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

Okonko IO[1], Adejuwon OA[2], Okerentugba PO[1] and Innocent-Adiele HC[1]

[1]Medical Microbiology Unit, Department of Microbiology, University of Port Harcourt, P.M.B. 5323 Uniport Post Office, Choba, East-West Road, Port Harcourt, Rivers State, Nigeria;
[2]Department of Microbiology, Lead City University, Ibadan, Oyo State, Nigeria;
mac2finney@yahoo.com, iheanyi.okonko@uniport.edu.ng; Tel.: +234 803 538 0891

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.

[Okonko IO, Adejuwon OA, Okerentugba PO and Innocent-Adiele HC. **Circulating *Plasmodium falciparum* and HIV 1/2 as Co-infections among Blood Donors in Ibadan, Southwestern Nigeria.** *Nat Sci* 2012;10(9):42-47]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 6

Keywords: Antibody, HIV/malaria co-infection, seropositivity, blood donors, *Plasmodium*, prevalence, transfusional malaria

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 HIV-malaria 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^{0}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 Determine™ 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 Stat-

Pak® 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.

Table 2: Prevalence rates of *Plasmodium falciparum* and HIV as co-infection in relation to age

Age groups (years)	*Plasmodium falciparum* Infection				
	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)**

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

Table 3: Prevalence rates of *Plasmodium falciparum* and HIV as co-infection in relation to sex of the subjects

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

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 (Onyenekwe 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 co-infection. 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 co-infection 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.

ACKNOWLEDGEMENTS

We acknowledge the permission and assistance of the Miss OA Abraham, The Deputy Director, Department of Haematology, University College Hospital, Ibadan and the assistance of Mr. JA Alli, The Principal Medical Laboratory Scientist, Department of Medical Microbiology & Parasitology, University College Hospital, Ibadan. The authors sincerely appreciate the assistance of Miss. OS Salinsile, of the Department of Microbiology, Lead City University, Ibadan, who assisted in the collection of these samples and analysis.

CORRESPONDENCE TO:
Iheanyi O. Okonko;
Department of Microbiology,
University of Port Harcourt,
East/West Road, P.M.B 5323, Choba,
Port Harcourt, Rivers State, Nigeria;
E-Mail: mac2finney@yahoo.com;
iheanyi.okonko@yahoo.com;
Tel: +234-080-3538-0891

REFERENCES

1. Abdullahi K, Abubakar U, Adamu T, Daneji AI, Aliyu RU, Jiya N, Ibraheem MTO, and Nata'ala SU. 2009. Malaria in Sokoto, North Western Nigeria. *African Journal of Biotechnology; 8 (24)*: 7101-7105.
2. Abu-Raddad LJ, Patnaik P, Kublin JG. 2006. Dual infection with HIV and malaria fuels the spread of both diseases in sub-Saharan Africa. Science;314(5805):1603-1606.
3. Addissie A, Sellassie FE, Deressa W. 2007. Malaria and HIV co-infection in Hadya Zone, southern Ethiopia. Ethiop Med J. 45(1):9-17.
4. Atif SH, Farzana M, Naila S, Abdul FD. Incidence and Pattern of Malarial Infection at a Tertiary Care Hospital of Hyderabad. *World J. Medical Sciences,* 2009; *4 (1)*: 09-12
5. Baye G and Yohannes M. (2007). The prevalence of HBV, HCV and malaria parasites among blood donors in Amhara and Tigray regional states. *Ethiop.J.Health Dev.*;22(1):3-7.
6. Bigoga JD, Manga L, Titanji VP, Coetzee M, Leke RG. Malaria vectors and transmission dynamics in coastal southwestern Cameroon. *Malar J* 2007; Jan 17, *6:* 5.
7. Brentlinger PE, Behrens CB, Kublin JG. 2007. Challenges in the prevention, diagnosis, and treatment of malaria in human immunodeficiency virus infected adults in sub-Saharan Africa. Arch Intern Med. 167(17):1827-1836.

8. Cheesebrough M (2006). District Laboratory Practice in Tropical Countries, part 1. University Press, Cambridge, pp. 239-258.
9. Cohen C, Karstaedt A, Frean J, Thomas J, Govender N, Prentice E, Dini L, Galpin J, Crewe-Brown H. 2005. Increased prevalence of severe malaria in HIV-infected adults in South Africa. Clin Infect Dis.;41(11):1631-1637.
10. Erhabor O, Ok O, Awah I, Uko KE, Charles AT. The prevalence of Plasmodia parasitaemia among donors in the Niger Delta of Nigeria. *Trop Doc* 2007, *37*(1): 32–34.
11. Greenberg AE, Nsa W, Ryder RW, Medi M, Nzeza M, Kitadi N, Baangi M, Malanda N, Davachi F, Hassig SE. 1991. Plasmodium Falciparum malaria and perinatally acquired human immunodeficiency virus type 1 infection in Kinshasa, Zaire. A prospective, longitudinal cohort study of 587 children. N Engl J Med. 325(2):105-109.
12. Grimwade K, French N, Mbatha DD, Zungu DD, Dedicoat M, Gilks CF. HIV infection as a co-factor for severe falciparum malaria in adults living in a region of unstable malaria transmission in South Africa. *AIDS* 2004; *18*(3): 547–554.
13. Grimwade K, French N, Mbatha DD, Zungu DD, Dedicoat M, Gilks CF. Childhood malaria in a region of unstable transmission and high human immunodeficiency virus prevalence. *Pediatr Infect Dis J* 2003; *22*(12): 1057–1063.
14. Ibekwe AC, Okonko IO, Onunkwo AI, Ogun AA, Udeze AO. Comparative Prevalence Level of *Plasmodium* in Freshmen (First Year Students) of Nnamdi Azikwe University in Awka, South-Eastern, Nigeria. *Malaysian Journal of Microbiology,* 2009; *5(1):51-54*
15. Mayor A, Aponte JJ, Fogg C, Sauté F, Greenwood B, Dgedge M, Menedez C, Alonso PL. The epidemiology of malaria in adults in a rural area of southern Mozambique. *Malar J* 2007; Jan 17, *6:* 3.
16. Mens P, Spieker N, Omar S, Heijnen M, Schallig H, Kager PA. Is molecular biology the best alternative for diagnosis of malaria to microscopy? A comparison between microscopy, antigen detection and molecular tests in rural Kenya and urban Tanzania. *Trop Med Int Health* 2007; *12*: 238–244.
17. Nega A. Malaria in the Ecology of Health and disease in Ethiopia. Ethiopian Medical Journal. 1993;41-341-52.
18. Okonko IO, Soleye FA, Amusan TA, Ogun AA, Udeze AO, Nkang AO, Ejembi J, Faleye TOC. Prevalence of Malaria Plasmodium in Abeokuta, Nigeria. *Malaysian Journal of Microbiology,* 2009; 5(2): 113-118
19. Okonko IO, Donbraye-Emmanuel OOB, Donbraye E, Alli JA, Adekolurejo OA, Ojezele MO, Babalola ET, Mejeha OK, and Amusan TA. 2010. Malaria parasitaemia among patients in Ibadan, Southwestern Nigeria. *Journal of Applied Biosciences 29: 1774-1780.*
20. Onyenekwe CC, N. Ukibe, S.C. Meludu, A. Ilika, N. Aboh, N. Ofiaeli, M. Ezaeni and A. Onochie. 2007. Prevalence of malaria as co-infection in HIV-infected individuals in a malaria endemic area of southeastern Nigeria. *J Vector Borne Dis.,* 44: 250–254
21. Perlmann P, Troye-Blomberg M (2000). Malaria Immunology. Perlmann P and Troye-Blomberg M (editors). *Basel, Krager, 80*: 229-242.
22. Plebanski M, Hill AV (2000). The Immunology of Malaria Infection. *Curr. Opin. Immunol. 12(4)*: 437-441.
23. Salawu L, Murainah HA. (2006). Pre-donation screening of intending blood donors for antibodies to infectious agents in a Nigerian tertiary health institution: a pilot study. *African Journal of Medicine and Medical Sciences; 35(4)*:453-6.
24. UNICEF (2003). Malaria and HIV/AIDS. UNICEF Malaria Technical Note # 6; February 2003; pp1-7.
25. Van Geertruyden JP, Menten J, Colebunders R, Korenromp E, D'Alessandro U. 2008. The impact of HIV-1 on the malaria parasite biomass in adults in sub-Saharan Africa contributes to the emergence of antimalarial drug resistance. Malar J. 7:134.
26. World Health Organization (WHO). A global strategy for malaria control. World Health Organization (WHO); Geneva; 1993.

6/6/2012