

## Malaria in HIV/AIDS Patients at Different CD4+ T Cell Levels in Limbe, Cameroon

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### Abstract

HIV infection has resulted in an increased risk of severe malaria and death, because the odds of parasitaemia and risk of malaria fever increase with decreasing CD4+ T cell count and increasing viral load. A cross-sectional study was conducted on 203 HIV/AIDS patients to determine the pattern of malaria infection, anaemic status and the outcome of ARV therapy vis-à-vis malaria treatment at different CD4+ T cell levels of the patient. Participants were HIV patients aged  $\geq 20$  years attending the HIV treatment centre of Limbe Regional Hospital, Cameroon. Clinical manifestations of malaria in patients were determined using a structured questionnaire. Their CD4+ T cell count and haemoglobin level were determined using the FACS count method. Malaria prevalence and density were determined from Giemsa-stained blood films. Clinical manifestations of malaria increased with decreasing CD4+ T cell counts. There was a negative correlation between malaria severity and decreasing CD4+ T cell counts. A significantly greater proportion ( $p < 0.01$ ) of patients had moderate anaemia. Cases of anaemia increased significantly ( $p < 0.001$ ) with decreasing CD4+ T cell counts. Declining immunity increases vulnerability to malaria infection and highly active ARV combination therapy has great potential to reduce HIV-related malaria.

**Keywords:** Malaria; HIV/AIDS; Patients; CD4+ T cell counts; ARV therapy; Limbe; Cameroon

### Introduction

Malaria and Human Immunodeficiency Virus (HIV) are among the most important global health problems of our time [1,2]. They have overlapping distribution in tropical areas especially in sub-Saharan Africa [3]. HIV infection leads to an increased risk of complicated and severe malaria and death, because the odds of parasitaemia and risk of malaria fever increase with decreasing CD4+ T cell count and increasing viral load [1,3]. HIV infection usually induces cellular depletion and early abnormalities of CD4+ T cells, decreases CD8 T cell counts and function (cellular immunity) and causes deterioration of specific antigen responses, humoral immunity [3]. In addition, there may be drug interactions and convergent toxicity between the drugs used to treat each of these diseases. Hence, with the increasing prevalence of HIV infection, malaria infection is fast becoming a diagnostic and therapeutic problem.

Both malaria and HIV/AIDS are co-endemic in Cameroon. Reported figures on the prevalence of malaria/HIV co-infections vary from one part of the country to another ranging from 29.4% in Douala, the economic capital [4] to 2.24% in Bamenda, the regional capital of the North West Region [5]. Some studies have suggested that repeated infections with malaria are associated with a rapid decline in CD4+ T lymphocytes over time while co-infections of malaria with HIV lead to more episodes of symptomatic [6] and even complicated malaria including death [7-10]. However, there is generally limited data on these aspects of malaria infection in HIV/AIDS patients in relation to CD4+ T lymphocyte count levels in Southwest Cameroon and such data is needed for proper control measures to be planned by health authorities. This study was therefore aimed at determining the pattern of malaria infection in HIV/AIDS patients at different CD4+ T cell count levels attending the HIV/AIDS Treatment Centre of the Limbe Regional Hospital in Southwest Cameroon.

### Materials and Methods

#### Study site

This study was carried out at the Limbe Regional Hospital in

Southwest Cameroon from February to June, 2009. Limbe is situated at the foot of Mount Cameroon and is bounded to the west by the Atlantic Ocean. Temperatures range from 23°C-32°C while the annual rainfall and relative humidity exceed 4,000 mm and 80% respectively. There are two seasons, the rainy and the dry seasons which start from Mid-March to October and November to Mid-March respectively. These climatic conditions favor the development of the malaria vectors and consequently malaria transmission. Limbe is a seaside resort and serves as the center of the oil industry of Cameroon. The Limbe port is one of the most important commercial ports of the country. As an urbanized centre promiscuity is common and as such both malaria and HIV/AIDS are co-endemic in the city. This made it a suitable city to study malaria in HIV/AIDS patients.

#### Study population

Subjects recruited into the study were those whose informed consent or that of their guardian had been sought. They were non-sickle cell patients aged 20 years and above and who had been tested and confirmed to be positive for HIV at the Voluntary Counselling, Testing and Treatment Centre of the Limbe Regional Hospital. The ethical clearance for this work was obtained from the Ethics Committee of the Delegation of Public Health, South West Region, Cameroon. Only those who gave their written informed consent were included in the study. These patients, who came for their routine CD4+ T cell count, were also examined for malaria parasite infection and haemoglobin

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level. They were administered a questionnaire that sought to identify the clinical manifestations of malaria.

### Collection and processing of blood

2 ml of blood were collected by venepuncture. Thick blood films were prepared, stained with 10% Giemsa stain for 20 minutes and examined for malaria parasites by microscopy. Each film was assessed independently by two microscopists. The parasite density was estimated by counting the number of asexual parasites against a minimum of 200 white blood cells (WBCs). Assuming a WBC count of 8000/ $\mu$ l of blood, the parasitaemia per  $\mu$ l of blood was then calculated using the formula:

$$\text{Number of parasites counted/Number of WBCs} \times 8000/\mu\text{l} [11].$$

### Determination of CD4+ T cell count

The CD4+ T cell count was done by the Florescence Activated Cell Sorter count method on patient's whole blood [12]. The software identified the T-lymphocyte population and calculated the absolute cell counts for CD4+ cells. The software similarly generated data on haemoglobin concentration.

### Data analysis

The data were analysed using the software package SPSS (version 11). The distribution of patients according to parasite density, haemoglobin concentration and clinical manifestations of the disease were compared by chi-square test after categorizing them following the WHO classification of CD4+ counts [13].

## Results

### Characteristics of the study population

Two hundred and three patients (26.1% males and 73.9% females), aged  $\geq 20$  years, whose HIV status had been confirmed constituted the sample studied. Some 89% of the patients had CD4+ T cell counts  $< 500/\mu$ l. Most of the study participants (89.7%) were in the 20-49 years age bracket.

### Distribution of patients according to clinical manifestations of malaria

The clinical symptoms frequently reported by patients were chills and rigours, followed by fever, headache, body pain, nausea and vomiting (Table 1). These manifestations were more frequently reported by patients with CD4+ T cell counts  $< 200/\mu$ l than by those with CD4+ T cell counts  $> 200/\mu$ l. Neurocerebral manifestations were reported exclusively in patients with CD4+ T cell counts  $< 200/\mu$ l.

### Relationship between malaria parasitaemia, antiretroviral (ARV) therapy and different CD4+ T cell levels

Overall, 58.9% of patients on ARV and 51.1% of those not on ARV were positive for malaria parasite infection. There was no significant difference in the distribution of parasite load of patients in the different categories of CD4+ T cell counts (Table 2). However, patients with lower CD4+ T cell counts generally had higher parasitaemia, irrespective of whether or not they were on ARV (Table 3).

Most of the patients (88.5%) on antiretroviral therapy had CD4+ T cell counts  $< 500/\mu$ l. The majority (84.4%) of the patients had been on treatment for at least one year. However, the percentage distribution of patients according to CD4+ T cell count level was not affected by duration on the therapy.

Clinical manifestations of malaria	Number examined	% distribution of patients according to CD4 T cell count/ $\mu$ l			Level of significance	
		< 200	200-499	$\geq 500$	x <sup>2</sup> value	p value
Fever	66	37.0	31.4	22.7	1.802	0.406
Chills and rigour	74	38.0	38.4	27.3	1.005	0.605
Headache	67	34.8	32.6	31.8	0.130	0.937
Body pain	58	29.4	30.2	22.7	0.486	0.783
Anorexia	61	0.4	27.1	18.2	3.846	0.146
Nausea	51	28.3	26.7	9.1	3.557	0.169
Vomiting	36	22.8	15.1	9.1	3.119	0.210
Abdominal pain	26	14.1	12.9	9.1	0.399	0.819
Neurocerebral manifestations	3	3.3	0.0	0.0	3.543	0.170

Table 1: Clinical manifestations of malaria in relation to CD4+ T cell categorization of patients.

Category of CD4 T cell count (No. per $\mu$ l)	Patients on ARV				Patients not on ARV	
	Number examined	% distribution of patients by parasite status		Number examined	% distribution of patients by parasite status	
		Positive	Negative		Positive	Negative
<200	75	65.3	34.7	17	52.9	47.1
200-499	78	51.3	48.7	8	50.0	50.0
$\geq 500$	20	65.0	35.0	2	50.0	50.0
Overall	173	58.9	41.0	27	51.9	48.1
		x <sup>2</sup> =3.461, p=0.177			x <sup>2</sup> =0.022, p=0.989	

Table 2: Status of the malaria parasite of patients in relation to CD4+ T cell count.

### Relationship between anaemia and different CD4+ T cell levels

The anaemic status and severity in patients classified according to CD4+ T cell category is shown in table 4. The majority (65.8%) of the anaemic patients had CD4+ T cell counts of  $< 200/\mu$ l, and the difference in the percentage distribution of anaemic patients between the different CD4+ T cell count categories was highly significant ( $\chi^2=41.142$ ,  $p<0.001$ ). Although anaemic severity was not statistically significant between patients of different CD4+ T cell counts, a significantly greater percentage (64%) of patients had moderate anaemia ( $p<0.01$ ).

### Relationship between total WBC count and CD4+ T cell levels

Most of the patients had WBC counts within the normal range, and this was true for all the CD4+ T cell count levels (Table 5). However, it was observed that the number of patients with a lower or higher than normal WBC count increased with increasing CD4+ T cell count, but the differences were not significant.

## Discussion

Repeated malaria infections are associated with rapid decline in CD4+ T cells over time [10]. Meanwhile, the main target of the HIV is the CD4+ T cells which the virus attacks and kills, resulting in a progressive immune decline from the normal range of 500-1500 cells/ $\mu$ l to  $< 200$  cells/ $\mu$ l if the virus is not interrupted by ARV therapy [14]. This agrees with our observation which revealed that up to 17 (63%) of patients who were not on ARV therapy had CD4+ T cell counts  $< 200$  cells/ $\mu$ l as against only 2 (7.4%) who had CD4+ T cell counts  $\geq 500$  cells/ $\mu$ l.

The 20-49 year age bracket which constituted 89.7% of the study participants is incidentally the sexually active group, an indication that HIV is spread mostly through sexual intercourse. This is consistent with a study in Zimbabwe which showed that 87% of HIV infections were transmitted through heterosexual intercourse, with lower proportions through blood transfusion (2%), intravenous drug use (1%) and mother to child (10%) [15].

Category of CD4 T cell count (No. per µl)	Patients on ARV				Patients not on ARV				
	Number examined	% distribution of study patients by parasitaemia severity			Number examined	% distribution of study patients by parasitaemia severity			
		No parasites found/µl	1-500/ µl	>500/ µl		No parasites found/µl	1-500/ µl	>500/ µl	
<200	75	34.7	37.3	28.0	17	47.1	35.3	17.6	
200-499	78	48.7	29.5	21.8	8	50.0	25.0	25.0	
≥500	20	35.0	40.0	25.0	2	50.0	50.0	0.0	
Overall	173	41.0	34.1	24.9	27	48.1	33.3	18.5	
				$\chi^2=3.554, p=0.470$					$\chi^2=0.921, p=0.923$

Table 3: Percentage distribution of study patients by level of parasitaemia.

Category of CD4 T cell count (No. per µl)	Anaemic status			Anaemic severity							
	No. examined	% distribution of study patients according to anaemic status		No. examined	% distribution of study patients according to degree of anaemia						
		anaemic	Not anaemic		Severe anaemia (Hb<7g/dL)	Moderate anaemia (7 ≤ Hb<10g/dL)	Mild anaemia (10 ≤ Hb ≤ 11g/dL)				
<200	90	81.1	18.9	73	2.7	65.8	31.5				
200-499	85	38.8	61.2	33	3.0	63.6	33.3				
≥ 500	20	25.0	75.0	5	0.0	40.0	60.0				
Overall	195	56.9	43.1	111	2.7	64.0	33.3				
				$\chi^2=41.142, p<0.0001$				$\chi^2=1.774, p=0.777$			

Table 4: Anaemic status and severity as affected by CD4+ T cell count category.

Category of CD4 T cell count (No. per µl)	No. examined	% distribution of patients according to WBC count		
		Low (<2.6×10 <sup>9</sup> /L)	Normal range (2.6-8.3×10 <sup>9</sup> /L)	High (>8.3×10 <sup>9</sup> /L)
<200	89	12.3	79.8	7.9
200-499	83	6.0	86.7	7.2
≥ 500	20	0.0	95.0	5.0
Overall	192	8.3	84.4	7.3
$\chi^2=4.660, p=0.324$				

Table 5: WBC count as affected by CD4+ T cell count category.

Recent studies in Botswana, Zimbabwe, Swaziland and South Africa have proven that HIV infections have increased the incidence of clinical malaria by more than 28% [16]. Although the differences in clinical manifestations of malaria were not significant by level of CD4+ T cell count, it was evident that the percentage of patients experiencing the clinical manifestations of malaria increased with decreasing CD4+ T cell levels. This is an indication that if HIV progression is not interrupted by ARV therapy, malaria death rate may likely increase in malaria endemic areas [14]. The number of patients who manifested the different clinical signs varied. This could be attributed to the fact that most of the patients were already on treatment, and some of the manifestations were probably the side effects of the drugs and not due to the malaria parasite itself, especially as some of the side effects of the drug mimic those of malaria. For example, stavudine causes anaemia, nausea and headache, and Abacavir causes fever [15].

Although the distribution of patients by parasitaemia severity did not differ significantly between the CD4+ T cell count levels, there was a negative correlation between malaria severity and CD4+ T cell count in patients, with CD4+ T cell counts decreasing as the proportion of patients increased. This is consistent with a study carried out in Uganda which revealed that malaria incidence for CD4+ T cell counts ≥ 500/µl, 200-499/µl and <200/µl was 57, 93 and 140 per 1000 person year respectively [8]. Thus, AIDS and malaria diseases are both controlled by immunity, and decreasing immune status as found in HIV patients will cause an increase in malaria severity.

None of the patients in our study population had high parasitaemia. This can be explained by the fact that most patients were those already on antiretrovirals and co-trimoxazole prophylaxis, which has been recommended for all HIV-infected patients in sub-Saharan Africa, has been found to reduce malaria incidence [17].

HIV patients have been found to be anaemic as a result of chronic inflammation of the HIV infection, malnutrition induced by infection, and opportunistic infections like malaria. Furthermore, the antiretroviral stavudine has been found to cause anaemia. Because of

these findings, all HIV patients are systematically placed on folic acid/iron. Our investigations revealed that 56.9% of the patients in the study population were anaemic as against 43.1% who were not. It is possible that those who were not anaemic were patients who adhered to their treatment for at least 6 months while those who were anaemic could be patients who were still on pretreatment work up (since 65.8% of them had CD4+ T cell counts of <200 cells/µl) or those who found it difficult to adhere to treatment or had therapeutic failure.

Total WBC count has been found to be a function of the immune status of HIV patients [18]. Patients with a higher than normal WBC count are considered to be those in the acute phase of the infection or with a recent bacterial or parasite infection. Those with a lower than normal WBC counts are probably patients at the AIDS defining stage. ARV therapy is also meant to boost the patient's immune status and, consequently, correct such abnormalities [18]. This is consistent with the observation in our study that with increasing CD4+ T cell count, probably due to ARV therapy, patients with abnormally low or high WBC counts had the counts brought to normal value.

It is concluded from this study that declining immunity caused by HIV infection increases the vulnerability to malaria infection in terms of clinical malaria and parasitaemia. Most patients manifested anaemia with moderate severity. Highly active antiretroviral therapy has great potential to reduce HIV-related anaemia. Cotrimoxazole prophylaxis, recommended for adults and children living with HIV in Africa, is also effective in reducing clinical malaria irrespective of baseline CD4+ T cell count.

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