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Malaria infection in HIV/AIDS patients and its correlation with packed cell volume (PCV)

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Abstract

Background & objectives: The study was designed: (i) to determine the prevalence of malaria parasites; (ii) to determine the relationship between parasitaemia and age/sex; (iii) to correlate the PCV levels with parasitaemia; and (iv) to determine the influence of protection against natural transmission on the prevalence of malaria.

Methods: Participants were recruited at the Plateau State Human Virology Research Laboratory (PLASVIREC), Robert Gallo House at the Plateau State Specialist Hospital, Jos and grouped into: (i) Malaria and HIV co-infection group (n = 64); and (ii) HIV infected group without concurrent malaria infection (n = 136). Standard laboratory procedures were used for the HIV and *Plasmodium* parasites screening, malaria parasite density, and packed cell volume.

Results: The results showed a significant difference (p <0.05) among the sexes and age groups. About 64 (32%) of the individuals had *Plasmodium* infection (30% *Plasmodium falciparum*, 0.5% *P. malariae,* and 1.5% mixed infections of *P. falciparum* and *P. malariae*). Malaria parasites were more common among the rural dwellers and in the age group of 21–30 yr. Regression analysis showed a negative association of malaria parasitaemia and PCV among the malaria–HIV positive and malaria-HIV negative (r^2 = 0.529; p <0.001).

Interpretation & conclusion: In the present study, PCV might be of useful indicator and if not monitored could lead to AIDS establishment especially where high malaria parasitaemia is noted. The findings further suggest that the defined stage of HIV infection in the study, malaria co-infection may moderate the impact of HIV infection on PCV.

Key words HIV co-infection – malaria parasitaemia – PCV malaria

Introduction

Two of the greatest challenges facing Africa today are human immunodeficiency virus (HIV) infection and malaria, yet the interaction between these two parasitic infections has been little studied. An interaction between HIV infection and malaria could work in either direction, i.e. HIV infection might reduce immunity to clinical malaria resulting in more frequent infection among the semi-immune and non-immune, or malaria might enhance the progression of HIV infection to clinical AIDS[1]. Sub-Saharan Africa has >70% of the over 42 million persons infected with HIV/AIDS worldwide and it is now the leading cause of death in the region. Nigeria, the most populous country in Africa, has over four million persons living with HIV/AIDS and a national seroprevalence of 5.8% (the end of 2001)[2], with the north-central region harbouring the highest HIV infection levels in the country[3].

Various reports[4–6] stated that malaria is a powerful

stimulator of the immune system and the subjects exposed frequently to malaria have enhanced serum levels of immunoglobulins and an accelerated rate of IgG turnover. Other authors[7,8] also reported that malaria infection might have an adverse effect on HIV infection both by stimulating T-cell turnover and by impairing T-cell cytotoxic function.

Malaria parasitaemia differs in instances of asymptomatic and clinical malaria, and the degree of parasitaemia may influence the pathological and biochemical presentations of individuals presenting with either of these conditions[9–13]. Reports have shown that in clinical cases of malaria, anaemia is a prominent factor[14,15], which is possibly caused by destruction of infected blood cells by the reticuloendothelial system and haemolysis of infected cells[16,17].

The present study was designed: (i) to determine the prevalence of malaria parasites in HIV/AIDS patients; (ii) to determine (if any) the relationship between parasitemia and age/sex; (iii) to correlate the packed cell volume (PCV) levels with parasitaemia; and (iv) to determine the influence of protection against natural transmission on the prevalence of malaria.

Material & Methods

Study area: The study was conducted at the Plateau State Human Virology Research Laboratory (PLASVIREC), Robert Gallo House, at the Plateau State Specialist Hospital, Jos (located at latitude 9° 55' to 10°N and longitude 8°52' to 9°E) from September–November 2006. The area has two seasons, the dry season (November–March) and the rainy season (April–October). Malaria transmission is usually intense as the end of the rainy season approaches. Other geographical indices of the area have also been reported elsewhere[18].

Ethical clearance: The committee for ethical clearance of the Plateau State Specialist Hospital, Jos approved the study (Ref. PSSH/ADM/454/IX). Informed consent was obtained from all study participants according to the guidelines of the Plateau State Specialist Hospital, Jos, Plateau State, Nigeria.

Subject selection and sample collection: The study population was 200 patients confirmed to be HIV-seropositive by standard laboratory techniques and in addition, presenting clinical signs and symptoms of malaria. HIV patients who had no malarial parasite, served as control. PCV of 20 healthy volunteers were used as the normal range values.

The following criteria were used for the selection of the study participants: (i) patients must have not received any antimalaria drugs for past two-months period; (ii) patients must have clinical signs of malaria; (iii) patients must not be <10 yr and >50 yr of age; (iv) patients must have been screened as positive for HIV using $HIV^{1/2}$ STAT-PAK^{TM} (Manufactured by CHEMBIO 3661 Horseblock Road, NY, USA) and further confirmed by Determine $HIV^{1/2}$ (Abbott Laboratory, Minato-Ku, Tokyo, Japan); and (v) patients must have been screened and found not to have Hepatitis-B or any previous history of liver disease. The patients' age, sex, and occupation were also obtained. A 5 ml blood sample was obtained by vene-puncture from each of these patients into an ethylene diamine tetra-acetic acid (EDTA) anticoagulant bottles.

Examination of samples: Thick and thin films were prepared from each subject's blood sample. The thin films were fixed with absolute methanol and both thick and thin films were stained with Giemsa after which they were examined microscopically with oil immersion under x 100 objective. The parasite counting was done using the thick blood films while the thin blood films were used for *Plasmodium* species identification. Malaria parasites were counted according to the method described above[19–22]. The parasite count in relation to the leucocytes count were converted to parasite per microliter of blood using the following mathematical formula.

$$\text{Parasitaemia } (\mu l^{-1}) = \frac{\text{Number of asexual parasites} \times 8000}{\text{Number of leucocytes}}$$

Where, 8000 = Putative mean number of leucocytes/μl blood.

The number of parasites was counted against 200 leucocytes and expressed as number of parasites/µl blood. The PCV was determined by micro-haematocrit centrifugation (at a speed of 1200 g for 5 min) of EDTA-whole blood collected into a capillary tube (Marienfield City, Germany). The volume of the PCV red cells was measured in a micro-haematocrit reader as a relative mass of packed red cells present in a sample of whole blood (percent).

Statistical analysis: All data were statistically analysed using chi-square, regression, and *t*- test for comparison between the infected and uninfected subjects.

Results

Prevalence of malaria parasites with respect to sex and age is as shown in Table 1 and 64 (32%) of the 200 patients examined had malaria parasites. Of the 64 infected individuals, there were 41 (37.96%) females and 23 (25%) males. The age group of 21–30 yr was recorded with highest percentage of malaria infection 35 (17.5%). A significant difference occurred among the sexes (χ^2 =3.84; df = 1; p <0.05)

Table 1. Prevalence of malaria with respect to sex and age

Parameter	No. examined (%)	No. with malaria infection (%)	*p*-value
Sex			
Male	92 (46)	23 (25)	
Female	108 (54)	41 (37.96)	<0.05
Total	200 (100)	64 (32)	
Age (yr)			
<20	30 (15)	11 (5.5)	
21–30	82 (41)	35 (17.5)	
31–40	46 (23)	12 (6)	
41–50	22 (11)	2 (1)	
>50	20 (10)	4 (2)	<0.05
Total	200 (100)	64 (2)	

Age grouping: (χ^2 =12.18; df = 4; p <0.05); Sex: (χ^2 = 3.84; df = 1; p <0.05).

Table 2. Prevalence of malaria with respect to occupation

Occupation	No. examined (%)	No. positive (%)
Commercial sex workers (CSW)	30 (15)	7 (3.5)
Civil servants	60 (30)	17 (8.5)
Farmers	78 (39)	30 (15)
Others (Students)	32 (16)	10 (5)
Total	200 (100)	64 (32)

and between the age groups (χ^2 =12.18; df = 4; p < 0.05). The highest prevalence rate 30 (15%) of malaria parasites with respect to occupation was among the farmers who are mostly rural dwellers while the lowest was among the commercial sex workers (Table 2).

Regarding the parasite density (Table 3), 13 (20.31%) of those with parasite density >5000 μl^{-1} are females while only 4 (6.25%) are males. Eleven (17.19%) of the age group 21–30 yr had parasite density >5000 μl^{-1}, while 1 (1.56%) in the age group >50 yr had >5000 μl^{-1}. Statistical analysis showed that there was no significant difference among the sexes (χ^2= 2.71; df = 1; p >0.05) and age groupings (χ^2=5.38; df=4; p >0.05). Collective parasite density of the infected patients was 228.24×10^3 μl^{-1}. Of this density, the age group of 21–30 yr had the highest with 135.92×10^3 μl^{-1} (59.55%), while the least was in the age group 41–50 yr with 1.4×10^3 μl^{-1} (0.61%) (Table 4).

The regression plot is shown in Fig.1 and the PCV level was observed to be closely associated with the presence of malaria parasitaemia (y =30.935–0.002x; r^2=0.529; p <0.001). A significant difference (p <0.05) was observed between the infected and uninfected as shown on Table 5. The mean total PCV for the infected was 24.81% and uninfected 34.81%.

Discussion

Malaria infection among the age grouping and vari-

Table 3. Levels of parasitaemia with respect to sex and age grouping

Parameters	Distribution: Parasite densities (parasite/μl^{-1})			
	<1000	1000–5000	>5000	Total
Sex				
Male	13 (20.31)	6 (9.38)	4 (6.25)	23 (35.94)
Female	17 (26.56)	11 (17.19)	13 (20.31)	41 (64.06)
Total	30 (46.88)	17 (26.56)	17 (26.56)	64 (100)
Age(yr)				
≤ 20	5 (7.81)	3 (4.69)	3 (4.69)	11 (17.19)
21–30	14 (21.88)	10 (15.62)	11 (17.19)	35 (54.69)
31–40	6 (9.38)	4 (6.25)	2 (3.12)	12 (18.75)
41–50	2 (3.25)	0 (0)	0 (0)	2 (3.13)
>50	3 (4.69)	0 (0)	1 (1.56)	4 (6.25)
Total	30 (46.88)	17 (26.56)	17 (26.56)	64 (100)

Age grouping ($\chi^2 = 5.38$; df = 4; $p > 0.05$); Sex: ($\chi^2 = 2.71$; df =1; $p > 0.05$).

Table 4. Parasite density index of patients with respect to sex and age

Age (yr)	Distribution: Parasite density (parasite/μl)						Total
	<1000		1000–5000		>5000		
	Males	Females	Males	Females	Males	Females	
10–20	1.92×10^3	1.88×10^3	5×10^3	10×10^3	18×10^3	6.4×10^3	43.2×10^3
21–30	4.56×10^3	7.76×10^3	11.2×10^3	21.2×10^3	0×10^3	91.2×10^3	135.92×10^3
31–40	2.72×10^3	2.36×10^3	14.92×10^3	0×10^3	5.2×10^3	15×10^3	40.2×10^3
41–50	1.4×10^3	0×10^3	0×10^3	0×10^3	0×10^3	0×10^3	1.4×10^3
>50	1.36×10^3	0.96×10^3	0×10^3	0×10^3	0×10^3	5.2×10^3	7.52×10^3
Total	11.96×10^3	12.96×10^3	31.12×10^3	21.2×10^3	23.2×10^3	117.8×10^3	228.24×10^3

Table 5. *t*-test for infected and non-infected subjects' PCV result

Group statistics

Content	Status	N	Mean deviation	Standard error mean	Standard
	Infected	64	24.8125	7.36115	0.92014
Non-infected	136	34.8088	5.07501	0.43518	

Independent samples test

Content	Levene's test for equality of variances		*t*-test for equality of means						
	F	Significance	t	df	Significance (2-tailed)	Mean difference	Standard error difference	95% confidence interval of the difference	
								Lower	Upper
Equal variances assumed	9.764	0.002	–11.178	198	0	–9.99632	0.89425	–11.75979	–8.23285
Equal variances not assumed			–9.821	92.183	0	–9.99632	1.01786	–12.01783	–7.97481

Regression summary—PCV *vs* Parasitaemia
Count: 64; No. missing: 0; r: 0.727; r^2: 0.529; Adjusted r^2: 0.521; RMS residual: 5.094.

ANOVA table—PCV *vs* Parasitaemia

	DF	Sum of squares	Mean square	F-value	*p*-value
Regression	1	1804.745	1804.745	69.542	<0.0001
Residual	62	1609.005	25.952		
Total	63	3413.750			

Regression coefficients—PCV *vs* Parasitaemia

	Coefficient	Std. error	Std. coeff.	*t*-value	*p*-value
Intercept	30.935	0.972	30.935	31.831	<0.0001
Parasitaemia	– 0.002	2.072E-4	– 0.727	– 8.339	<0.0001

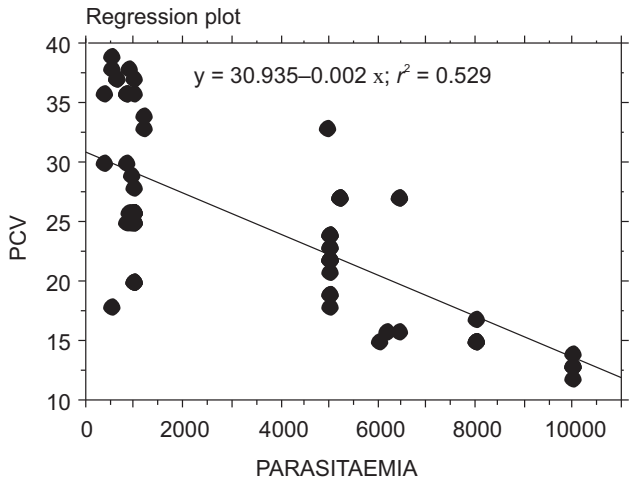

Fig. 1: Regression plot for parasitaemia and PCV

ous sexes was of significant value (p <0.05). Females were found to be more infected with malaria as compared to their male counterparts. This could be as a result of cooking at late evenings which are common in Plateau state and as such they were exposed to more mosquito bites resulting in the high transmission. Also, among the various age groups it was revealed that the age group of 21–30 yr had more of malaria parasitic infection than the other age groups and this was also confirmed with the high cumulative density index. This agrees with earlier report from Tanzania[23,24] and from Congo[25,26] in HIV-negative individuals that malaria infection prevalence's are inversely related to age; thus, those between the ages of 21–30 yr in our study had higher malaria infection while the older ones are at lower risk. Studies from Cameroon[14,15] both reported in HIV-negative individuals that young children presenting with severe malaria parasitaemia have shown to present with PCV as low as 25%.

The study also showed farmers who are mostly rural dwellers had the highest infection rate of *Plasmodium* infection. This could be attributed to their consistent bite by mosquitoes and the lack of insecticide treated nets to curb the menace. With such an intertwined infection, an intense decrease in PCV could therefore be envisaged. This confirms the earlier report[27] that rural areas have environmental conditions more favourable to transmission of the disease than urban areas; as malaria was found to be more prevalent among pregnant women from rural areas and they were at 5.18 times at greater risk than those from urban areas.

In association of malaria parasitaemia and PCV, the study revealed a close association which resulted in the decrease in the PCV of those infected with malaria as compared to those who were only HIV-positive. The mean total of PCV of the malaria infected patients was 24.81% and uninfected ones was 34.81% which falls below the normal lower limits of 36–38%[28]. Packed cell volume of less than 33% has been reported[29], and also PCV of <25% has been associated with severe malaria infection in children[15]. The observation that malaria patients near significantly lower PCV levels compared to the control confirm the earlier reports of a study among HIV-negative individuals[30,31]. In some studies on malaria infection in non-HIV pregnant women by[32,27]; they both reported that strong negative association between malaria parasites density and PCV exists in these subjects. But the report of Onyenekwe *et al*[33] stated that PCV was not significantly different between the malaria-HIV co-infected group and HIV infected group, which is at variance with the results

of our study. This could be expected because while Onyenekwe *et al*[33] worked purely among urban dwellers in southeastern Nigeria who would have been exposed to anti-retroviral drugs, good balanced diet and so on, our studies involved those from both rural and urban areas who have or have not been on good balanced diet and so on.

The finding, therefore, suggests that at the defined stage of HIV infection, malaria co-infection may moderate the impact of HIV infection on PCV. Malaria infection in HIV infected subjects in endemic area may therefore be implicated as contributing to adverse effect on PCV in HIV co-infected subjects and could therefore be useful guide in designing insecticide treated net (ITN) programme for HIV/AIDS patients.

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