

## Clinical Correlation of Differential CD4 T Cells Count with Malaria Among HIV Positive Patients

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### ABSTRACT

**Background:** It is logical to assume that HIV positive subjects are more at risk to malaria infection than HIV negative subjects.

**Objective:** The objective of this comparative cross-sectional study is to determine malaria infection in HIV positive and HIV negative subjects, and relate malaria infection to differential CD4 T cell counts in subjects who are infected with HIV.

**Method:** Giemsa staining technique was used to stain thin and thick blood film of consenting HIV positive and HIV negative controlled subjects, and observed under the microscope for the presence of malaria parasites. A positive test to malaria was defined as the presence of malaria parasites irrespective of species and density. CD4 T cell analysis was carried out on HIV positive subjects, using the Partec cyflow machine following the standard operating procedure.

**Result:** Among the two hundred and ten (210) HIV positive subjects, 10(4.8%) were positive for malaria. Out of the one hundred (100) HIV negative control subjects, 3(3%) were positive for malaria. Malaria recorded 6(2.9%) positive in CD4 counts  $\leq 300$  cells / $\mu$ l, 3(1.4%) positive in CD4 counts 301-833 cells / $\mu$ l, 1(0.5%) in CD4 counts 834-1365 cells /  $\mu$ l, and 0(0%) in CD4 counts 1366+ cells / $\mu$ l. We analyzed our findings using the SPSS version 21 and results show there was no significant difference in malaria infection between HIV infected and HIV non-infected subjects. Nonetheless, we also observed that 6 out of the 10 malaria positive subjects were those whose CD4 counts were  $\leq 300$  cells / $\mu$ l. This may suggest that either malaria or low CD4 Counts affects the outcome of the other.

**Conclusion:** Our findings statistically revealed that was no significant difference in malaria infection between HIV infected subjects and HIV negative control subjects.

**Keywords:** CD4 Count, HIV, Malaria

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## INTRODUCTION

The worldwide estimated malaria case by the World Health Organization in 2017 was 219 million and most of these cases occurred in the Africa region (1). In 2017, 36.9 million people were said to be living with HIV/AIDS worldwide and 940,000 died of HIV-related illnesses including malaria (2). Both malaria and HIV still remain deadly and are of public health concerned especially in Africa (3). There are studies in the past that showed malaria and HIV had no association (4, 5). But a recent study showed an association between malaria and HIV (6) and others showed that HIV patients who are infected with malaria had increased viral loads in their blood (7, 8).

## METHODS

### Ethical Considerations

Ethical clearance (HMB/ADM/423/T/76) was obtained from the ethical committee of Plateau State Hospitals Management Board. Written Informed consent was sought from subjects before participating in the study.

### Study Population

The study population consisted of HIV/AIDS patients who are on antiretroviral treatment (Tenofovir, Lamivudine, Efavirenze). Participants were required to sign a consent form after duly being informed about the study. Apparently healthy individual who tested negative for HIV antibody, were also included as control subjects in the study. Those who were on antimalarial drugs two weeks to the commencement of the study were excluded from the study.

### Study design

This was a hospital based comparative cross-sectional study that was carried out between May and June 2018. Participants were selected based on simple random sampling. This was done by asking

participants to pick pieces of papers with written numbers 1, 2, and 3, and placed in a box. Subjects who picked number 1 were enrolled in the study. Enrolled participants were asked to complete a structured questionnaire written in English language and those who couldn't understand English were assisted.

### Sample collection and analysis

Thin and Thick blood films were prepared from blood by pricking the patient's finger with a sterile, non-reusable lancet. Thin film was prepared from 2 to 5 $\mu$ l of blood spread on a clean, grease free glass slide, then fixed with methanol and allowed to dry before staining. Thick film was prepared from 12 to 15 $\mu$ l of blood uniformly spread on a clean, grease free glass slide and allowed to dry without fixing.

Thin and thick blood films were flooded with 10% Giemsa stain for 10 minutes, then rinsed with buffered water and allowed to air-dry. The stained films were observed with oil immersion objective of the light microscope and observed at different slide fields for trophozoites and other forms of malaria parasites.

Control subjects were screened for HIV antibodies using the Alere Determine TM HIV-1/2 (Waltham, Massachusetts, USA) according to the manufacturer's standard operating procedure for confirmation.

CD4 T cell enumeration was carried out using the partec cyflow machine following the standard operating procedure as describe by the manufacturer.

### Statistical analysis

Data generated was analyzed using the SPSS version 21 (SPSS Inc. Chicago, Illinois, United states). Chi-Square test for independence was used to determine the relationship between the two groups. A probability  $\leq 0.05$  was considered statistically significant.

## RESULTS

A total of 210 HIV positive and 100 HIV negative subjects, were screened for the presence of malaria parasite in their blood. The mean age for HIV positive subjects was 25±6 and median with age range of all participants was 28(15-65).

Among the 210 HIV positive subjects, 10(4.8%) were positive for malaria and out of the 100 HIV negative control subjects 3(3%) were positive for malaria.

Table 1: Malaria in relation to HIV Positive and HIV negative subjects.

	HIV Pos (%)	HIV Neg (%)	Total (%)
Malaria +	10 (3.2)	3 (1.0)	13 (4.2)
Malaria -	200 (64.5)	97 (31.3)	297 (95.8)
	210 (67.7)	100 (32.3)	310 (100)

$\chi^2=0.1767$ ,  $df=1$ ,  $p=0.6742$ ; OR:1.617 (95% CI: 0.4348 – 6.010)

Statistical analysis showed there was no relationship between malaria and HIV positivity, with no significant difference ( $p=0.6742$ ) of malaria between HIV positive and HIV negative subjects.

CD4 cells counts in relation to malaria infection among HIV positive patients is summarized in Table 2 below

CD4 (cells/mm <sup>3</sup> )	No. examined	No. positive (%)
≤300	77	6(2.9)
301-833	111	3 (1.4)
834-1365	19	1 (0.5)
1366+	3	0(0.0)
Total	210	10(4.8)

Table 2: The table above showed the highest malaria prevalence was among those whose CD4 counts were ≤300 cells/mm<sup>3</sup>.

## DISCUSSION

This study recorded a prevalence of 4.8% malaria parasitemia among HIV positive patients, while the controls had 3.0%. There was no significant difference between malaria infection in HIV positive subjects and HIV negative control subjects.

The malaria prevalence in this study is lower compared to a similar study in Jos, North-Central Nigeria, which recorded malaria prevalence in HIV infected patients as 24% and 9% in controls (9).

The low malaria prevalence rate in this study, as compared to most studies in Nigeria and the African region, could be due to the fact that the life cycle of mosquito and the dynamics in the transmission of the disease has been shown to be strongly influenced by variations in climate (10, 11, 12). As such, the low prevalence rate in this study was probably because this work was carried out during the dry season (October-May) which coincides in North central of

Nigeria with very low malaria transmission (13).

Previous studies in Kenya and Uganda have shown that the risk of malaria infection doubles in patients who were HIV positive, with higher malaria density associated with increased HIV immunosuppression (14).

In this study, there was no association between malaria infection in HIV positive patients and HIV negative controls. However, we observed that six(6) out of the Ten (10) malaria infected subjects in the HIV patients, are those whose CD4 counts were  $\leq 300$  cells/ $\mu$ l. This may suggest that CD4 immunosuppression may have increased the risk of malaria infection or vice versa.

### Conclusion

Although there was no relationship between malaria and HIV positivity, our study showed that malaria infection was found to be more prevalent among low CD4 counts than higher CD4 counts.

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