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Circulating *Plasmodium falciparum* and HIV 1/2 as Co-infections among Blood Donors in Ibadan, Southwestern Nigeria

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ABSTRACT: Blood serves as a vehicle for transmission of blood-borne pathogens including hemoparasities. In Nigeria, screening of blood for blood-borne pathogens does not fulfill the standard protocols and screening for malaria parasites is not practiced. Determination of the prevalence of circulating *Plasmodium falciparum* and HIV as co-infections in a population in general, and blood-donors in particular will certainly help in reviewing the screening procedures and making health policy decisions. In view of the problem of transfusional malaria, the prevalence of malaria Plasmodium in consenting blood donors was assessed. Whole blood was used for the diagnosis of P. falciparum malaria using Malaria P. falciparum Rapid Test Device. The screening for HIV antibodies was carried out using Chembio HIV-1/2 Stat-Pak® and Abbott Determine HIV-1/2® test. All tests were done according to the manufacturers' specifications. Overall prevalence rate of asymptomatic P. falciparum malaria was 17.5% (n=35) and HIV as co-infection was 22.9% (n=8), while the remaining 27(77.1%) had no HIV. The study showed no significant difference between malaria infection either for age groups (17.8% vs 16.6%, P>0.05) or sexes (16.6% vs 22.6%, P > 0.05). However, it showed a significantly higher prevalence of HIV as co-infection among blood donors within ages less than 40 years than their counterparts in age groups 40 years and above (29.6% vs. 0.0%; P = 0.001). It also showed a significantly higher prevalence of HIV as co-infection among female donors than males (42.9% vs. 17.9%; P = 0.001). This study however confirmed the presence of P. falciparum malaria infection and HIV as co-infection among blood donors in Ibadan, Nigeria. This could be attributed to lack of adequate accommodation and poor sanitary conditions in the area under study. General surveillance and public health education to stop the spread of the infection among blood donors in Ibadan and indeed the whole society is advocated.

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1. INTRODUCTION

The epidemic of human immunodeficiency virus (HIV) in areas of the tropics where *Plasmodium falciparum* is endemic has generated serious concern about potential interactions between the two infections (Greenberg et al., 1991). Malaria remains one of the leading causes of morbidity and mortality worldwide, causing about 3000 deaths per day (Okonko et al., 2010).

Malaria and HIV are the major priority medical challenges currently facing sub-Saharan Africa, and yet little has been known on the clinical and public health implications of their co-infections (Addissie et al., 2007). As HIV spreads, it interacts with other infectious diseases, facilitated by the increase in numbers of immunosuppressed individuals and because its own clinical course can be altered by other infections (UNICEF, 2003).

Many countries in sub-Saharan Africa currently report high prevalences of both human

immunodeficiency virus (HIV) and Plasmodium falciparum malaria (Brentlinger et al., 2007). The likelihood of HIV-malaria coinfection may affect clinical management of patients. The extent to which standard clinical guidelines address HIVmalaria coinfection is unclear (Brentlinger et al., 2007). In more recent years, three key issues have focused much of the research effort (UNICEF, 2003): Does HIV/AIDS increase susceptibility to malaria infection or increase severity of acute malarial episodes? Does malaria infection accelerate progression of HIV/AIDS? And what is the impact of malaria and HIV co-infection during pregnancy? Mounting evidence has revealed pathological interactions between HIV and malaria in dually infected patients, but the public health implications of the interplay have remained unclear (Abu-Raddad et al., 2006). HIV-related immune-suppression increases the risk of malaria (infection, disease and treatment failure) and probably the circulating parasite biomass, favoring the emergence of drug resistance parasites (Van Geertruyden et al., 2008).

In view of its status as a state capital, Ibadan metropolis is growing rapidly, and medical cases requiring blood transfusion has expectedly increased. The quality of donor blood particularly with respect to infectious diseases including malaria and HIV would therefore attract great concern. In this study, the prevalence of circulating *Plasmodium falciparum* and possible co-infections with HIV-1/2 antibodies was examined in blood donors.

2. MATERIAL AND METHODS 2.1. Study Area

The study area is the Blood Grouping & Serology Unit, University College Hospital (UCH), located at the municipal area of Ibadan, which is made up of five local government areas. Ibadan is the capital city of Oyo State located in the forest zone of southwestern Nigeria. Ibadan city lies on the longitude 3°5' East of Greenwich meridian and latitude 7°23' North of the Equator. Besides being the largest indigenous city in Africa south of Sahara, the city is an important trade and educational centre. It also houses one of the largest and foremost teaching hospitals in Africa. However, the city is characterized by low level of environmental sanitation, poor housing, and lack of potable water and improper management of wastes especially in the indigenous core areas characterized by high density and low income populations.

2.2. Study Population

A total of 200 blood samples were collected from the Blood Grouping and Serology Unit, University College Hospital, Ibadan, South-Western, Nigeria.

2.3. Demographic Information

The study groups were stratified by Age and sex. Screenings of other infectious agents were done before bleeding them (Salawu and Murainah, 2006). Table 1 summarizes the age and sex characteristics of Nigerian donors used in this study.

 Table 1: Demographical Characteristics/Parameters

 of the Blood Donors

Parameters	No. Tested
Age Group (years)	
18-39	152
40 and above	48
Sex	
Males	169
Females	31
Total	200

2.4. Sample Collection

The method of sample collection employed was venepuncture technique (Cheesbrough, 2006). When sufficient blood had been collected, the blood was transferred into an EDTA bottle. This was centrifuged and the plasma was then pipetted into sterile ependorf tubes and stored at -20° C until ready for use.

2.5. Detection of *Plasmodium falciparum*

Whole blood was used for the diagnosis of P. falciparum malaria using parallel Malaria Plasmodium falciparum Rapid Test Device (manufactured by Global device, USA and IND^R Diagnostica, USA). The malaria P.f. Rapid Test Device (Whole Blood) is a qualitative, membrane based immunoassay for the detection of P.f antigen in whole blood. The principle is based on a rapid chromatographic immunoassay for the qualitative detection of circulating P. falciparum antigen in the whole blood. This method utilizes Gold conjugate to selectively detect *Plasmodium* antigen. The procedure was as described by the manufacturer. After 15 min the results were read. The test device has inherent quality control that validates the result. The presence of two pink lines at the region of the control and test sample signifies presence of P. falciparum malaria infection while the presence of only one pink line in the control region signifies absence of P. falciparum. The Malaria P.F. Rapid Test Device (Whole Blood) has tested with thin or thick blood smears on clinical samples. The results show that the sensitivity of the Malaria P.f. rapid test Device (Whole Blood) is >99.0% relative to blood smears. The malaria P.f Rapid test Device (Whole Blood) uses an antibody that is highly specific for malaria P.f. antigen in Whole Blood The results show that the specificity of the Malaria P.f. Rapid Test device Whole Blood is >99.0% relative to blood malaria.

2.6. Detection of Antibodies to HIV-1 AND -2 in Human Plasma

Two different methods were used namely Chembio HIV-1/2 Stat-Pak[®] by CHEMBIO DIAGNOSTICS SYSTEMS, INC. Medford, New York 11763 USA; sensitivity 100% (129/129) and specificity 100% (207/207)] and Abbott DetermineTM HIV-1 & 2 test kit [by ABBOTT JAPAN CO.,LTD. Minto-Ku, Tokyo, Japan; sensitivity 100% and specificity 100%]. These are an *in vitro* visually read immunoassay and immunochromatographic test for the qualitative detection of antibodies to HIV-1 & 2 in human plasma. For the Chembio HIV-1/2 StatPak[®] and Abbott determineTM HIV-1 & 2, the procedure as described by the manufacturers was used for the analysis. The results of the tests were read at zero minute after the addition of the running buffer. This method has inherent quality control that validates the results. The presence of two pink/purple lines in the region of test sample and control indicates HIV seropositive reaction while a single pink/purple line at the control region indicates HIV seronegative reaction. HIV seropositive results' using these two methods was used to classify participants as presenting with HIV infection.

2.7. Data Analysis

The prevalence for *Plasmodium falciparum* and possible co-infection with HIV was calculated by using patients with positive samples as numerator and the total numbers of patients enrolled in this study as denominator. The data generated from this study were presented using descriptive statistics. The data was subjected to Fisher's Exact Test for comparison of proportions to determine any significant relationship between infection rate, age and gender using SPSS computer software version 19.0 for Windows.

3. RESULTS ANALYSIS

A total of 200 blood donors from different households in different communities and locations of Ibadan were enrolled in the study. Rapid ELISA techniques at the Medical Microbiology and Virology Unit, Lead City University, Ibadan, Nigeria identified 35(17.5%) with *Plasmodium falciparum* infection. Out of the 35 (17.5%) asymptomatic *P. falciparum* positive subjects; 8 (22.9%) had HIV-1/2 antibodies as co-infection while the remaining 27(77.1%) had no HIV (Table 2).

3.1. Prevalence rates of *Plasmodium falciparum* and HIV as co-infection in relation to age

Table 2 showed the prevalence of *Plasmodium falciparum* and HIV as co-infection in relation to the ages of the blood donors in Ibadan, Nigeria. It showed that there was no significant difference between age groups for either malaria infection (17.8% vs 16.6%, P>0.05). However, donors less than 40 years of age had a significantly higher prevalence of HIV co-infection (29.6% vs. 0.0%; P = 0.001) as shown in Table 2.

3.2. Prevalence rates of *Plasmodium falciparum* and HIV as co-infection in relation to sex of the subjects

Table 3 shows the prevalence of *Plasmodium falciparum* and HIV as co-infection in relation to the sexes of blood donors in Ibadan, Nigeria. It showed that there was no significant difference between sexes for malaria infection (16.6% vs 22.6%, P >0.05). However, female donors had a significantly higher prevalence of HIV co-infection (42.9% vs. 17.9%; P = 0.001) as shown in Table 3.

	Plasmodium falciparum Infection					
Age groups (years)	No. Tested (%)	No. Positive (%)	Co-infection with HIV (%)	No. Negative (%)	No. positive for HIV (%)	
18-39	152(76.0)	27(17.8)*	8(29.6)**	125(82.2)	20(16.0)*	
40 and above	48(24.0)	8(16.6)*	0(0.0)**	40(83.3)	7(17.5)*	
Total	200(100.0)	35(17.5)	8(22.9)	152(82.5)	27(17.8)	
Kov * = Not	Significant (P>0.04	5)• ** = Significa	nt			

Key: * = Not Significant	(<i>P>0.05</i>); ** = Signifi	cant
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 Table 3: Prevalence rates of Plasmodium falciparum and HIV as co-infection in relation to sex of the subjects

Plasmodium falciparum Infection					
No. Tested (%)	No. Positive (%)	Co-infection with HIV (%)	No. Negative (%)	No. Positive for HIV (%)	
169(49.8)	28(16.6)*	5(17.9)**	141(83.4)	24(17.0)*	
31(50.2)	7(22.6)*	3(42.9)**	24(77.4)	3(12.5)*	
200(100.0)	35(17.5)	8(22.9)	165(82.5)	27(17.8)	
	169(49.8) 31(50.2)	No. Tested (%) No. Positive (%) 169(49.8) 28(16.6)* 31(50.2) 7(22.6)*	No. Tested (%) No. Positive (%) Co-infection with HIV (%) 169(49.8) 28(16.6)* 5(17.9)** 31(50.2) 7(22.6)* 3(42.9)**	No. Tested (%) No. Positive (%) Co-infection with HIV (%) No. Negative (%) 169(49.8) 28(16.6)* 5(17.9)** 141(83.4) 31(50.2) 7(22.6)* 3(42.9)** 24(77.4)	

Key: * = Not Significant (P>0.05); ** = Significant

4. DISCUSSION

This study examined the prevalence of circulating *Plasmodium falciparum* and possible co-infections with HIV-1/2 antibodies in blood donors. In Nigeria, screening of blood for blood-borne pathogens

do not fulfill the standard protocols for blood donations and screening for malaria parasites is not practiced during blood donation. The diagnosis of *P. falciparum* infection using *P. falciparum* antigen has been widely accepted as a rapid antigen test for *P. falciparum* malaria (Onyenekwe et al., 2007). Its accuracy has also been put at 86–99% compared with microscopic detection of malaria parasites in smears (Mens et al., 2007) as with very high specificity. It has been recommended for use where microscopic detection of malaria parasites in smears is not possible. However, one of the limitations is that the malaria antigen may still be detected after treatment has been effected with successful clearance of parasites from blood (Onyenekwe et al., 2007).

The result of this study showed 17.5% prevalence of malaria among blood donors, which is higher than the prevalence reported elsewhere. The possible reason for the high prevalence of malaria parasites among blood donors in this study is the sampling period (March to September, 2011) as explained by the seasonal changes in mosquito density (Nega, 1993). The prevalence of P. falciparum malaria in these subjects is a reflection of the prevalence of this species of malaria in the population. Similar prevalence has been reported from Nigeria amongst blood donors by Erhabor et al. (2007) who observed a prevalence rate of 10.2% amongst the blood donors. Similar, prevalence has also been reported in Cameroon showing changes in prevalence with age (Bigoga et al., 2007) and from rural area in southern Mozambique (Mayor et al., 2007). The 17.5% prevalence reported for malaria in this study is higher than the 10.0% reported by Cohen et al. (2005) among HIV-infected adults in South Africa.

The study also showed that among the asymptomatic *P*. falciparum malaria-infected subjects, the prevalence rate of HIV/malaria co-infection was 22.9%. This prevalent rate is closely similar to that observed amongst previous studies (Onvenekwe et al., 2007). Contrastingly, female donors had a significantly higher prevalence of HIV co-infection (42.9% vs. 17.9%; P = 0.001). This was doubled compared with their male counterparts with 17.9% prevalence of HIV/malaria coinfection. Donors less than 40 years of age had a significantly higher prevalence of HIV co-infection (29.6% vs. 0.0%; P = 0.001). Thus, this very high rate of HIV co-infection in malaria-infected subjects calls for public health concern (Onyenekwe et al., 2007). Studies reported elsewhere from malaria endemic area with unstable transmission throughout the year showed that subjects with malaria infection had 10.1% and 29.9% prevalence for HIV respectively (Grimwade et al., 2003, 2004). In a study by Addissie et al. (2007), the HIV serostatus assessment revealed that 4.2% of the P. falciparum patients were seropositive for a single ELISA HIV test. The 22.9% reported for HIV/malaria co-infection in this study is lower than the 33.0% prevalence of HIV infection reported by Cohen et al. (2005) in South Africa and the 33.0% of the patients

who were reported to be nonimmune to malaria (Cohen et al., 2005).

Conflicting reports exist regarding the impact of HIV on the risk of severe malaria (Cohen et al., 2005). Co-infection might also have facilitated the geographic expansion of malaria in areas where HIV prevalence is high. Hence, transient and repeated increases in HIV viral load resulting from recurrent co-infection with malaria may be an important factor in promoting the spread of HIV in sub-Saharan Africa (Abu-Raddad et al., 2006). In a study by Van Geertruyden et al. (2008), a model shows that in 2005 HIV-1 increased the overall malaria parasite biomass by 18.0% in southern Africa. According to Van Geertruyden et al. (2008), the HIV-1 epidemic by increasing the malaria parasite biomass in sub-Saharan Africa may also increase the emergence of antimalarial drug resistance, potentially affecting the health of the whole population in countries endemic for both HIV-1 and malaria. HIV-infected nonimmune adults are at increased risk of severe malaria. This risk is associated with a low CD4+ T cell count. This interaction is of great public health importance (Cohen et al., 2005).

The study showed that there was no significant difference between sexes and age groups for malaria infection (16.6% vs 22.6%, P >0.05) and (17.8% vs 16.6%, P>0.05) respectively. Malaria can affect all the age humans groups and both male and female sexes. This is consistent with previous reports (Atif *et al.*, 2009; Abdullahi *et al.*, 2009); however, it was not consistent with previous studies which documented higher prevalence of malaria in females (Ibekwe *et al.*, 2009; Okonko *et al.*, 2009). There is no scientific evidence to prove the higher prevalence being related to gender as susceptibility to malaria infection is not influenced by gender (Abdullahi *et al.*, 2009).

Generally, with regards to the age distribution of the blood donors, our findings showed that the highest prevalence of malaria was found in age groups less than 40 years of age. This also correlate well with the findings of Atif *et al.* (2009) who reported infection rate to be higher among young adult in Pakistan. According to some authors (Perlmann and Troye-Blomberg, 2000; Plebanski and Hill, 2000; Abdullahi *et al.*, 2009), there is slow acquisition of active immunity to malaria. Thus, high prevalence of malaria infections in older adults suggests that they have lost some degree of immunity as a result of poor living conditions other than lifelong exposure.

This study has shown that a good percentage of the blood donors were infested by P. falciparum. Majority of the blood donors in this study belonged to the urban areas of Ibadan. This is contrary to the findings of Atif et al. (2009) who reported malaria infections to be prevalent among majority of patients belonging to the rural areas of Hyderabad, in Pakistan. The relatively high prevalence of malaria infection in this study may have been triggered by the failure of antimalarial drugs. The high prevalence of *P. falciparum* observed in this study is worrisome because high-density urban African populations are not often considered particularly vulnerable to malaria infection. Prompt and accurate diagnosis of malaria is the key to effective disease management and therefore it is one of the main interventions of the global malaria control strategy (WHO, 1993).

One of the limitations of this study is that the study was conducted using one tertiary health centre, though located in the strategic location of the township; these results may reflect what is happening in other heath centres in the metropolis. However, the results obtained are within limits compared to similar researches (Ibekwe *et al.*, 2009; Abdullahi *et al.*, 2009; Okonko *et al.*, 2009, 2010). This base-line data could be useful in effective planning of tailor-made prevention and control measures in the Ibadan and other similar townships in the Southwestern States of Nigeria.

5. CONCLUSION

The HIV prevalence among P. falciparum positive blood donors was not different from HIV prevalence in the general population. In conclusion, the high rate of malaria and HIV/malaria co-infection in the blood donors was quite worrisome. This is a reflection of the high rate of asymptomatic malaria parasitaemia and HIV/malaria co-infection in endemic malaria regions. The implication of this with regard to blood transfusion is enormous. One in three blood transfusions carries the risk of transmitting malaria parasites and HIV as coinfection to the recipients. However, identification of the species of malaria parasite is very important for its effective and curative treatment as resistance to chloroquine and other anti-malarial drugs has been reported previously (Atif et al., 2009). Further studies with carefully designed methodologies are recommended. Further research is urgently needed to define best practices for prevention, diagnosis, and management of HIV-malaria coinfection in this region.

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