values, differential values, odds ratios, and their corresponding 95% confidence intervals (CIs). Statistical significance was determined using the Pearson's Chi-square test.

RESULTS

Of the 67 subjects recruited in this study, 38 (38%) were men, whilest 29 (29%) were women. Their ages ranged from 20-56 years with a mean age of 34.84±9.86 standard deviation (SD). The mean age of men was 35.16±10.69SD, while women had a mean age of 34.41±8.81SD. The highest occurring age among the subjects was 29 years. The age range of 30-39 years had the highest subjects recruited into the study, with a total of 25 (25%). More than half of the participants in the study were married, 35%; widowed, 3% while 29% were single. All the subjects had formal education. Post-secondary education was seen to be the highest education amongst participants (29%) while secondary and primary education was 23% and 15% respectively. Unskilled jobs were highest among the study participants (43%), while skilled and professional jobs were 9% and 15% respectively (Table 1).

Table 1: Socio-demographic characteristics of participants (n=67).

Age in years	Males (%) 38	Female (%) 29	Total (%) 67
20-29	15	8	23
30-39	11	14	25
40-49	9	6	15
50 and above	3	1	4
Marital Status			
Single	20	15	35
Married	17	12	29
Widowed	1	2	3
Non skilled	18	25	43
Skilled	9	6	15
Professional	6	3	9

Prevalence of falciparum malaria infection according to HIV status and gender

Five HIV patients on HAART had *falciparum* malaria. No *falciparum* malaria was detected in HIV negative participants. A prevalent rate of malarial infection was determined in HIV positive patients (Table 2).

Table 2: Prevalence of *falciparum* malarial infection according to HIV status (n= 67).

HIV status	No. of samples	No. positive cases (%)	χ^2	P-value
HIV positive				
Male	13	2 (5.38)	0.02	0.88
Female	22	3 (4.7)		
Total	35	5 (14.2)		
HIV negative (Control)				
Male	25	0 (0)		
Female	7	0 (0)		
Total	32	0 (0)		

The risk factors associated with HIV patients

Age group of 30-39 had the highest number of malaria positive and negative cases. $CD4^+$ counts 81 and 105µl were of 2 +ve cases of *falciparum* malaria respectively, while 424, 450, 488µl were of 3 +ve of *falciparum* malaria. There was no significant difference between risk factors and *falciparum* malarial infection in HIV patients (Table 3).

Prevalence of falciparum malarial infection according to highly active antiretroviral therapy

Patients who were administered with nevirapine had a case of malarial infection 4.37%, patients on lamivudine had 0.14% of malarial infection, while patients on efavirenz/zidovudine had 5.3%.

The prevalence of malarial infection among HIV patient son HAART differed significantly ($P < 0.01$) (Table 4).

Table 3: Risk factors to *falciparum* malarial infection in HIV patients (n = 35).

Variables	No. of samples 35	No. of Malaria positive cases 5 (%)	No. of Malaria negative cases 30 (%)	χ^2	df	P-value	Odd ratio	95% C.I.
$CD4^+$ counts (cells/µl)								
<200	9	2 (7.7)	7 (29.6)	0.056	1	0.81	1	0.43-30
≥200	26	3(4.0)	23 (30.2)				0.46	
Age in years								
20-29	9	0 (0)	9 (35.0)	6.13	3	0.17	13.11	0.35-27.5
30-39	17	3 (6.1)	14 (28.0)					
40-49	2	2 (14.0)	3 (21.0)					
50 and above	0	0 (0)	4 (35.0)					

Table 4: Prevalence of *falciparum* malarial infection according to highly active antiretroviral therapy (n=35).

HARRT received	No. tested for *falciparum* malarial infection	No. positive cases in %	χ^2	P-value
Nevirapine	8	1 (4.37)	37.0	0.01
Lamivudine	14	2 (0.14)		
Efavirenz/Zidovudine	13	2 (5.3)		

Molecular screening of falciparummalarial infection in HIV patients

Lanes 11, 21, 23, 24, 30 show visible bands of *Plasmodium falciparum* genes in HIV positive patients. Dd_2 and $3D_7$ are the Mutant and Wild type controls, respectively (Figure 1).

Molecular screening of falciparum malarial infection in HIV negative participants (control)

Non presence of *falciparum* malarial infection was observed in all agarose gel wells. Lane M represents the quick- load 100bp molecular ladder while Dd_2 and $3d_7$ is the mutant and Wild type controls respectively (Figure 2).

Prevalence of falciparum malarial infection among HIV patients according to gender (n=35)

Of the 67 subjects recruited in the study, 38 were of males while 29 were females, only 5(7.0%) HIV positive patients had *parasitaemia*.

High prevalence of *parasitaemia* was seen in male subjects who had a prevalence rate of 10.3% (Figure 3).

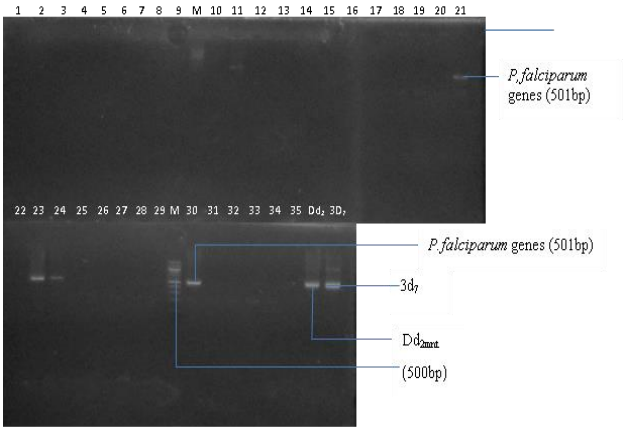


Figure 1: Molecular screening of *falciparum* malarial infection in HIV positive patients.

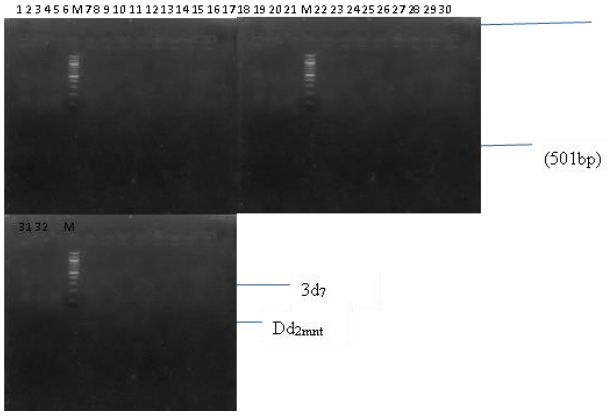
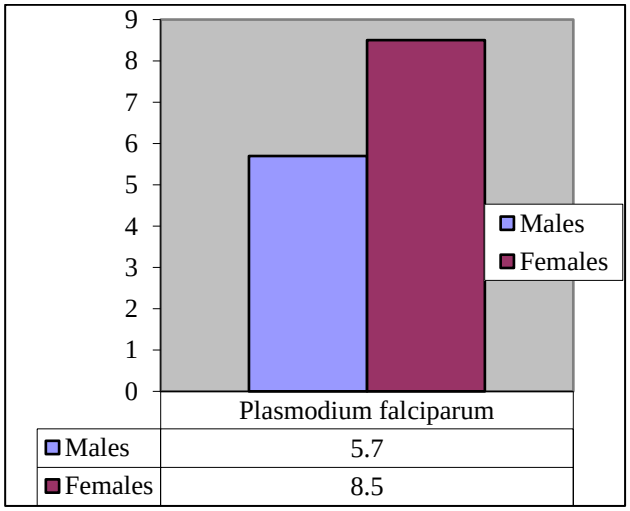


Fig 2: Molecular screening of falciparum malaria infection in HIV negative patients (controls).



χ^2 = 37, P-value = 0.01 *P<0.05

Figure 3: Prevalence of *falciparum* malarial infection among HIV Patients according to gender.

DISCUSSION

Malaria continues to be a public health challenge to HIV patients in developing African countries. HIV individuals living in endemic areas are at increased risk to malaria infection, its clinical course, and additionally impairs antibody response to malaria infective antigens.

In this study, 35 HIV adult patients and 32 HIV negative adults were screened for *falciparum* malarial infection, five positive cases of *falciparum* malaria was detected in HIV positive adult patients, while 32 non HIV adult participants had no *falciparum* malaria. Upon microscopic examination, 2 (5.7%) of *falciparum* malarial infection was detected, while Nested PCR Assay detected 3 (8.7%) *falciparum* malarial infection in the DNA sample extracts of HIV patient, a prevalence rate of 14.2% was evaluated in this study. The prevalence of *falciparum* malaria did not differ significantly between the HIV patients and the non HIV participants (P = 0.88)

Of the five positive malaria cases detected in HIV patients, 8.5% were females and 5.7% were males. This apparently showed that females who received HAART were mostly affected by *falciparum* malaria. The high prevalence of *falciparum* malaria in females could be as a result of malaria transmission determined in large part by social, economic and cultural factors, which intersect with sex-specific and gender-specific vulnerabilities to impact women's ability to prevent malaria infection. Women's traditional household roles, such as cooking the evening meal outdoors or waking up before sunrise to prepare the household for the day, may also put them at greater risk of malaria infection.[14] There was a significant difference between gender and *falciparum* malaria (P=0.01).

Investigation to risk factors of malaria parasite in HIV patients in this study showed that there was no significant difference in relationship between the level of $CD4^+$ count, age group and malaria parasite (P=0.81, 0.17). Of the subjects in this study, 31% adhered to the usage of long lasting insecticide treated nets. The non-utilization of long-lasting insecticide treated bed net by four HIV positive adult patients is also a reason for the transmission of *falciparum* malaria. These four subjects did not sleep under insecticide treated nets, and complained that they were not comfortable with bed nets because they felt it caused body heat thus affecting their sleep at night. Although one subject was comfortable with sleeping under long lasting insecticide treated net, the subject still had *falciparum* malaria. The possible reason to this may be that this subject stayed out late at nights most times.

A prevalence rate of 14.2% was determined in HIV patients; HIV patients in this study area followed the HAART regimen (lamivudine, zidovudine, nevirapine and efavirenze) daily. This was higher than a previous study by Akinbo et al, who reported a prevalence rate of 7.5% in Benin City Nigeria and 4% reported in Uganda.[6] Although Akinbo et al, stated that the possible reason for the prevalent rate in his study was that some drugs in the

HAART regimen have anti-*Plasmodium* activity in his report, nevirapine and lamivudine were stated to inhibit the growth of *Plasmodium* in *vitro*.[6,15,16] Nevirapine and lamivudine were among the HAART regimens given to the HIV patients. This was dissimilar to our study; the possible reason to high prevalent rate in this study may be that drugs in HAART regimen interfered with the action of ACT drugs against *falciparum* malaria. Studies have shown artesunate and artemether are metabolized to dihydroatemisinin by Cytochrome P3A4 (CP3A4), this may decrease antimalarial concentration and its efficacy, especially when antiretroviral drugs like nivrapine, lamivudine and efavirenze are taken daily by HIV patients. This can be seen as a treatment failure of ACT dugs, due to interference of anti-retroviral drugs.[16] Thus, in this study there was a significant difference between HAART and *falciparum* malaria as in infection in HIV patients (P=0.01).

CD4+ T-cell counts are used as a measure of immunity and HIV disease progression, and counts <200cells/µL increase the risk of opportunistic infections.

Although investigation in this study showed that majority of HIV patients on HAART had CD4+ T-cell counts >200cells/µL, three of these healthy HIV patients had *Plasmodium falciparum* infection. CD4+ T-cell counts <200cells/µL HIV patients were at a risk of acquiring malarial infection.

This indicates that effective HAART drugs should be administered to those HIV patients who are still yet to recover from low immunity to *falciparum* malarial infection.

CONCLUSION

This study revealed the prevalence rate of 14.2% *falciparum* malarial infection in HIV patients. This was high compared to previous studies in Benin City, Nigeria and Uganda. Gender affected the prevalence of *falciparum* malarial infection in HIV patients. Although majority of HIV patients had healthy $CD4^+$ T-cell counts, some patients who had $CD4^+$ T-cell counts <200 cells/µL were infected with *falciparum malarial* infection. This indicated that some HAART did not prevent *falciparum* malarial infection. An effective HAART should be administered to HIV patients for prevention malarial infection.

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Ethical approval: The study was approved by the ethical Review Board of the University of Uyo Teaching Hospital, Uyo

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