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Prevalence of *Plasmodium falciparum* Infection among HIV Patients Attending Selected Hospitals in Niger State, Nigeria

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Abstract
Malaria and Acquired Immune Deficiency Syndrome (AIDS) are among the world's greatest health problems and are concentrated in the tropical and sub-tropical regions of the world. Together, they are the cause of death of millions of people each year, hence they become a burden and a threat for development in India, Southern Asia, South America and Africa including Nigeria. The prevalence of malaria among HIV patients attending selected hospitals in Niger State, Nigeria was determined. A total of 300 HIV patients within the age range of 2-69years were enrolled, of which 217 were females and 83 were males. Four (4) mL of venous blood was drawn from each participant. The blood samples were examined for the presence of Plasmodium falciparum using thin and thick films while the CD_4count was determined using flow cytometer (Partec Cyflow). Plasmodium falciparum was detected in 43 out of 300 participants with a prevalence of 14.3%.The prevalence was observed to be higher in males (18.07%) than in females (12.90%) and in the age group 1-10years (28.57%). Statistically, there was no association between Plasmodium falciparum infection with sex (p = 0.223) or age (p =0.253). The CD_4 counts of all the participants ranges between 28-2000 cells/µL with a mean of 1111.40 ± 739.589. Patients with Plasmodium falciparum infection had lower CD_4 counts than those that were negative. Conclusively, HIV individuals with severe immunosuppression (CD_4 counts <200 cells/µL) are at higher risk of Plasmodium falciparum infection. Hence, HIV individuals should take all necessary measures to prevent the infection to avoid the negative impact.
Keywords: Plasmodium falciparum, anaemia, HIV, malaria, CD_4, Niger State.

INTRODUCTION

Malaria is a parasitic disease caused by five *Plasmodium* species in humans namely *Plasmodium falciparum* (*P. falciparum*), *P. ovale*, *P. malariae*, *P. vivax* and *P. knowlesi*. *Plasmodium falciparum* is the most virulent, predominant species and one of the leading causes of morbidity and mortality in the developing countries. It is estimated that 50% of the world's population are at varying degrees of risk of the *plasmodium falciparum* infection (Adeoti *et al.*, 2015).Acquired Immune Deficiency Syndrome is caused by Human Immunodeficiency Virus (HIV). This virus is also an intracellular pathogen which mostly attacks immune cells of the body such as Clusters of Differentiation at position four (CD_4) cells and macrophages. It depletes these cells thereby putting the patients at risk of opportunistic infections and malignancy (Berg *etal.*, 2014; WHO, 2020).

These two deadly diseases, Malaria and AIDS, are among the world's greatest health problems and are concentrated in the tropical and sub-tropical regions of the world (WHO, 2017). In 2018, World Health Organization (WHO) approximated the clinical cases of malaria to 228 million with deaths of 405,000 individuals worldwide. About 90% of these cases occur in sub Saharan Africa (SSA) (WHO, 2019). The same year, 37.9 million cases of HIV, 770,000 deaths and 1.7 million new cases were recorded worldwide in which sub Saharan Africa and Nigeria accounts for 61% and 13% of the cases respectively (UNAIDS, 2018).

The co-infection of *Plasmodium falciparum* and HIV may result in quick progression and severity of both diseases in developing nations due to overlap in the geographical distribution. Malaria in HIV patients leads to an increase in viral load and decline in CD_4/CD_8 counts and function thereby causing severe changes in the immune system (Dada, 2015), resulting to faster disease progression from HIV to AIDS (Kwenti, 2018). HIV, on the other hand can increase the risk and severity of *Plasmodium falciparum* infection and parasite burden which may facilitate high rate of malaria transmission and increased risk of congenital infection (Ouedraogo *et al.*, 2015).

HIV infection can also impair the efficacy of antimalarial treatment, increase adverse events, and select for parasites with drug-resistant mutations due to re-infection with new malaria strains, rather than recrudescence of prior infection (Kamya *et al.*, 2006). Although the prevalence of co-infection between *Plasmodium falciparum* and HIV had been reported in some parts of Nigeria, there are limited reports of these co-infection in Niger State, Nigeria. This study was therefore conducted to determine the prevalence of *Plasmodium falciparum* infection and its effect on CD_4 count in HIV patients attending selected Hospitals in Niger State, Nigeria.

MATERIALS AND METHODS

Study Area

This study was conducted in three selected hospitals in Niger State, Nigeria namely: General Hospital Bida, General Hospital Minna and General Hospital New Bussa. Niger State is divided into three senatorial zones namely Zone A (Niger South), Zone B (Niger East) and Zone C (Niger North) hence one hospital was selected from each zone.

Study Design

This was a hospital-based cross-sectional study involving consenting HIV positive individuals attending General Hospitals Bida, General Hospital Minna and General Hospital New Bussa in Niger State, Nigeria.

Ethical Approval

Ethical approval was obtained from the Ethical committee of Niger State Hospital Management Board/General Hospital, Minna, Niger State (HMB/GHM/136/VOL.III/590). Consent of participants or guardians of the children was also obtained prior to collection of samples.

Blood Sample Collection and Processing

Using sterile vacutainer needle/holder, 4mL of blood samples were drawn aseptically by venipuncture from the patients and transferred into a sterile Ethylene Diamine Tetra Acetate (EDTA) anticoagulant bottle. The samples were mixed immediately using vortex mixer (Cheesbrough, 2009).

Detection of Malaria Parasites

Thin and Thick Blood Film

From each participant's blood sample, 2µL of blood was placed on a labelled clean, grease-free glass slide and spread immediately using a smooth-edged slide spreader to make a thin film, as for thick smear, 6µL of blood was used. The slides were allowed to air dry and the thin smears were fixed with methanol. The slides were then flooded with 3% Giemsa working solution (pH 7.2) for 60minutes, washed and allowed to air dry. The stained blood films were viewed under x100 oil immersion objective lens. A total of 200 fields per film were examined (Cheesbrough, 2009; WHO, 2015).

Determination of CD_4Count

The CD_4 count was determined using a two-color single platform flow cytometer (Partec Cyflow) within 6hours of sample collection. Twenty microliter (20µL) CD_4 phytoerythrin (PE) antibody was dispensed into labelled Partec (Rohren) tubes and 20µL of well mixed whole blood was added, mixed with a vortex mixer and incubated in the dark for 15minutes at room temperature. The mixture was mixed at 5minutes interval during incubation. After incubation, 800µL of CD_4 diluting buffer was added to the mixture of antibody and samples mixed gently before analyzing (Cyflow Partec, 2010).

Data Analysis

All the data collected were entered into Microsoft Excel files and analyzed using STATA statistics/ Data analysis. Univariate analysis which includes descriptive statistics such as percentages and exploration of the distribution of all variables (age, sex, malaria parasite and CD_4 count) was performed. Pearson's correlation co-efficient was used to test the association between categorized variables. The mean values of the parameters were tested using simple linear regression analysis. All tests were performed at 95% confidence interval ($P \geq 0.05$).

RESULTS

The overall prevalence of *Plasmodium falciparum* is illustrated in Figure 1. A prevalence of 14.3% (43/300) was obtained. Of the 300 HIV patients enrolled in the study, 217 (72.3%) of them were females. The age of the participants ranges between 2-69years. The prevalence of *Plasmodium falciparum* infection among the participants who were enrolled in the study based on age and sex is presented in Table 1. The result obtained revealed variations in the prevalence of *Plasmodium falciparum* infection in all the age groups studied. The age group 1-10years had the highest prevalence of 28.57% while the least prevalence of 9.10% was observed in the age group 41-50years. The findings also revealed a prevalence of 18.07% among males (15/83) while that of females was 12.90% (28/217). There was no statistically significant association between the prevalence of *Plasmodium falciparum* infection with the age and sex of the participants ($p > 0.05$).

Table 2 shows the result of categorized CD_4 count and the occurrence of *Plasmodium falciparum* among the study participants. Participants withCD_4 count <200cells/µL had a higher prevalence (38.9%)of *Plasmodium falciparum* infection. Majority of the HIV positive patients co-infected with *Plasmodium falciparum* had CD_4 count <200cells/µL followed by those withCD_4 count >500cells/ µL. The association between categorized CD_4 count and distribution of *Plasmodium falciparum* is statistically significant (P < 0.05).

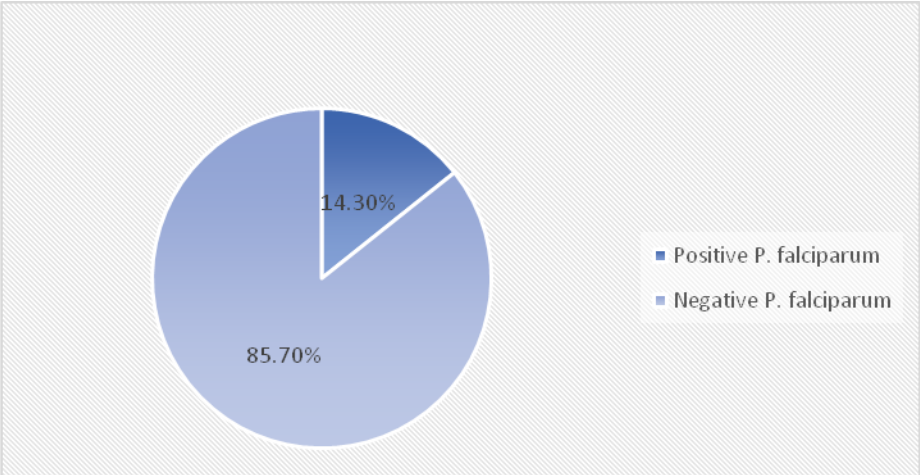

Figure 1: Prevalence of *Plasmodium falciparum* infection among study participants attending selected Hospitals in Niger State, Nigeria

Table 1: Prevalence of *Plasmodium falciparum* infection among the HIV patients based on Age and Sex

Variable	Number examined	Prevalence (%)	x^2	df	P-value
Age group (Yrs)					
1 -10	14	4 (28.57)	6.967	5	0.223
11 - 20	19	3 (15.79)			
21 - 30	64	12 (18.75)			
31 - 40	109	12 (11.01)			
41 - 50	66	6(9.10)			
>50	28	6 (21.43)			
Sex					
Males	83	15 (18.07)	1.306	1	0.253
Females	217	28 (12.90)			

Key: x^2 = Chi-square, df = Degree of freedom, Yrs.= Years.

Table 2: Occurrence of *Plasmodium falciparum* and CD_4Count among the Study Participants

CD_4 Counts (cells /µL)						
PF	1 - 199	200 - 500	>500	df	x^2	P - value
	No. (%)	No. (%)	No. (%)			
PF Pos	21(38.9)	7 (12.7)	15 (7.9)	2	33.165	0.000
PF Neg	33 (61.1)	48 (87.3)	176 (92.1)			
Total	54 (100.0)	55 (100.0)	191 (100.0)			

Key: *PF* - *Plasmodium falciparum*, Pos - Positive, Neg - Negative, df - Degree of freedom.

DISCUSSION

Malaria is known to cause an increase in transitory viral load while HIV causes more clinical malaria and increases the severity of malaria (Abu-Raddad *et al.*, 2006; Tay *et al.*, 2015). The overall prevalence of *Plasmodium falciparum* infection among HIV patients in this study was14.3%. The prevalence (14.3%) obtained in this study may be due to the period of study (dry season) which coincides with low malaria parasite transmission in the north-central parts of Nigeria(Nmadu *et al.*, 2015). It could also be due to the number of participants that were enrolled, frequency of exposure to malaria parasite infection, as well as care seeking attitude of the people living with HIV.

This prevalence is similar to 14.2% reported by Amadi *etal*. (2018) in University of Uyo, Nigeria and 15.5% reported by Tagoe and Boachie (2012)in Ghana. The prevalence obtained in this finding is however lower than59.2% reported byAbioye *etal*. (2014) in Kaduna, Nigeria and 33.0% reported by Gennaro *etal*. (2018) in Mozambique.

The prevalence of *Plasmodium falciparum* infection (14.3%) in this study is higher than 4.8% reported by Dada (2015) in Ondo State, Nigeria. The finding from this study is also in variance with the prevalence of 7.3% reported by Njunda *et al*. (2016)in Cameroun which was attributed to health-seeking attitude of the HIV patients and use of cotrimoxazole (CTX) based chemoprophylaxis which is recommended for the protection against opportunistic infection in all people living with HIV in Cameroon.

The prevalence of *Plasmodium falciparum* infection among HIV patients in relation to age groups revealed that the highest prevalence of 28.57% was observed among patients within the age group of 1-10years. This highest prevalence observed may be as a result of their low immunity which makes them susceptible to malaria. The result of this research is in conformity with those of Onankpa *et al*. (2017) who reported high prevalence of *Plasmodium falciparum* infection in HIV children (6-10 years). It is however at variance with the findings of Okonko *etal*. (2012) in Ibadan and Ahmed *et al*. (2016) in Abuja that reported lower prevalence in HIV children.

In this study, males had relatively higher prevalence of *Plasmodium falciparum* infection (18.07%) than their female counterparts (12.90%). This may be attributed to their occupation and travel routines that expose them to higher risk of being bitten by mosquito than HIV positive women. This finding is in conformity with the previous studies of Iroezindu *et al*. (2012) in Jos, Nigeria and Hwida *et al*. (2019) in Sudan who recorded high prevalence of *Plasmodium falciparum* infection in males. However, this finding is in contrast with that of Abioye *et al*. (2014) in Kaduna, Nigeria who reported that HIV positive females were at higher risk of contracting malaria compared to their HIV positive male counterparts. There was no statistically significant association between co-infection of *P. falciparum*/HIV with age and sex.

CD_4 counts are used as a measure of HIV infection progression and counts less than 200 cells/µL increases the risk of opportunistic infections. This statement is further supported by an important finding in our study that revealed higher prevalence of *Plasmodium falciparum* infection among HIV patients that have CD_4 count less than 200 cells/µL. This may also be attributed to the weakening of the immune response of the patients which causes decrease in CD_4 count and also exposes them to manifestation of malaria disease (CDC, 2009). This finding is in agreement with the findings of Jegede *etal*. (2017) in Kano, Nigeria and Bouyou-Akotet *et al*. (2018) in Central Africa that recorded high prevalence of malaria in HIV patients with low CD_4 count below 200cells/µL.

CONCLUSION

A prevalence (14.3) of *Plasmodium falciparum* infection was observed among HIV positive participants in Niger State, Nigeria.The prevalence was not associated with age or sex ($p > 0.05$). Higher prevalence of *Plasmodium falciparum*infection was obtained in participants with CD_4 count below 200cells/µL and a negative correlation between the *Plasmodium* parasitaemia and the CD_4 count is indicative of the role of the immune response in the protection against malaria in the target population.

RECOMMENDATIONS

Routine screening and treatment of malaria in HIV individuals should be adopted as part of the management policy to check the co-infection. Also, health education on malaria transmission and prevention methods should be provided.

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