# Prevalence of malaria and anaemia among HIV-infected patients in Benin City, Nigeria

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## Abstract

**Objective:** To determine the prevalence of malaria and anaemia in HIV-infected persons and the effect of age, gender and $CD4^+$T cell counts thereon.

**Methods:** Blood samples were collected from 491 patients (240 female) attending an out patient clinic. Malaria parasitaemia was diagnosed by microscopy while anaemia was defined as haemoglobin concentration <130g/L in males and <120g/L in females. The $CD4^+$T cell count was estimated by flow cytometry.

**Results:** HIV infection was a risk factor for malaria infection (OR: 16.31; 95% CI: 7.41-35.87; p < 0.0001). $CD4^+$T cell counts was equally a significant risk factor in malaria infection among HIV-infected patients (OR: 1.96; 95% CI: 1.28-3.02; p = 0.002). The prevalence of anaemia was significantly affected by HIV-infection (OR: 25.12; 95% CI: 11.42-55.28; p < 0.0001) while age was not associated with increased risk of malaria infection (p=0.13).

**Conclusions:** A prevalence of 46.0% of malaria infection among HIV-infected was observed. HIV-infected patients were more likely to develop malaria and anaemia, while $CD4^+$T cell counts < 200cells/μL was associated with an increased risk of malaria infection among HIV-infected. Age and gender did not affect the prevalence of malaria. HIV status should be considered early in the diagnostic evaluation of patients with suspected malaria and anaemia.

**Key words**: HIV, malaria, anaemia, Nigeria
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## Introduction

More than 40 million people are living with HIV/AIDS while the majority (more than 25 million) are in sub-Saharan Africa and up to 2.4 million deaths were recorded in 2005 (1). Each year 500 million infections and up to 2.7 million deaths are attributable to malaria (2). Malaria and HIV are among the two most important global health problems of our time, together they cause more than 4 million deaths per year. Malaria and HIV/AIDS are both diseases and causes of poverty and they share determinants of vulnerability (3). Both diseases kill millions of people each year and both are scourges of developing nations in Africa, India, Southeast Asia and South America (4). Since HIV- infection interferes with cellular immune function, protection against malaria depends on cellular immunity, which may be impaired in HIV-infected persons with low $CD4^+$ T cell counts (5).

Anaemia is one of the complications in both malaria and HIV infections and contributes to its morbidity and mortality (6). Previous studies have revealed malaria as fueling the spread of HIV in sub-Saharan Africa, while HIV is been implicated as playing a role in boosting adult malaria infection rates (7). This has not been well studied in this locality, therefore we determined the prevalence of malaria and anaemia in HIV-infected persons as well as the effect of age, gender and $CD4^+$ T cell counts thereon.

## Methods

### Study population

The study was conducted in the University of Benin Teaching Hospital (a tertiary hospital with a referral status), Benin City, Edo State, Nigeria, between March 2008 and March 2009. A total of 350 HIV-infected and 141 HIV non-infected patients as controls (251 males and 240 females) were studied. Age of study subjects ranged from 20 to 70 years (mean: 34.3 ± 9. 4 years). All enrolled subjects were out patients that were on their first hospital visit before commencement of HAART therapy. Verbal informed consent was obtained from each subject before specimen collection. The study was approved by the Ethical Committee of the University of Benin Teaching Hospital.

### Specimen collection and processing

About four to five milliliters of blood was obtained from each patient, dispensed into ethylene diamine tetra-acetic acid (EDTA) container and mixed. Malaria was diagnosed by examination of a stained thick blood film. Thick blood films were made from each blood sample and allowed to air-dry. Slides were stained in 3% Giemsa stain for 30 minutes, rinsed in tap-water and allowed to air-dry. The stained films were examined for malaria parasites by microscopy using a x100 oil immersion objective lens. A total of 200 fields per film were examined (8).

Haemoglobin estimation was determined using a Sysmex KX – 21 haematology analyzer (Sysmex Corporation, Kobe, Japan). Anaemia was defined as a haemoglobin concentration less than 130 g/L in males and 120 g/L in females (9). A $CD4^+$T cell count was analyzed using flow cytometry (Partec, Gmbh, Germany). Briefly, into a Partec test tube 20μl CD4 PE antibody and 20 μl of well mixed whole EDTA blood were added, mixed gently and incubated in the dark for 15 minutes at room temperature. This mixture was mixed during incubation every 5 minutes. Eight hundred μl of CD4 buffer was added to the mixture of antibody and sample and mixed gently and $CD4^+$T cells counted.

### Statistical analysis

Data were analyzed using chi square ($X^2$) test or Fisher's exact test as appropriate and odd ratio analysis, using the statistical software INSTAT®.

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## Results

A total of 168 (34.2%) out of 491 patients had malaria, while 161 (46.0%) out of 350 HIV-infected patients had malaria and 7 (4.97%) out of 141 HIV non-infected patients had malaria. Generally, HIV-infection was a significant risk factor for malaria (OR: 16.31; 95% CI: 7.41-35.87;p<0.0001) (Table 1). The prevalence of malaria was not significantly affected by gender (OR: 0.70; 95% CI: 0.46-1.07;

p = 0.097) (Table 1). $CD4^+$ T cell count was a significant risk factor for malaria infection among HIV-infected patients (OR: 1.96; 95% CI: 1.28-3.02; p = 0.002) (Table 2). The prevalence of anaemia was significantly affected by HIV infection (OR: 25.12; 95% CI: 11.42-55.28; p < 0.0001) (Table 2).

Increasing age was not associated with increased risk of malaria infection (p = 0.13). Although females had a higher prevalence of malaria than males, this was not statistically significant (p=0.097). Age was a significant risk factor for development of anaemia in HIV-infected patients (p = 0.040). The 20 – 29 and 50 – 59 year age groups showed the highest prevalence of anaemia (64.5% and 64.7% respectively) with the 40 – 49 year age group showing the lowest prevalence of anaemia (42.9%) in relation to age.

**Table 1:** Prevalence of malaria and anaemia

| | N | No. of positive cases | OR | 95% CI | *p* |
|---|---|---|---|---|---|
| **Malaria** | | | | | |
| HIV infected | 350 | 161 (46.0%) | 16.31 | 7.41-35.87 | <0.0001 |
| Non-HIV | 141 | 7 (5.0%) | | | |
| **Anaemia** | | | | | |
| HIV infected | 350 | 198 (56.6%) | 25.12 | 11.42-55.28 | < 0.0001 |
| Non-HIV | 141 | 6 (4.3%) | | | |

**Table 2:** Risk factors for malaria among HIV patients

| | N | No. of Positive cases | OR | 95% CI | P value |
|---|---|---|---|---|---|
| **$CD4^+$count cells/µL** | | | | | |
| < 200 | 195 | 104 (53.3%) | 1.96 | 1.28-3.02 | 0.003 |
| ≥ 200 | 155 | 57 (36.8%) | | | |
| **Gender** | | | | | |
| Male | 195 | 82 (42.1%) | 0.70 | 0.46-1.07 | 0.097 |
| Female | 155 | 79 (51.0%) | | | |
| **Age (yrs)** | | | | **Malaria** | |
| 20 – 29 | 121 | 56 (46.3%) | | | |
| 30 – 39 | 85 | 32 (37.7%) | | | |
| 40 – 49 | 63 | 30 (47.6%) | | | |
| 50 – 59 | 68 | 39 (57.4%) | | | |
| ≥ 60 | 13 | 4 (30.8%) | | | 0.126 |

## Discussion

Malaria may be helping to spread the HIV-virus that causes AIDS (3). HIV-infected patients are at higher risk for malaria because of their weakened immune systems. Sub-Saharan Africa carries a high burden of both diseases thus co-infection is common in many areas there (10). This study focused on determining the prevalence of malaria and anaemia among HIV-infected patients as well as the effect of age, gender and $CD4^+$ T cell counts on its prevalence. A prevalence of 46.0% of malaria infection among HIV-infected patients was observed in this study. This is a relatively high prevalence that may be due to poor control measures. In countries like Togo, long-lasting insecticide treated nets have been distributed throughout the country (11). This kind of control programme has not been done in Nigeria, particularly, Benin City. There is therefore a need for an effective control programme to stem the tide of high malaria prevalence.

HIV infection was a risk factor for acquiring malaria infection in our study. This is consistent with previous findings (12). HIV-infected adults are more likely to develop malaria (13, 14), again consistent with our findings. The reason for this may be due to their weakened immune system induced by the HIV virus. However, Berg et al reported no significant association between HIV infection and malaria (15).

HIV infection has also been reported as an important risk factor for anaemia (12,16). Similarly, in our study HIV-infected patients were observed to have on 11 – 55 fold increased risk for acquiring anaemia. Bone marrow suppression by the HIV virus has been reported as a mechanism of anaemia among HIV-infected patients (17). $CD4^+$ T cell counts is used as a measure of immunity and HIV disease progression (18) and counts less than 200cells/µL increases the risk of opportunistic infections. In our study $CD4^+$ count of <200cells/µL was associated with an increased risk of malaria infection among HIV infected patients. This is in agreement with the findings of Whitwort et al (14) but not with that of Laufer et al (12). A decline in $CD4^+$ T cell counts below 200cells/µL increases immunosuppression and the risk of contracting an opportunistic infection.

Age and gender did not significantly affect the prevalence of malaria, a finding consistent with a previous report (15). It seems that the level of immunosuppression among HIV-infected patients determines the prevalence of malaria. However, in relation to anaemia, in our study age affected its prevalence among HIV-infected patients with those between 40 – 49 years of age having the lowest prevalence. The reasons for this are not clear. Among HIV patients with malaria, an anaemia prevalence of 70.7% was observed in this study (data not shown). The combined effect of malaria and HIV infection on erythropoiesis and red cell survival may be responsible for the higher prevalence of anaemia observed.

In conclusion, we observed a prevalence of 46.0% of malaria infection among HIV infected patients. HIV-infected patients are more likely to develop malaria and anaemia, while a $CD4^+$T cell count of < 200cells/µL was associated with an increased risk of malaria infection among HIV-infected patients. Age and gender did not affect the prevalence of malaria. The knowledge of HIV status may be valuable in the diagnostic evaluation of patients with suspected malaria and anaemia.

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