Prevalence of asymptomatic malaria in HIV-infected subjects on cotrimoxazole antimalarial prophylaxis attending a tertiary health care center in southern Nigeria: a cross-sectional study

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Abstract

Introduction **Co-infection has become a major contributor to increased burden of morbidity and mortality in HIV infection. The aim of this study was to assess the prevalence of asymptomatic malaria in the HIV-infected subjects on antimalarial prophylaxis and provide information to improve management of HIV subjects.**

Methods **This was a cross-sectional study with a purposive sampling. Microscopy method was used for the confirmation of malaria parasitemia status. The study was performed in University of Calabar Teaching Hospital, Calabar, Cross River State, Nigeria, a major tertiary health institution within the period of January to June 2016 involving 100 participants.**

Results **The majority (65%) of the study participants were females. The majority of the studied population belonged to the age range 33-38 years old. Most (45%) of the patients had CD4 count ≥500 cells/µL. The prevalence of asymptomatic malaria was found to be 13% (13/100). The distribution of asymptomatic malaria based on gender and age were found not to be statistically significant (P>0.05). Subjects with CD4 count in the range of 200-499 cells/µL had the highest prevalence (24.39%) of asymptomatic malaria.**

Conclusions **Considering that all the studied participants were on antimalarial prophylaxis, it signals a public health concern to employ more intensive preventive methods in addition to antimalaria prophylaxis.**

Keywords **Asymptomatic malaria, prevalence of malaria, malaria in HIV, malaria-HIV coinfection.**

Introduction

Human immunodeficiency virus (HIV)/AIDS, despite campaigns such as ("Getting to zero", "90-90-90", "Doing it", "Let's stop HIV together", "Act against HIV" and more), has remained a public health concern. As of 2016, 36.7 million persons were living with HIV worldwide. Approximately 0.8% of adults in the age range of 15 to 45 years are estimated to be living with HIV globally although the epidemic burden differs significantly among countries and regions. Sub-Saharan Africa has maintained the status of the most severely affected region with 1 in every 25 adults (4.2%) living with HIV, consequently representing approximately two-thirds of people living with HIV globally.[1] Nigeria accounts for 9% of persons living with HIV according to the 2014 Gap report, making Nigeria the second largest disease burden with 3.2 million persons living with HIV as of 2016 after South Africa, which has 7.1 million persons living with HIV constituting 19% of the global epidemic.[2,3]

Malaria has maintained its status as one of the most serious infectious disease of global concern. It is a vector-borne parasitic disease found in 91 countries globally (predominantly the tropics) transmitted through the bite of female *Anopheles*

Received: 14 October 2019; revised: 01 January 2020; accepted: 12 January 2020.

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Published March 2020

ISSN 2248 – 2997
ISSN – L = 2248 – 2997

mosquito.[4-6] Only 6 out of the more than 120 *Plasmodium* species that infect birds, mammals and reptiles, are known to regularly infect humans; *Plasmodium falciparum*, *P. vivax, P. malariae, P. ovale curtisi, P. ovale wallikeri* and *P. knowlesi*.[6] Nevertheless, the predominant species globally are *Plasmodium falciparum* and *P. vivax*. In view of the World Malaria Report of 2017, there were 216 million cases of malaria in 2016 of which 91% of the deaths were from the African region.[5] Children below 5 years of age, pregnant women and persons with immune compromised status such as HIV/AIDS bear the greatest burden of the illness.

In view of the considerable geographical overlap of HIV and malaria in Sub-Saharan Africa, coinfections are prevalent. Recent data show that combined malaria and HIV cause more than 2 million deaths annually.[7] The morbidity and mortality associated with malaria is expected to be increased by HIV infection, since immunosuppression affects the immune response to *Plasmodium*, determining more frequent occurrence of clinically severe malaria. However, daily cotrimoxazole prophylaxis has been recommended by World Health Organization (WHO) as a major strategy of preventing infection (including malaria)/opportunistic infections in HIV infected subjects in Sub-Saharan Africa.[8]

The aim of this study was to evaluate the prevalence of asymptomatic malaria in HIV-infected subjects on cotrimoxazole prophylaxis to provide information to improve management of patients with HIV infection.

Methods

Study area and design

This cross-sectional study was performed in University of Calabar Teaching Hospital, Calabar, Cross River State, Nigeria, a major tertiary health institution, within the period of January to June 2016. The studied subjects were all HIV-infected subjects on antiretroviral therapy (ART) and on anti-malaria prophylaxis with cotrimoxazole. Purposive sampling technique was used: patients living with HIV were consecutively recruited into the study as they came for consultation to collect ART.

Study population

HIV-infected patients attending the University of Calabar Teaching Hospital HIV Clinic for their routine ART served as the study subjects. All HIV positive patients who were on anti-malaria prophylaxis (cotrimoxazole) and without clinical malaria symptoms who gave their consent were included. Children (minors) in the study were recruited following written, informed consent from the parent/caregiver. HIV-infected subjects who were not on anti-malaria prophylaxis were excluded. An asymptomatic malaria subject was defined as a person with no current symptoms and/or signs of malaria, who showed laboratory confirmation of parasitemia.

Sample size determination

The sample size was calculated based on an expected malaria parasitemia prevalence of 2.11% with 5% alpha-type error (95 confidence interval).[9] The expected prevalence was based on the assumption that the prevalence of this study will be comparable with the last prevalence of the most identical study design in Nigeria as at the time of study none had been done in Calabar. The literature review showed varying prevalence of asymptomatic malaria, between 2.11% and 7.8% giving sample sizes of 32 and 110, respectively. Considering the similarity of design, 2.11% was used for the calculation. Considering attrition, 100 subjects were selected.

Sample collection

Four (4) milliliters of blood were collected into K_2EDTA bottle from the subjects that gave their consent. The blood sample was used for CD4 count and microscopy for malaria parasitemia assay and was analyzed within 1 hour of sample collection.

Microscopy for malaria parasites

Thick and thin blood films were prepared and stained with Giemsa stain and examined under the microscope using 100X objective with

oil immersion according to the guidelines of the World Health Organization.

Determination of CD4 count

The CD4 count was analyzed using Partec cytometer by Partec CyFlow, Munster, Germany.

Data analysis

The data collected were analyzed using SPSS version 20 software (IBM Corp., Armonk, NY, USA) and Stata software. Frequencies and percentages were used to summarize categorical demographic and clinical variables. Chi square test for association, Chi square test for trend analysis and Fisher exact tests were used to assess association between categorical variables. Statistical significance was assessed using an alpha value of 0.05.

Ethical clearance

This study was approved by the Health Research Ethical Committee (HREC) of the University of Calabar Teaching Hospital. Informed consent was obtained from the study participants or from the parent (caregiver) in cases of children (minors).

Results

One hundred HIV-infected subjects in all were recruited. Females constituted 65% of the study subjects while males were 35%. The majority (20% and 19%) of the studied subjects were in the age ranges of 33-38 years and 27-32 years, respectively. The average age was found to be 39.3±10.4 years. Most (86%) of the studied subjects had CD4 count ≥200 cells/μL. Further stratification showed 14%, 41% and 45%, of the subjects with a CD4 count of <200 cells/μL, 200-499 cells/μL and ≥500 cells/μL, respectively (Table 1).

Table 2 shows the distribution of asymptomatic malaria based on gender. The overall prevalence of asymptomatic malaria in the study was observed to be 13%. The prevalence of asymptomatic malaria was found to be 8.6% (n=3), 15.4% (n=10) in the males and females, respectively. However, gender was not significantly ($p>0.05$) associated with asymptomatic malaria.

Table 3 shows the distribution of asymptomatic malaria based on age. The majority (50.0%, 18.2% and 15.8%, respectively) of the asymptomatic malaria subjects were in the 15-20, 21-26 and 27-32 years age range. We observed that age was not associated with the distribution of asymptomatic malaria ($p\geq0.05$).

Table 1. Demographic characteristics of the HIV-infected subjects studied

Parameter	Number	Percentage
Gender		
Male	35	35
Female	65	65
Age (years)	39.3±10.4*	
Age group (years)		
15-20	2	2
21-26	11	11
27-32	19	19
33-38	20	20
39-44	17	17
45-50	15	15
51-56	16	16
CD4 category		
CD4 <200 cells/μL	14	14
CD4 200-499 cells/μL	41	41
CD4 ≥500 cells/μL	45	45

*Value represents mean ± standard deviation.

Out of the hundred HIV-infected subjects screened, those with CD4 ≥500 cells/μL, 200-499 cells/μL and <200 cells/μL, had prevalence of asymptomatic malaria of 6.7% (n=3), 24.4% (n=10) and 0.0% (n=0), respectively (Table 4). The CD4 category was found to be associated with the prevalence of asymptomatic malaria prevalence. However, the trend was found not to be linear.

Discussion

The demographic data of this study revealed more females than males living with HIV (1:1.8; M vs F). A higher incidence of HIV infection among females is well documented.[10-12] Prenatal screening via prevention of mother-to-child transmission programme in women of childbearing age might lead to earlier detection and subsequent presentation for ART in females, partly explaining the reason for an apparently higher incidence of HIV in females than in

Table 2. Distribution of asymptomatic malaria based on gender

Gender	No. examined	No. infected (%)	No. uninfected (%)	P-value
Males	35	3 (8.6)	32 (91.4)	0.534*
Females	65	10 (15.4)	55 (84.6)	
Total	100	13 (13.0)	67 (67.0)	

*Fisher's exact test.

Table 3. Distribution of asymptomatic malaria based on age

Age group (Years)	No. examined	No. infected (%)	No. uninfected (%)	P-value
15-20	2	1 (50.0)	1 (50.0)	0.692[a]
21-26	11	2 (18.2)	9 (81.8)	
27-32	19	3 (15.8)	16 (84.2)	
33-38	20	2 (10.0)	18 (90.0)	
39-44	17	2 (11.8)	15 (88.2)	
45-50	15	2 (13.3)	13 (86.7)	
51-56	16	1 (6.3)	15 (93.8)	

*Fisher's exact test.

Table 4. Distribution of asymptomatic malaria based on CDC staging of HIV

CDC category	No. examined	No. infected (%)	No. uninfected (%)
Category 1 (CD4 ≥500 cells/µL)	45	3 (6.7)	42 (93.3)
Category 2 (CD4 200-499 cells/µL)	41	10 (24.4)	31 (75.6)
Category 3 (CD4 <200 cells/µL)	14	0 (0.0)	14 (100.0)

$X^2(2)=8.391$, p=0.015. X^2 trend(1)=0.190, p=0.663

X^2 – Chi square. **X^2 trend** – Chi square test for trend analysis (Chi square linear-by-linear association/Mantel-Haenszel test of trend).

males.[13] Also, the female genital anatomy has been postulated to favor transmission of the virus in females compared to males.[14]

The prevalence of asymptomatic malaria among HIV-infected subjects in this study was approximately 13.0%. Although comparatively low and similar to 17.78% earlier reported in the general population, this observation is worrisome considering that all the subjects were on anti-malaria prophylaxis. Cotrimoxazole has been shown to reduce malaria prevalence by two-fold; however, it is not very effective for malaria treatment.[4,15] This is possibly due to poor adherence to prophylaxis drug. Although the drugs are free for HIV subjects and the subjects collect them on routine visits, poor adherence has been well documented among subjects on antimalaria prophylaxis.[9,16-20] Reasons reported for poor adherence include forgetfulness, perceived side effects, not seeing mosquitoes, peer advice, and lack of knowledge of the consequences of non-adherence.[20] This value is similar to 7.8% and 7.3% from studies in Kogi, Nigeria and Cameroon, respectively.[8,21] However, the value is higher than 2.11% reported in a study from Benin, Nigeria.[9] Conversely, the value is lower than 47.7%, 24%, 32.2%, 18.5% reported in Lagos, Jos, Kano, Nnewi and Osun, respectively, being studies in Nigeria, and 15.5%, 20% and 61.7% in studies in Ghana, and South Africa, respectively.[16-20,22,23] Malaria parasitemia in HIV infection is worrisome considering the fact

that the patients are already immune compromised, and HIV and *Plasmodium* interrelate with the host's immune system, yielding complex activation of immune cells giving rise to dysregulated levels of antibodies and cytokine generation. Malaria also, has been reported to increase HIV replication both in vitro and in vivo.[24]

The difference in the malaria-HIV co-infection in our study and the other studies could be due to geographical differences in the studied populations, differences in the level of malaria endemicity, health seeking attitude of the patients with HIV infection, disparity in adherence to prophylaxis therapy and the study designs and sample sizes. Our study participants were all on cotrimoxazole prophylaxis and were comprised only of asymptomatic subjects whereas the studies in Jos (n=100), Kano (n=363), Benin (n=285), Osun (n=200), Ghana (n=220) and South Africa (n=336), were comprised of both symptomatic and asymptomatic malaria infections.[17-20,22,23] Furthermore, the studies were silent on the percentage of the studied participants that were on anti-malarial prophylaxis except for the studies in Benin, Kano and Mozambique where 100%, 53.5% and 35.4%, respectively of the studied subjects were on anti-malarial prophylaxis.[9,18,21] The study in Gabon was solely on asymptomatic malaria but only 38.3% of the HIV-infected subjects were on cotrimoxazole prophylaxis.[15] Trimethoprim-sulfamethoxazole, which is the active ingredient of cotrimoxazole, has been documented to be effective against malaria as well as other opportunistic infections.

We observed higher prevalence of asymptomatic malaria among the female HIV-infected subjects, however, this difference was not statistically significant. The finding is in consonance with previous reports.[8,18] Conversely, Akotet and colleagues reported a significantly higher value in the females in a similar study in Gabon.[15] This difference is possibly due to disparity in the health seeking behavior in both genders in the two study areas, disparity in adherence to preventive measures such as antimalarial treatment/prophylaxis and use of insecticide treated net which this study didn't assess.[15]

The prevalence of HIV and asymptomatic malaria co-infection observed between the different age groups showed no significant difference as similarly observed by Njunda and colleagues.[12]

CD4 cell count less than 200 cells/μL signifying the advanced stage of HIV infection (AIDS) was observed in 14% of the studied subjects. The prime reason for this low proportion of AIDS is possibly early detection and treatment stemming from the facts that the majority of the participants were females in whom prenatal screening had aided early detection, and the effects of various intervention programmes on HIV in the form of voluntary counseling and testing in the region.[13]

A higher proportion of the patients (24.39%) of the HIV subjects with asymptomatic malaria infection had CD4 count within 200-499 cell/μL, while none, and lower prevalence, were seen in those with CD4 <200 cells/μL and >500 cells/μL, respectively. This observation partly differs from previous reports in which the highest prevalence of malaria parasitemia was recorded in the <200 cells/μL CD4 category.[12,15,17] The reason for this variation could be attributed to the choice of participants (study design). Our study assessed HIV-infected subjects on their routine clinic visit for antiretroviral therapy, asymptomatic for malaria, whereas the other studies assessed both symptomatic and asymptomatic malaria infection. A CD4 cell count less than 200 cells/μL represents the advanced stage of HIV infection (AIDS) and is associated with higher risks of opportunistic infections and disease progression. Consequently, most (if not all) the subjects with CD4 count less than 200 cells/μL would be symptomatic and would be in the family medicine department for treatment rather than in the HIV Clinic for routine ART. In HIV-positive adults, opsonizing antibodies and variant surface antigens antibodies against malaria parasites are generally decreased with lower CD4; opsonizing antibodies are also negatively correlated with the viral load, suggesting that functional antibodies to malaria

antigens are particularly affected by the degree of immune suppression.[25]

This study has a number of potential limitations. We were not able to accompany microscopic blood smear assessment of malaria parasitemia with a PCR assay; the latter would have been more sensitive. However, microscopic blood smear remains the gold standard in resource-limited laboratories.

Conclusions

A prevalence of 13.0% for asymptomatic malaria was observed in this study. Considering the already immune compromised status of the subjects, the value raises a public health concern. This calls for more intensive preventive measures such as use of insecticide treated nets and environmental fumigation, considering the fact that all the subjects were on antimalarial prophylaxis. There is also need to assess the possibility of drug resistance development.

Authors' contributions statement: EFE conceived the study, designed the study, performed analytical procedures, analyzed data and edited the initial manuscript; TYM participated in the analytical procedure, and analyzed data. HUO analyzed data, performed statistical analysis, performed literature search, and wrote the initial manuscript draft. All authors read and approved the final version of the manuscript.

Conflicts of interest: All authors – none to declare.

Funding: This study was funded by the authors.

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Please cite this article as:
Ekere EF, Mirabeau TY, Okoroiwu HU. Prevalence of asymptomatic malaria in HIV-infected subjects on cotrimoxazole antimalarial prophylaxis attending a tertiary health care center in southern Nigeria: a cross-sectional study. GERMS 2020;10(1):44-50. doi: 10.18683/germs.2020.1184