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Epidemiological Studies of Malaria Parasite on HIV Patients Attending General Hospital Awo-Omamma, Oru East, Imo State

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Abstract.

HIV and malaria are the two most prevalent and deadly diseases in the world. Malaria and HIV accounted for about 255 million cases in 2017, with malaria having 86% of this distribution and HIV having 14% of the distribution. Given the overlap of their geographic distribution and resultant rates of coinfection, interactions between the two diseases pose major public health problems. This study was aimed at investigating the epidemiology of malaria – HIV co-infection in respect to sex and age and its association with CD4+ count and viral load. 230 HIV sero-positive participants and 100 HIV sero-negative participants (control) were employed for this study. 52 (22.6%) of the HIV infected participants tested positive for malaria while only 9(9.0%) of the non-HIV participants tested positive to malaria. The prevalence of malarial infection in HIV positive individuals was shown to be higher in females (23.9%) compared to the prevalence for males (18.5%). In respect to age, the age group of 30-39 showed the highest prevalence (35.3%) of co-infection. A high prevalence of 47.7% was recorded in participants with CD4+ below 200 cells/µl compared to the prevalence of 7.6% in participants with CD4+ greater than 200 cells/µl. A high prevalence (49.2%) was also detected in patients with viral load of above 10,000 copies/µl compared to that of those with viral load less than 10,000 copies/µl (12.6%). The findings in this work show a high prevalence of malaria in HIV patients in Awo-Omamma, Oru East, Imo state. This should be considered a great concern to public health. Thus, more effort should be put in research to curb this health issue.

Keywords: Malaria, HIV; Malaria parasites; CD4+, Viral load, Antiretroviral therapy.

Introduction

HIV and malaria are the two most prevalent and deadly diseases in the world. Malaria and HIV accounted for about 255 million cases in 2017, with malaria having 86% of this distribution and

HIV having 14% of the distribution. Given the overlap of their geographic distribution and resultant rates of coinfection, interactions between the two diseases pose major public health problems. Together they accounted for about 2 million deaths in 2017 (WHO Malaria Report, 2018; UNAIDS, 2018), and millions more are adversely affected each year. Malaria and HIV/AIDS are both diseases of poverty and contribute to poverty by affecting young people who would otherwise enter the workforce and contribute to the local economy (Dawaki*et al*., 2016; Njoku-Obi *et al*.,2016).

Malaria is caused by the protozoan parasite *Plasmodium* and is transmitted by Anopheles mosquitoes. It is endemic in most tropical and subtropical regions of the world (WHO 2019). Malaria is most endemic in sub-Saharan Africa. In 2017, 5 countries accounted for nearly half of all malaria cases worldwide: Nigeria (25%), the Democratic Republic of the Congo (11%), Mozambique (5%), India (4%) and Uganda (4%) (WHO, 2019).

Human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS) is a spectrum of conditions caused by infection with the human immunodeficiency virus (HIV). Areas of the world with high rates of malaria also carry a heavy burden of HIV. There are 36.9 million people living with HIV worldwide, with 25.8 million in sub-Saharan Africa alone (UNAIDS, 2018).

Generally, Malaria – HIV Co-infection prevalence is 0.7%–72% in Sub Saharan Africa: 0.7%–47.5% in nonpregnant adults, 1.2%–27.8% in children, and 0.94%–37% in pregnant women. (Kwenti, 2019). A meta-analysis performed by Naing *et al*. (2016) revealed overall pooled prevalence of Malaria – HIV Co-infection in Sub Saharan Africa was 19%, 26% in adults, 12% in pregnant women, and 9% in children. A study by Jegede*et al.,* (2017) in Kano, Nigeria revealed a prevalence of 27.7% of Malaria – HIV Co-infection. Another study in Mozambique revealed a prevalence of 33% (232 out of the 701 patients enrolled) (Francesco *et al.,* 2018). In areas with stable malaria transmission, HIV increases the risk of malaria infection and clinical malaria in adults, especially in those with advanced immunosuppression. In settings with unstable malaria transmission, HIV-infected adults are at increased risk of complicated and severe malaria and death.

This study was therefore conducted to determine the epidemiology of malaria infection among HIV-infected individuals in Awo-omamma, Oru east, Imo State.

METHODS

Study Area

Awo-Omamma, in the Northeast of Niger Delta is an oil-rich town on the banks of Njaba River in Nigeria's South East Imo State. Situated in Oru-East, Awo-omamma is some 25 kilometers from Owerri and 62.5 kilometers from Onitsha. It lies in tropical rain forest, with hot and rainy seasons.

Awo-Omamma is bounded in the North by Amiri, Imo State in Oru-East, and Mgbidi and Otulu Nigeria both in Oru-West. In the East it shares boundaries with Okwudor in Njaba LGA. In the West Awo-omamma is bounded by Akabo, Oguta LGA, Awa, Oguta LGA, Abiaziem and Ngbele communities in Oguta LGA, and in the South by EziamaObiato and Njaba River.

Study Design

This is a cross-sectional study involving 230 diagnosed HIV-infected adults and 100 HIV negative controls.

Data Collection

We used a detailed anonymous pre-tested questionnaire to capture participants' basic characteristics and clinical information with the help of trained healthcare workers. This information included: clients' code, age, sex and viral load count.

Detection of Malaria parasites using the Thick film:

Blood sample is extracted from a patient into the EDTA bottle. A thick film was made on the clean grease free slide, then allowed to air dry. The dried film was covered with a well diluted Giemsa stain for 30 minutes and then washed off with clean water. The back of the slide was cleaned and allowed to air dry before the addition of oil immersion. The slide was examined systematically using a microscope with x100 objective.

Cells containing late stage of trophozoites of *P. falciparum* often include irregular reel mauve dots known as maurer dots. Some other components including blue cytoplasm, purple chromatin and black or brown pigment granules may also be seen.

Detection of antibodies to HIV-1 and HIV-2 in human serum:

The procedure as described by the manufacturer was used for the analysis. Briefly, 50 µl of participants' plasma samples separated from corresponding whole blood samples in EDTA was applied to appropriately labelled sample pad. After 15 min of sample application, the result is read. This method has inherent quality control that validates the results. Two visible red colours in the region labelled control and patient represents HIV seropositive reaction while a single red colour in the region labelled control represents HIV seronegative reaction.

CD4 Estimation—PartecCyflow Technique

We put 20µl of CD4+- PE monoclonal antibody in labelled Partec (Rohren) tubes and added 20µl of well mixed EDTA blood. This content was mixed together several times for 2 minutes and incubated in the dark for 15 minutes at room temperature with intermittent mixing every 5 minutes. After incubation, 800µl of CD4 diluting buffer was added to each preparation, mixed properly before analyzed on the Cyflow counter as describe by equipment manufacturer (CyflowPartec Manual, 2010).

Ethical Clearance

Approval to carry out the research was obtained from the medical director of the General Hospital Awo-omamma, the participants who were volunteers and from the department of Microbiology and Industrial Microbiology, Imo State University, Owerri.

RESULTS

Socio-Demographic Profile of Study Respondents

A total of 330 participants were employed for this study. A total of 99 males and 231 females. Most of the participants were between the ages of 40 – 49 years while the least of the participants were about 20 – 29 years of age. HIV Confirmation test confirmed that 230 of the respondents were HIV sero-positive while 100 were HIV sero-negative. 23.5% (54) of the HIV sero-positive patients were males while 76.5% (176) of the patients were females. Most of the HIV sero-positive patients were between 40 – 49 years of age (45.2%) while the least were in the age range of 20 – 29 years. The 100 negative patients were presented as the control subjects. 45% of the control subjects were males while 55% were females. Most of the control subjects were less than 20 years of age while the least were between 20 – 29 years of age. More details are shown in **Table 1**.

Table 1: Demographic Characteristics of Study Population

Socio-Demographic Parameters	Respondents (%)		
	HIV Positive (N = 230)	HIV Negative (N = 100)	Total (N = 330)
Sex			
Male	54 (23.5)	45 (45.0)	99 (30)
Female	176 (76.5)	55 (55.0)	231 (70)
Age			
<20	12 (5.2)	27 (27.0)	39 (11.8)
20 – 29	7 (3.0)	15 (15.0)	22 (6.7)
30 – 39	68 (29.6)	16 (16.0)	84 (25.4)
40 – 49	104 (45.2)	24 (24.0)	128 (38.8)
50 and Above	39 (17.0)	18 (18.0)	57 (17.3)

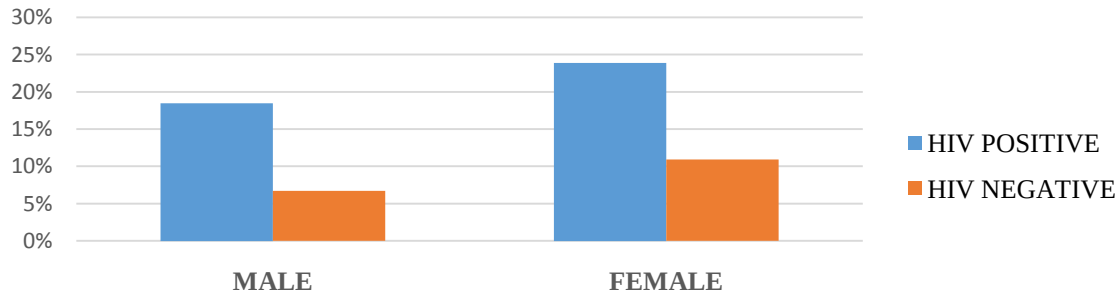
Sex Related Malaria Prevalence

Of the 230 HIV sero-positive patients examined, a total of 52 tested positive to malaria parasite infection with an overall prevalence of 22.6%. 18.5% (10) of the 54 male HIV patients were infected with malaria while 23.9% (42) of the 176 female HIV patients tested positive to malaria. The control group (100) had a total malaria prevalence of 9.0%. Of the 45 male control subjects, 3 (6.7%) tested positive to malaria infection while 6 (10.9%) tested positive to malaria in the female control group. Comparison of the prevalence rates in the HIV sero-positive group and in the control group shows significant differences in both groups with that of the HIV group being significantly higher. This result also shows that females are more predisposed to malaria infection. This is explained further in **Table 2** and **Figure 1**.

Table 2: Prevalence of Malaria Parasite Infection based on Sex

SEX	HIV POSITIVE		HIV NEGATIVE	
	Number examined (N = 230)	Number Infected (N = 52)	Number examined (N = 230)	Number Infected (N = 52)
Male	54 (23.5%)	10 (18.5%)	45 (45.0%)	3(6.7%)
Female	176 (76.5%)	42 (23.9%)	55 (55.0%)	6 (9.1%)
Total	230 (100.0%)	52 (22.6%)	100 (100.0%)	9 (9.0%)

Figure 1: Prevalence of Malaria Parasite Infection based on Sex



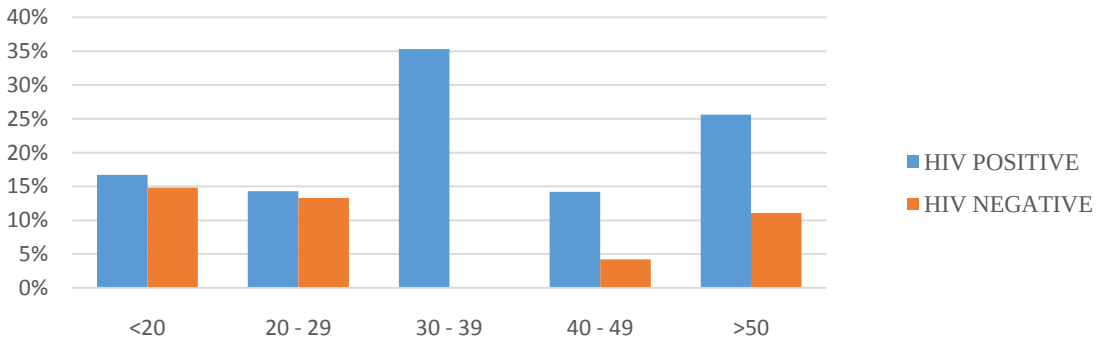
Age Related Malaria Prevalence

The age related prevalence among the HIV sero-positive showed that malaria and HIV co-infection was most prevalent in patients between 30 – 39 years of age (35.3%), followed by those above 50 years (25.6%) and the patients below 20 years. The least prevalence of the co-infection was in patients between 20 – 29 years and patients between 40 – 49 years with prevalence rates of 14.3% and 14.2% respectively. In the control group, prevalence of malaria infection based on age was observed in this order from highest to lowest: less than 20 years (14.8%), 20 – 29 years (13.3%), above 50 years (11.1%), 40 – 49 years (4.2%) and 30 – 39 years (0.0%). This result reveals that HIV patients between the ages of 30 – 39 are at most risk of malaria infection while HIV patients between 40 – 49 years have the least risk of malaria infection. Comparison of the prevalence rates in the HIV sero-positive group and in the control group shows significant differences in both groups with that of the HIV group being significantly higher. This age related prevalence of malaria infection is detailed in **Table 3** and **Figure 2**.

Table 3: Prevalence of Malaria Parasite Infection based on Age

	HIV POSITIVE		HIV NEGATIVE	
AGE	**Number examined (N = 230)**	**Number Infected (N = 52)**	**Number examined (N = 230)**	**Number Infected (N = 52)**
<20	12 (5.2%)	2 (16.7%)	27 (27.0%)	4 (14.8%)
20 – 29	7 (3.0%)	1 (14.3%)	15 (15.0%)	2 (13.3%)
30 – 39	68 (29.6%)	24 (35.3%)	16 (16.0%)	0 (0.0%)
40 – 49	104 (45.2%)	15 (14.2%)	24 (24.0%)	1 (4.2%)
50 and Above	39 (17.0%)	10 (25.6%)	18 (18.0%)	2 (11.1%)
Total	230 (100.0%)	52 (22.6%)	100 (100%)	9 (9.0%)

Figure 2: Prevalence of Malaria Parasite Infection based on Age



Effect of Malaria Infection on $CD4^+$ Level

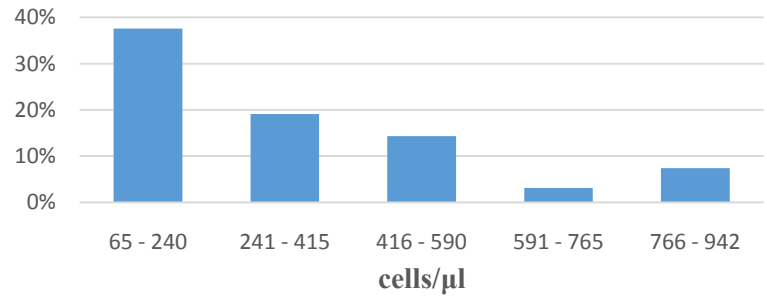
Average CD4 count range: 500 – 1500 cells/µl

Table 4 below evaluates the effect of CD4 level on malaria infection in HIV patients by comparing the prevalence rates between the different groups stratified by CD4 count range. All HIV patients had CD4 cell count ranging from 65 cells/µl to 942 cells/µlHIV patients with CD4 count between 65 – 240 cells/µl had the highest malaria prevalence (37.6%). This is followed by patients with CD4 count between 241 – 415 cells/µl (19.1%). Patients with CD4 count between 416 – 590 cells/µl and 766 – 942 cells/µl had prevalence rates of 14.3% and 7.4% respectively. The lowest prevalence was observed in patients with CD4 count between 591 – 765 cells/µl (3.1%). This table reveals that risk of malaria co-infection in HIV patients decrease with increase in CD4 count. The only exception being in the patients with CD4 level between 766 – 942 cells/µl where they had a higher malaria prevalence than those with CD4 count between 591 – 765 cells/µl. This could be attributed to the fact that some of the patients with a high CD4 also had high viral load counts which is also a predisposing factor to malaria infection. More details in **Table 4** and **Figure 3**.

Table 4: Prevalence of Malaria Infection in HIV Patients based on CD4 count

CD4 CELL COUNT	NO. TESTED (%)	NO. POSITIVE (%)
65 - 240 cells/µl	101 (43.9)	38 (37.6)
241 – 415 cells/µl	21 (9.1)	4 (19.1)
416 – 590 cells/µl	49 (21.3)	7 (14.3)
591 – 765 cells/µl	32 (13.9)	1 (3.1)
766 – 942 cells/µl	27 (11.8)	2 (7.4)
Total	230 (100)	52 (22.6)

Figure 3: Prevalence of Malaria Infection in HIV Patients based on CD4 count



Effect of Malaria Infection on Viral Load

Viral load above 100,000 copies/µl is described as high/risky viral load while viral load below 10,000 copies/µl is described as low/safe viral load.

The table below evaluates the effect of viral load on malaria infection in HIV patients by comparing the prevalence rates between the different groups stratified by viral load count range. All HIV patients had viral load count ranging from 001 copies/µl to 314510 copies/µl. HIV patients with viral load count between 100,00 – 200,000 copies/µl had the highest malaria prevalence (54.5%). This is followed by patients with viral load count above 200,000 copies/µl (51.7%). Patients with viral load count between 10,001 – 100,000 copies/µl and 1,001 – 10,000 copies/µl had prevalence rates of 25.8% and 11.7% respectively. The lowest prevalence was observed in patients with viral load less than 1000 copies/µl (10.8%). This table reveals that risk of malaria co-infection in HIV patients increase with increase in viral load count. Exceptions may be attributed to the fact that some of the patients with a high viral load count also had high CD4 counts which is also a predisposing factor to malaria infection.

Table 5: Prevalence of Malaria Infection in HIV Patients Based on Viral Load Count

VIRAL LOAD	NO. TESTED	NO. POSITIVE (%)
<1,000 copies/µl	37 (16.1)	4 (10.8)
1,001 – 10,000 copies/µl	111 (48.2)	13 (11.7)
10,001 – 100,000 copies/µl	31 (13.5)	8 (25.8)
100,000 – 200,000 copies/µl	22 (9.6)	12 (54.5)
>200,000 copies/µl	29 (12.6)	15 (51.7%)
Total	230 (100)	52 (22.6)

Figure 4: Prevalence of Malaria Infection in HIV Patients based on Viral Load

DISCUSSION

The prevalence of malaria amongst the HIV sero-negative (control) subjects was 9.0%. This prevalence was much lower compared to the prevalence of 22.6% in HIV sero-positive subjects. This suggests that HIV infection exposes patients to a higher risk of malaria parasitaemia infection.

Amongst the HIV sero-positive subjects, the prevalence rate of malaria as a co-infection was 22.6%. This is in line with a study by Gumel*et al.*, (2019) in Zaria, Kaduna which reported a prevalence rate of 22.9%. This prevalence is higher compared to the prevalence of 18.5 % for malaria-HIV co-infection reported in the general population of Nigeria (Ojurongbe*et al.*, 2014). Contrastingly, this prevalence is lower compared to the 29.2% reported in Abuja (Mustapha *et al.*, 2017). These disparities could be attributed to the different climatic conditions in the three geopolitical zones. Swampy regions could encourage the growth of mosquitoes in an area and therefore increase malaria transmission in that area.

Furthermore, the prevalence observed in this study is higher compared to similar studies performed in other countries; 11.75 % in Ghana (Tay *et al.*, 2015), 7.3 % in Cameroon (Njunda*et al.*, 2016) and 20.33% in DR Congo (Kamanyanu*et al.*, 2015). The difference between the prevalence observed in these studies and ours could be due to the geographical differences in the study populations and the differences in the level of malaria endemicity. Malaria is highly endemic in Nigeria as Nigeria suffers the highest burden of malaria in the world (Dawaki*et al.*, 2016).

The females were observed to have a higher prevalence of malaria (23.9%) than the males (18.5%). This corresponds with similar studies which show a higher prevalence rate in the female population (Gumel*et al.*, 2019). This may be as a result of cooking at late evenings which are common in rural areas and as such they were exposed to more mosquito bites resulting in the high transmission. Some studies however, contradict this observation by showing a higher malaria – HIV co-infection in the male population (Akinbo*et al.*, 2016; Iroezindu*et al.*, 2012).

Also, among the various age groups it was revealed that those between the ages of 30 – 39 had more of malaria parasitic infection than the other age groups. This agrees with earlier report by Dada (2015) which showed a high prevalence (68.2%) among this age group.
CD4 cell count less than 200 cells/µl (signifying the terminal stage of HIV infection (AIDS) was seen in 37.4% patients. Majority of the patients (78.9%) with malaria infection in this study had

CD4 cells count less than 200 cells/µl. This can be attributed to the well establishment that CD4+ T lymphocyte cells <200 cells/µl is associated with a higher risk of malaria parasitaemia (Akinbo*et al.,* 2016; Tay *et al.,* 2015).

This study revealed that 49.2% of HIV patients with viral load >10,000 copies/µl had malaria co-infection. This is much higher compared to the prevalence of the co-infection in patients with viral load <10,000 copies/µl (21.6%). A similar study by Kakisingi*et al.,* (2016) also revealed that patients with viral load <10,000 copies/µl had a higher prevalence (46.4%) of malaria co-infection. It is well established that malaria leads to an increase in HIV viral load and hence increases the probability of transmission of HIV thereby increasing HIV prevalence (Franke *et al.,* 2010).

CONCLUSION

In conclusion, the findings of this study indicates that the prevalence of malaria co-infection among HIV patients in Awo-Omamma, Imo State is high and of public health concern. It also suggests that age and sex are risk factors for HIV-malaria co-infection. The study also revealed that low CD4+ count and high viral load is associated with malarial infection. This indicates that low CD4+ count increases the risk of malaria transmission and that malaria infection increases the viral load in HIV patients.

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