

## Some Haematological Parameters in Plasmodial Parasitized HIV-infected Nigerians

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### ABSTRACT

**Background:** Nigeria is highly burdened by malaria and HIV-infection, yet researchers know little about the impact of this co-infection on the haematological profile of HIV-infected adult Nigerians. This case control study is an attempt to investigate the effect that HIV/malaria co-infection has on some haematological parameters of HIV-infected Nigerians.

**Methods:** Complete blood count (CBC) of 30 plasmodium parasitized HIV-infected subjects and 70 non-parasitized controls were studied.

**Results:** Of the 30 parasitized subjects, 28 (93.3%) were positive for falciparum malaria and 2(6.7%) for vivax malaria. The incidence of anaemia, thrombocytopenia, neutropenia and leucopenia were significantly higher in parasitized subjects compared to non-parasitized controls 66.7%, 60%, 36.7% and 63.3% versus 32.9%, 42.9%, 24.3% and 24.3% respectively. A statistically significant difference was observed between the haemoglobin, platelet count and the erythrocyte sedimentation rate (ESR) of parasitized and non-parasitized individuals ( $p < 0.05$ ) respectively. A significant positive correlation was observed between the level of parasitaemia and anaemia ( $r = 0.37$ ,  $p < 0.04$ ) in parasitized subjects. The incidence of anaemia was two times higher in parasitized subjects compared to non-parasitized controls (66.7% versus 32.9%). Red cell morphology showed a normocytic and normochromic picture in 40% and 67.1% of parasitized and non-parasitized individuals respectively. Microcytic, hypochromic picture was observed in 60% and 23% respectively in parasitized and non-parasitized individuals. Striking eosinophilia was seen in 5 (16.7%) of parasitized and 3 (4.3%) of non-parasitized individuals.

**Conclusion:** Incidence of cytopenia appear significantly higher in parasitized subjects compared to non-parasitized control and bring to bare the need for regular anti-malaria prophylaxis for HIV-infected patients particularly in Nigeria.

**KEYWORDS:** Plasmodium parasitaemia; Haematological parameters; HIV-infected; Nigerians.

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### INTRODUCTION

There is no doubt that the two most serious and common infections in Africa are malaria and the acquired immunodeficiency syndrome (AIDS)<sup>1,2</sup>. Malaria is a global public health problem resulting in considerable morbidity and mortality<sup>1,2</sup>.

Malaria remains the most serious and widespread protozoan infection of humans. Over 40% of the world population is at risk of contracting malaria, which is endemic in 91 countries mostly, developing<sup>3</sup>.

The prevalence of HIV has been increasing steadily in Nigeria from 1.8% in 1991 to 3.8% in 1993, 4.5% in 1999 and 5.8% in 2001<sup>4</sup>.

Haematological abnormalities that have been reported to invariably accompany infection with malaria include anaemia, thrombocytopenia, leucopenia eosinophilia and leucocytosis<sup>5-7</sup>.

Cytopenia resulting from the myelosuppressive effects of HIV, the secondary effects of opportunistic infection and other complications of AIDS is common in HIV/AIDS infected persons<sup>8-10</sup>.

Nigeria is highly burdened with malaria and HIV, yet researchers know little about treating both diseases simultaneously. The impact of this co-infection on the haematological profile of HIV-infected Nigerians is unknown. The aim of the study was to investigate the haematological changes that may occur in acute plasmodia infection in HIV-infected patients and to alert physicians caring for HIV-infected Nigerians on the need to update information so that rational decision on the haematological complications associated with HIV/Malaria co-infection could be taken. This may go a long way to reduce the mortality and morbidity associated with the co-infection and by so doing optimize medical cost.

### MATERIALS AND METHODS

#### Subjects

The study design utilized in this study is a case control study involving one hundred HIV-infected (plasmodium parasitized  $n = 30$  and non parasitized  $n = 70$ ) randomly selected from patients recruited into phase three of the antiretroviral therapy programme in the Haematology Department of the University of Port Harcourt Teaching Hospital a 500 bed tertiary health facility and one of the designated antiretroviral therapy centre in the heart of the oil and gas industry, in the

Niger Delta geopolitical zone of Nigeria.

## Methods

A case was defined as a malaria positive thin and or thick blood film<sup>11</sup> in an HIV- infected. While control was an HIV- infected with a malaria negative thin and thick blood film. Complete blood count (CBC), plasmodium specie, parasite load, social demographic data (age and sex) were recorded. Written informed consent was obtained from all participants. All hematological investigations were carried out manually as described by Dacie and Lewis<sup>11</sup>.

CD4 lymphocyte count enumeration was carried out using the Dynal beads technique (Dynal Biotec Oslo, Norway), an alternative method to flow cytometry in resource -limited settings based on CD4 T-cell isolation using anti CD4 monoclonal antibody coated paramagnetic polymer beads. HIV status of participants were confirmed using a double enzyme immunoassay (EIA) of WHO approved Immnocomb HIV 1 & 2 kits (Orgenics, Israel) and Genscreen HIV 1 & 2 kits (Bio Rad, France) both are qualitative and differential test for the detection of antibodies to HIV-1 and 2 in human serum or plasma.

## Statistical Analysis

Data was analyzed using a statistical package on personal computers SPSS version 10. Statistical analysis included descriptive statistics of mean and bivariate analysis of t- test and chi- square. Correlation was compared using linear regression analysis. Differences were considered significant when  $p \leq 0.05$

## RESULTS

A total of 30 HIV- infected patients who fulfilled the inclusion criteria of a positive malaria thin or thick film with mean age  $35.2 \pm 1.29$  years (age range 14-50) and made up of 14 males and 16 females constituted the subjects for this case control study. Seventy HIV- infected plasmodium negative individuals made up of 37 females and 33 males, mean age  $35.5 \pm 1.10$  (age range 18-54) years were included as controls. Of the 30 parasitized subjects 28 (93.32%) had falciparum malaria while 2 (6.7%) had *plasmodium vivax*. No mixed infection was detected.

Analysis of complete blood count showed a statically significant difference between the haemoglobin, platelet and erythrocyte sedimentation rate (ESR) of plasmodium parasitized subjects and non-parasitized controls ( $p < 0.05$ ) as shown in table I. The incidence of anaemia defined as haemoglobin value of  $< 13\text{g/dl}$  for males and  $< 12\text{g/dl}$  for females<sup>12</sup>, leucopenia defined as total white cell count of  $< 3.0 \times$

$10^9/\text{L}$ , thrombocytopenia defined as platelet count of  $< 150 \times 10^9/\text{L}$ <sup>13</sup> and neutropenia defined as differential neutrophil count of  $< 38.7\%$ <sup>14</sup> was significantly higher in plasmodium parasitized subjects; 66.7%, 63.3%, 60% and 36.7% respectively compared to 32.9%, 24.3%, 42.9% and 24.3% respectively in non-parasitized controls as shown in Table II.

Anaemia was found positively correlated with the level of parasitaemia in parasitized subjects ( $r = 0.37$ ,  $p = 0.04$ ). Red cell morphology was variable; 40% and 67.1% of parasitized and non-parasitized showed a normochromic normocytic blood film while microcytic and hypochromic picture was observed in 60% and 23% respectively in parasitized and non-parasitized individuals. Striking eosinophilia as defined as differential eosinophil count of  $> 10\%$ <sup>10</sup> was seen in 5 (16.7%) of parasitized subjects and 3 (4.2%) of non-parasitized controls.

**Table I. Mean Haematological values in plasmodial parasitized subjects and non-parasitized controls**

Haematological Parameters	Plasmodium Parasitizedn =30	Non- parasitized n=70	p-value
Haemoglobin (g/dl)	$9.17 \pm 1.69$	$10.54 \pm 1.94$	$< 0.05$
WBC count ( $\times 10^9/\text{L}$ )	$4.51 \pm 1.88$	$4.45 \pm 1.79$	$> 0.05$
Platelet count ( $\times 10^9/\text{L}$ )	$160 \pm 33.99$	$174.67 \pm 51.94$	$< 0.05$
ESR (mm/hr)	$93.1 \pm 39.84$	$74.4 \pm 37.9$	$< 0.05$
Neutrophil count (%)	$47.33 \pm 17.89$	$48.29 \pm 17.1$	$> 0.05$
Lymphocyte count (%)	$51.33 \pm 18.02$	$49.79 \pm 16.53$	$> 0.05$
Monocyte count (%)	$0.47 \pm 1.14$	$0.52 \pm .29$	$> 0.05$
Eosinophil count (%)	$1.3 \pm 3.29$	$1.3 \pm 3.56$	$> 0.05$

**Table II. Cytopenia in plasmodial parasitized and non-parasitized HIV-infected individuals**

Haematological derangement	Plasmodium n	Parasitized %	Non- parasitized n	%
Anaemia (Hb $< 13\text{g/dl}$ males, $< 12\text{g/dl}$ females)	20	66.7	23	32.9
Thrombocytopenia (platelet count $< 150 \times 10^9/\text{L}$ )	18	60	30	42.9
Neutropenia (differential Neutrophil count $< 38.7\%$ )	11	36.7	17	24.3
Leucopenia (WBC count $< 3.0 \times 10^9/\text{L}$ )	19	63.3	17	24.3

## DISCUSSION

HIV/AIDS remains the greatest health crisis in the world today with an estimated 40 million people now living with the virus, 95% of them in developing countries. In this case control study we have investigated the impact of HIV and malaria co-infection on the haematological parameters of HIV- infected Nigerians.

Haematological abnormalities are considered a hallmark of malaria and reported to be most pronounced in *plasmodium falciparum* infection<sup>15</sup>. The findings presented in this case control study show that in acute plasmodial infection in HIV-infected, there are several peripheral blood changes including anaemia, which was a common presentation. Anaemia (Hb <13g/dl for males and, < 12g/dl for females) was found to be two times higher in parasitized HIV- infected subjects (66.7%) compared to non- parasitized controls (32.9%) ( $p = 0.001$ ). Anaemia was normochromic and normocytic in 12 (40%) and 47 (67.1%) respectively in plasmodium parasitized and non-parasitized HIV- infected Nigerians while 18 (60%) and 23(32.1%) showed a microcytic hypochromic red cell picture. The pathogenesis of anaemia in plasmodial parasitized patients, are complex and multifactorial and are thought to result from haemolysis of parasitized red cell, exacerbated removal of parasitized red cells, depressed and ineffective erythropoiesis<sup>16</sup>.

*Plasmodium falciparum* malaria is one of the commonest causes of anaemia<sup>17</sup> and correlates with the severity of the infection. In our study we found plasmodium falciparum the commonest cause of malaria among parasitized HIV infected Nigerians 28 (93.3%) while 2 (6.7%) had *plasmodium vivax*. This observation is consistent with a previous report that found *plasmodium falciparum* the predominant cause of malaria in Nigeria<sup>18</sup>. We also observed a significant positive correlation between the level of parasitaemia and anaemia ( $r=0.37$ ,  $p = 0.04$ ). The anaemia seen in HIV- infected Nigerians had the characteristics of anaemia of chronic disease with mean haemoglobin of 10g/dl<sup>19</sup>. In this study we observed a mean haemoglobin value of  $9.17 \pm 1.67$  and  $10.54 \pm 1.94$  respectively for parasitized and non- parasitized HIV-infected Nigerians. Factors such as the direct myelosuppressive effect of HIV infection, the secondary effect of drug therapy and other pre-existing or co-existing medical problems has been incriminated in the HIV-infected population<sup>20</sup>.

In our study the incidence of leucopenia defined as total white cell count ( $< 3.0 \times 10^9/L$ ) was more than two times higher in plasmodium parasitized HIV-infected (63.3%) compared to non-parasitized (24.3%). Leucopenia appears a common finding in patients with falciparum malaria when WBC count may be as low as  $1-2 \times 10^9/L$ <sup>5</sup>.

Thrombocytopenia defined as platelet count ( $<150 \times 10^9/L$ ) was a characteristic finding among plasmodial parasitized and non-parasitized HIV-infected in this study 18 (60%) versus 30 (42.9%) respectively. Thrombocytopenia has been reported as a classical feature of malaria and low platelet count is usually seen in 85% of patients with uncomplicated malaria<sup>5</sup>.

Thrombocytopenia although a recognized manifestation of HIV infection was seen in 5% of patients, it seems up regulated as observed in this study by plasmodial co-infection<sup>21</sup>.

The differential leucocyte count indicated a normal neutrophil count in majority of parasitized and non-parasitized Nigerians 19 (63.3%) and 53 (75.7%). However 11 (36.7%) of parasitized and 17(24.3%) of non-parasitized individuals had neutropenia. Previous study however found malaria associated with neutropenia<sup>15</sup>.

In this study we observed a statistically significant difference between the haemoglobin, platelet count and erythrocyte sedimentation rate (ESR) of parasitized and non-parasitized HIV-infected Nigerians ( $p < 0.05$ ). In conclusion, this case control study has indicated that the incidence of haematological derangements in HIV-infected Nigerians is significantly higher in parasitized subjects compared to non-parasitized controls. With the recent strategic decision by the Nigeria government to introduce her citizens living with HIV to antiretrovirals, the next step presumably is the need for regular antimalarial prophylaxis for HIV-infected patients particularly in sub-Saharan Africa where both HIV and malaria are endemic.

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