Malaria and HIV co-infection and their effect on haemoglobin levels from three health-care institutions in Lagos, southwest Nigeria

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Abstract

Background: Malaria and human immunodeficiency virus (HIV) are two major infections with enormous public health consequence. Together, they are endemic in many developing countries with anaemia being the most frequent haematological consequence of the infections.

Objective: To determine the prevalence of malaria and HIV co-infection as well as anaemia among selected patients from three health-care institutions in Lagos, Nigeria.

Methods: A cross-sectional study of 1080 patients was carried out to determine the prevalence of malaria and HIV co-infection as well as anaemia. Blood sera from each of the patients were screened for malaria parasites, HIV-1 and HIV-2 using Giemsa stain, Cambridge Biotech Recombigen HIV-1/HIV-2 rapid device, respectively while haemoglobin estimation was performed using cyanmethemoglobin method.

Results: Our data showed that the total number of malaria infected patients were significantly higher in HIV sero-positive patients 47.7% (31/65) when compared with their HIV sero-negative counterparts 25.8% (262/1015) P = 0.047. The result also revealed that 25.8% (8/31) of the patients co-infected with malaria and HIV had anaemia as compared to 11.1% (29/262) infected with malaria alone. Multivariable logistic regression analysis showed that patients with dual infection of malaria and HIV were twice likely to be anaemic than those infected with malaria alone [adjusted OR 2.4, 95% CI, 1.3 to 2.7, P = 0.014].

Conclusion: Our data indicated a higher prevalence of malaria in HIV infected patients and also revealed that patients co-infected with malaria and HIV were more likely to be anaemic.

Keywords: Malaria, malaria and HIV co-infection, anaemia, parasite density

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Introduction

Malaria remains one of the leading causes of morbidity and mortality globally and nearly half of the global populations are at risk of malaria infection. Malaria and human immunodeficiency virus (HIV) infection accounted for over 3 million deaths in 2007 and millions more are adversely affected each year[1]. However, current report indicates a major global reduction in malaria cases between 2000 and 2010 due to control efforts[2]. Nigeria has the largest population in Africa with a population of over 150 million and HIV prevalence of 4.6% in 2008[3]. It is estimated that 2.95 million individuals live with HIV/AIDS in Nigeria[4] and integrated control efforts are immeasurably needed[5, 6].

The prevalence of malaria and HIV infection overlaps in most endemic regions and co-infection of these infections have important public health implications. This geographical overlap of these infections has generated global interest in terms of their potential interactions and an integrated control effort in most endemic regions is essential. While early population-based studies reported no association between malaria and HIV co-infection[7-9], recent study from east sub-Saharan Africa indicated malaria as a risk factor of concurrent HIV infection at the population level[10]. Malaria is believed to increase HIV replication *in vitro*[11] and *in vivo*[12]. In

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addition, evidence shows that malaria co-infection with HIV triggers malaria disease progression, increases the risk of severe malaria in adults[13, 14], increases risk of congenital infection[15] and this dual infection fuels the spread of both diseases especially in sub-Saharan Africa[16]. This compelling evidence has called for integrated health sciences for early, effective and preventive treatment of both infections[17]. In pregnant women, HIV infection increases the risk of high-density *Plasmodium falciparum* infection, higher risk of maternal anaemia and low-birth weight[18]. HIV and malaria each interact with the host's immune system, and this interaction often results in a complex activation of immune cells which cause dysfunctional levels of cytokine and antibody productions[19]. In addition, $CD4^+$ T cells have a major role in the development and maintenance of anti-malaria immunity, but HIV infections meddle with this immunity[20].

The majority of studies on malaria and HIV co-infection in sub-Saharan Africa have been from eastern and southern part of the continent.

In a cross-sectional study, we examined the prevalence of malaria and HIV co-infection as well as anaemia among selected patients from three health-care institutions in Lagos.

Methods

Study design and population

Patients from three health-care institutions in Lagos – General Hospital Ikeja, Sexually Transmitted Diseases Clinic Yaba, and the Central Public Health Laboratory Yaba – were selected for this cross-sectional study between 1996 and 1997. The health-care institutions selected for the study were as a result of their strategic functions: (a) referral centres for HIV positive and STD patients, (b) client-based facilities serving people who want to know their HIV and STD status, and (c) provider-based facilities providing specific medical services to the communities.

The study was designed with the primary interests of investigating parasitic infection among HIV infected patients. Details of the study design have been published elsewhere[21]. Briefly, the same blood samples from the published work on entero-parasitic infections mentioned above were used for the screening of HIV, malaria parasite and haemoglobin obtained with informed consent from 1080 patients out of 2000 patients targeted for the study. Consent could not be obtained from 920 patients who declined to participate. Those who refused to be enrolled were all given appropriate clinical services and were excluded from the study. Information on questionnaires administered on the recruited patients included age, sex, symptoms, frequency of malaria attack and any anti-malarial drug taken in the past two weeks prior to the study. Parents or guardians of younger patients assisted in filling questionnaires. Written informed consent or thumbprints were received from all recruited individuals and the study received ethical approval from the Federal Ministry of Health Authority.

Laboratory procedures

Thick and thin blood smears from each of the study individuals were made on grease-free slides and stained with Giemsa to determine species of malaria parasites and parasite density according to the earlier published protocol[22]. Parasite densities were estimated by counting the number of *P. falciparum* malaria parasites (parasite count) per 200 leukocytes per high power field (number of parasites/µl of blood)[23]. All stained slides were examined by microscopy and read by two competent microscopists using 100 power fields under oil immersion. Where there were discordances, a third microscopist re-examined the slide. A definitive malaria diagnosis was determined by a reddish chromatin dot with a purple or blue cytoplasm of the malaria parasite seen together while a slide was pronounced negative when 100 high power fields have been examined using x100 oil immersion objective lens.

Haemoglobin (Hb) estimation was performed using cyanmethemoglobin method[24]. Malaria anaemia was defined as haemoglobin <11 g/dL in the presence of microscopically detectable asexual parasitaemia, while severe malaria anaemia was defined as Hb <5 g/dL, with *P. falciparum* parasitaemia of >250,000 parasites/µl. Blood sera of each of the patients were screened for HIV-1 and HIV-2 using the Cambridge Biotech Corporation Recombigen HIV-1/HIV-2 (Galway, Republic of Ireland) rapid test device while confirmation of positive cases was done with Immunocomb 11 (Yavne, Israel) and Biorad Novapath HIV-1 Immunoblot (Biorad, Hercules, CA, USA) for HIV-2 and HIV-1, respectively.

Statistical analysis

The obtained data were analysed using the Epi-Info statistical software version 6.0 (CDC, GA, USA) and GraphPad InStat (GraphPad Software Inc., CA,

USA). Univariable analyses were based on Pearson's Chi-square test for comparison of proportions. Fisher's exact tests for contingency tables were used to test for significance in proportions of categorical data when the expected cell counts were less than 5. Binary logistic regression was applied for the analysis of associations with haemoglobin which was used as the dependent variable against the explanatory variables (malaria and HIV/malaria co-infection) in multivariable analysis controlling for age, sex, parasite density and parasitic infections. Odds ratios (OR) with 95% confidence interval (CI) were used to measure the strength of associations. All tests were two-tailed and *P* value < 0.05 was considered statistically significant.

Results

Among the 1080 selected patients, 293 (27.1%) were infected with malaria parasites and 31 (2.9%) with malaria/HIV co-infection. The patients consisted of 570 (52.8%) males and 510 (47.2%) females. *P. falciparum* and *P. malariae* were the only two types of malaria parasite species found in the blood smear of the sampled patients. Infection with *P. falciparum* 24.8% (268/1080) recorded the highest prevalence among the patients when compared with *P. malariae* 0.6% (7/1080) and 1.7% (18/1080) had mixed infection with both parasites (Table 1). There were no significant differences between the prevalence of *P. falciparum* malaria in HIV sero-positive and sero-negative patients. However, the prevalence of *P. malariae* was statistically significantly higher in HIV sero-positive patients though the sample size was small. In addition, mixed malaria infection was also statistically significantly higher among HIV sero-positive patients when compared with HIV sero-negative patients (6.2% versus 1.4%). The total number of malaria infected patients was significantly higher in HIV sero-positive patients (47.7%) than the HIV sero-negative patients (25.8%) as indicated in table 1.

Table 1: Prevalence and species of *Plasmodium sp* infection in relation to HIV status of the study participants

Plasmodium sp	Total n=1080 (%)	HIV- n=1015 (%)	HIV+ n=65 (%)	P value
P. falciparum	268 (24.8)	243 (23.9)	25 (38.5)	0.082
P. malariae	7 (0.6)	5 (0.5)	2 (3.1)	<0.001*
Mixed infection	18 (1.7)	14 (1.4)	4 (6.2)	0.017*
Total no. of malaria infected patients	293 (27.1)	262 (25.8)	31 (47.7)	0.047

P values for univariable analysis were based on Pearson's Chi-square test. * P values derived by Fisher's exact test.

Among the respondents, analysis from the questionnaire showed that there were no major differences in the prevalence of parasitaemia with patients who reportedly used anti-malarial drugs two weeks prior to the study irrespective of their HIV status. In total, 4.2% (45/1080) of the recruited patients reportedly used anti-malarial treatment prior to the study and still had parasitaemia. Out of which 40% (18/45) were HIV sero-positive and 60% (27/45) HIV sero-negative patients. Although there were no statistical significance with the categories of parasite density in relation to the patients' HIV status, the median parasite density in HIV sero-negative patients was higher than that of the HIV sero-positive patients as shown in table 2.

Table 2: Malaria parasite density in relation to HIV status

Parasite density (No. of parasites µ/l)	**No. of malaria infected patients with** HIV+ n=31 (%)	HIV- n=262 (%)	***P* value**
0-999	23 (74.2)	200 (76.3)	
1000-9999	8 (25.8)	57 (21.8)	0.64
>10,000	0 (0)	5 (1.9)	
Median (Interquartile range)	754.43 (215-9882)	962.24 (35-36,248)	0.21

Analysis from the questionnaire also indicated that there were no statistically significant differences in the frequency of malaria attack between the HIV sero-positive and HIV sero-negative patients while it tended to be higher among HIV sero-positives.

Since anaemia is the most frequent haematological consequence of malaria and HIV infection, their association was explored. No selected patient had severe malaria anaemia during the study. Taken together, only 3.4% (37/1080) of the recruited patients had anaemia at the time of the study. However, patients with malaria and HIV co-infection had higher odds of anaemia than patients with malaria alone in multivariable logistic regression analysis (11.1% versus 25.8%), adjusted OR 2.4 (95% CI, 1.3 to 2.7, P = 0.014) as shown in table 3.

Table 3: Multivariable logistic regression analysis of the risk of anaemia with malaria and HIV co-infection

Category	Malaria (n = 262)	Malaria/HIV co-infection(n = 31)	Adjusted OR (95% CI)	P value
Haemoglobin<11 g/dl	29 (11.1)	8 (25.8)	2.4 (1.3 to 2.7)	0.014
Haemoglobine>11 g/dl	233 (88.9)	23 (74.2)	0.8 (0.3 to 3.1)	0.78

Adjusted for age, sex, parasite density and parasitic infections. OR = odds ratio, CI = confidence interval

Discussion

Our results underscore the higher prevalence of malaria infection in HIV sero-positive patients than HIV sero-negative patients. In addition, our data showed a 2.9% prevalence of patients co-infected with malaria and HIV. While some interactions between malaria and HIV have been studied extensively, individuals with HIV are considered to be at high risk of malaria in endemic areas[25]. Accumulating data have shown the effects of HIV-1 infection in adults and children on malaria[13, 26] and also in pregnant women[15]. In multigravid women, HIV appeared to impair a pregnant woman's ability to control malaria parasitaemia thereby resulting in high parasite density when compared to HIV negative pregnant women[27].

The trademark of malaria infection is anaemia, especially with *P. falciparum* infection. The aetiology of anaemia is usually multifactorial due to malnutrition, iron deficiency, vitamins A, C and B_{12} deficiency, foliate deficiency, sickle cell anaemia, thalassemia, HIV, bothriocephaliasis, malaria and other parasites such as hookworm, *Ascaris* and *Schistosoma*[28, 29]. However, in sub-Saharan Africa, malaria is a major contributor of anaemia[30]. Though it is demanding to measure all parameters in one study, our data indicated that patients infected with both malaria and HIV were more likely to have anaemia than those infected with malaria alone. Similarly, a study from Zambia showed that HIV-1 infected malaria patients had a slower haematological recovery after successful parasite clearance[31]. In another study, assessing haematological predictors of increased severe anaemia in Kenyan children, the authors indicated that malaria/HIV-1 co-infection was characterized by more profound anaemia and increased mortality[32]. In addition, patients infected with HIV have more frequent and severe episodes of malaria[33].

Our data showed no differences in the prevalence of patients with parasitaemia between HIV sero-positive and sero-negative patients who used anti-malarial drug two weeks prior to the study. Data have shown that HIV infected individuals with low CD4 cell counts and anaemia had increased risk of anti-malarial treatment failure[34]. In another study, viral burden in some patients can be partly reduced with anti-malarial therapy.[35] Our results indicated a higher median parasite density in HIV sero-negative patients than the HIV sero-positive patients. One would have expected the reverse due to the immunocompromised state of the HIV infected patients. One important limitation of this study is its cross-sectional nature, limiting us to a small sample size with patients co-infected with malaria and HIV. In view of this, our results should be interpreted with reference to the observed. Perhaps, a cohort study of HIV-infected patients in malarial endemic areas will reveal more information on the interaction between the two infections. Another limitation of this study is the inability to carry out a CD4 count and viral load on HIV-infected individuals due to limited resources. However, the clinical and public health implication of HIV and malaria, especially in endemic regions, is overwhelming.

Conclusion

Our data indicated a higher prevalence of malaria in HIV infected patients and also revealed that patients with malaria and HIV co-infection were more likely to have anaemia than patients with only malaria infection.

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