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

Frederick O Akinbo[1,2], PhD; Christopher E Okaka[1], PhD; Richard Omoregie[3], MSc; Raphael Mordi[1], PhD; Osamwonyi Igbinuwen[1], M.Sc

[1]Departments of Pathology and [2]Animal and Environmental Biology, and [3]School of Medical Laboratory Sciences, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria

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

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®.

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.

Acknowledgements

References
1. Merwin J, Ekwaru JP, Liechty CA, Were W, Downing R, Ransom R, et al. Effect of co-trimoxazole prophylaxis, antiretroviral therapy, and insecticide-treated bednets on the frequency of malaria in HIV-1-infected adults in Uganda: a prospective cohort study. *Lancet* 2006; 367: 1256-61.
2. Bremen JG. The ears of hippopotamus: manifestations,determinants and estimates of the malaria burden. *Am J Trop Med Hyg* 2001; 64 (suppl 1-2): 1-11.
3. WHO : Malaria and HIV/AIDS. Interactions and complications. Conclusions of a Technical Consultation convened by WHO, 23–25 June 2004; World Health Organisation. WHO/HIV/2004.08.
4. Verhoeff FH, Brabin BJ, Hart CA, Chimsuku L, Kazembe P, Broadhead RL. Increased prevalence of malaria in HIV-infected pregnant women and its implications for malaria control. *Trop Med Int Health* 1999; 4: 5–12.
5. Rosenberg ZF, Fauci AS. Immunopathogenesis of HIV infection. *FASEB J* 1991; 5: 2382– 90.
6. Malamba S, Hladik W, Reingold A, Banage F, McFarland W, Rutherford G, et al. The effect of HIV on morbidity and mortality in children with sever malarial anaemia. *Malar J* 2007; 6: 143.
7. Laith JA, Padmaja P, James GK. Malaria and HIV fact sheet. European Alliance Against Malaria, 2006.

8. Omoregie R, Adedotun BB, Ogefere HO, Iduh P, Duru M. Comparison of the efficacy of malaria PF rapid test device, Giemsa-stained thick blood film and QBC in the diagnosis of malaria in Benin City, Nigeria. *Mary Slessor J Med* 2007; 7: 1–4.

9. Beutler E, Waalen J. The definition of anaemia: what is the lower limit of normal of the blood haemoglobin concentration? *Blood* 2006; 107: 1747–50.

10. WHO. Malaria and HIV interactions and their implications for public health policy. World Health Organisation Technical Consultation Report, 23–25 June,2004.

11. Eliades MJ, Wolkon A, Morgah K, Crawford SB, Dorkenoo A, Sodahlon Y, et al. Burden of malaria at community level in children less than 5 years of age in Togo. *Am J Trop Med Hyg* 2006; 75: 622–9.

12. Laufer MK, van Oosterhout JJ, Thesing PC, Thumba F, Zijlstra EE, Graham SS, et al. Impact of HIV-association immunosuppression on malaria infection and disease in Malawi. *J Infect Dis* 2006; 193: 872-8.

13. French N, Nakiyingi J, Luganda E, Watera C, Whitworth JA, Gilks CF. Increasing rates of malarial fever with deteriorating immune status in HIV-1-infected Ugandan adults. *AIDS* 2001; 15: 899–906.

14. Whitworth J, Morgan D, Quigley M, Smith A, Mayanja B, Eota H, et al. Effect of HIV-1 and increasing immunosuppression on malaria parasitaemia and clinical episodes in adults in rural Uganda: a cohort study. *Lancet* 2000; 356: 1051–6.

15. Berg A, Patel S, Langeland N, Blomberg B. Falciparum malaria and HIV-1 in hospitalized adults in Maputo,Mozambique:does HIV-infection obscure the malaria diagnosis? *Malar J* 2008; 7: 252.

16. Omoregie R, Omokaro EU, Palmer O, Ogefere HO, Egbeobauwage A, Adeghe JE, et al. Prevalence of anaemia among HIV patients in Benin City, Nigeria. *Tanz J Health Res* 2009; 11: 1 – 4.

17. Odunukwe N, Idigbe O, Kanki P, Adewole T, Onwujekwe D, Audu R, et al. Haematological and biochemical response to treatment of HIV-1 infection with a combination of nevirapine + stavudine + lamivudine in Lagos, Nigeria. *Turk J Haematol* 2005; 22: 125–31.

18. Oguntibeju OO, Van Der Heever, Van Schalkwyk FE. Effect of a liquid nutritional supplement on viral load and haematological parameters in HIV-positive/AIDS patients. *Brit J Biomed Sci* 2006; 63: 134–9.

Address for correspondence: Frederick O Akinbo**,** Department of Pathology, University of Benin Teaching Hospital/Department of Animal and Environmental Biology, University of Benin, PMB 1111, Benin City, Nigeria. E-mail: fgbengang@yahoo.com