ORIGINAL ARTICLE

Prevalence and predictors of placental malaria in human immunodeficiency virus-positive women in Nigeria

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Abstract

Background: Human immunodeficiency virus (HIV)-infected pregnant women have alterations in cellular and humoral immunity that increase the risks to placental malaria infection.

Aim: This study aimed at determining the prevalence and predictors of placental malaria among HIV-positive women in Nigeria.

Materials and Methods: It was a longitudinal cohort study of pregnant women receiving antenatal care at a tertiary hospital in Nigeria. Peripheral blood sample for packed cell volume estimation and placental blood sample for malaria parasite estimation were collected from each participant at a presentation in labor and upon delivery, respectively.

Results: The Prevalence of placenta malaria (68.6%) and anemia (66.7%) in HIV-positive women were significantly higher than the prevalence of placental malaria (35.3%) and anemia (44.1%) in HIV-negative control ($P < 0.001$ and $P = 0.001$ respectively). The employment status was the only sociodemographic factor significantly associated with the development of placental malaria in HIV-positive women (odds ratio: 21.60; 95% confidence interval: 7.1–66.2; $P < 0.001$).

Conclusion: The prevalence of placental malaria is very high among HIV-positive women in Nigeria. Scaling up free distribution of insecticide treated nets in the short term and employment opportunities of HIV-positive women, in the long run, may reduce the prevalence of placental malaria in our population.

Key words: Human immunodeficiency virus-positive women, Nigeria, placental malaria

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Introduction

Malaria infection during pregnancy is a major public health problem in the tropical and subtropical regions of the world.

Infection with human immunodeficiency virus-1 (HIV-1) and malaria are among the prevalent infectious diseases worldwide. They disproportionately affect children and adults in sub-Saharan Africa[1] where currently two-thirds of HIV-1 infections, 72% of acquired immunodeficiency syndrome (AIDS)–related deaths[2] and more than one million deaths from malaria occur.[1] Both HIV-1 and malaria are associated with an increased risk of adverse

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pregnancy outcomes, such as low birth weight, preterm birth, and stillbirth.[3] In the absence of prenatal health services and interventions to prevent mother-to-child transmission (PMTCT) of HIV-1, an estimated 15–30% of HIV-1 infected pregnant women transmit the virus to their infants antenatally and peripartum.[4,5] According to the latest National HIV/AIDS and Reproductive Health Survey (NARHS Plus II, 2012),[6] the national HIV prevalence rate is 3.4%, lower than 3.6% reported in 2007. The prevalence is highest in the South-South zone of the country (5.5%) and lowest in the South-East zone (1.8%). The prevalence in Lagos state and (federal capital territory) are 1.5% and 6.2%, respectively.[6]

Maternal malaria specifically increases the risk of severe illness, anemia, and death for the mother, and contributes to spontaneous abortion and stillbirth and is associated with preterm birth and low birth weight.[7]

In addition to their respective disease manifestations, HIV-1 and malaria may interact synergistically in co-infected individuals, and result in an increased risk of adverse health outcomes.[8,9] In studies among pregnant women, the reported frequency and severity of malaria[10-12] and risk of adverse pregnancy outcomes[13,14] were increased among HIV-1 infected women compared with HIV-1 uninfected. Infection with HIV-1 also has been identified as an independent predictor of malaria among pregnant women.[10,15] HIV-1 infection has been associated with an approximately two-fold increase in the risk of having placental malaria.[10] In addition, an estimated one-third to half of malaria infections were attributable to HIV-1 co-infection in a study among HIV-1 infected women in Kenya.[12]

In view of the above enormous burden of malaria during pregnancy, the World Health Organization (WHO), as part of the roll back malaria initiative, recommended insecticide-treated bed nets (ITNs), intermittent preventive treatment (IPT) with an effective antimalarial during the latter two trimesters of pregnancy, and good case management of malaria and/or severe anemia for all pregnant women living in sub-Sahara Africa where transmission of *Plasmodium falciparum* is stable.[16] Diagnosis of placental malaria is important because the condition increases the risk of low birth weight, congenital malaria, and death of the baby during the 1st year of life.[17] Since falciparum placenta malaria has been widely used as a standard indicator to characterize malaria in epidemiological studies,[18] and HIV-infected pregnant women have been shown to have significant alterations in both cellular and humoral immunity to malaria with increased risk of both malarial illness and placental malaria,[19,20] studying the prevalence and predictors of placental malaria in HIV-infected pregnant women may help elucidate the extent of the disease among this group of women in our environment as well as determining the factors associated with the disease. This may help the policy makers in designing strategies aimed at controlling placental malaria in HIV-positive women in our environment. All these underscored the need for this study which aimed at determining the prevalence and predictors of placental malaria among HIV-positive women in our population.

Materials and Methods

The study took place in Enugu, southeast Nigeria, between September 1, 2011, and March 31, 2013. The town is located in the hilly tropical rain forest approximately 230 m above sea level. The average annual temperature is between 23.1°C and 31°C with a rainfall of 1520–2030 mm. There are two major seasons, the rainy season (April–October) and the dry season (November–March). The area has a mixed rural and urban population of 3.2 million of which 52.1% are female. The area is hyperendemic for malaria which is predominantly caused by *P. falciparum*.

It was a longitudinal study of two cohorts of women selected on a 1:1 ratio from pregnant women attending the PMTCT clinic and the antenatal clinic (ANC) of the University of Nigeria Teaching Hospital (UNTH) Enugu, Nigeria. The first (study) group was made up of HIV-positive women who delivered at the UNTH labor ward. The second (control) group consisted of HIV-negative women deliberately matched for age, gravidity, employment status, educational level, and use of insecticide-treated nets. These two groups of pregnant women were consecutively recruited at the booking clinic after obtaining their written informed consents. They were provided with insecticide-treated nets and encouraged to always sleep under the nets. In addition, they were given IPT with sulfadoxine/pyrimethamine (IPT-SP) during pregnancy according to the WHO recommendation: At least 2 doses starting after 16 weeks gestation and separated by an interval of at least 1 month, for the HIV-negative women.[15] The HIV-positive women received at least 3 doses after 16 weeks gestation, also separated by at least 1 month intervals.[15] The doses of IPT-SP were directly observed in the PMTCT/ANC clinics. All the HIV-positive women also received antiretroviral drugs according to the national PMTCT guideline-the common first line "highly active anti-retroviral therapy (ART)" received being a combination of zidovudine (AZT), lamuvidine (3TC) and lopinavir; AZT, 3TC, and efavirenz (EFV); or 3TC, EFV, and tenofovir disoproxil fumarate.[21]

Following the presentation in labor, at the labor ward of the hospital, 2 ml of peripheral venous blood was collected from each participant using the antecubital vein of her preferred arm under aseptic technique. Each collected sample was quickly put into a sterile ethylenediaminetetraacetic acid (EDTA) bottle and then immediately transferred to the UNTH medical laboratory where two laboratory scientists

working independently and blinded to the women's HIV status, estimated the packed cell volume (PCV), using the microhematocrit centrifuge, and hematocrit reader. The mean of the PCV values for each participant was recorded and used for the study.

Furthermore, within 30 min of delivery, 2 ml of placental blood was collected into a sterile EDTA bottle by making a shallow incision into the maternal side of the placenta using sterile scissors in order to collect blood that pooled from the intervillous space. Each sample was also quickly transferred to the same laboratory where the two laboratory scientists working independently and blinded as above, made thin and thick blood smears. These were stained with Giemsa and then examined using ×100 objective lens. Identification of malaria parasite species was done using the thin blood smear while parasite density was estimated on the thick smear under oil immersion using ×100 objective lens. Definitive parasite count was made by counting the number of asexual forms of *P. falciparum* parasites against 200 leucocytes,[22] the estimation of parasite density per microliter of blood was achieved by multiplying the number of parasites counted by 40 under the assumption of a leukocyte count of 8000 cells per microliter of blood. In the rare event of discrepant results, the slides were reviewed by a third scientist whose findings served as a tie-breaker. The degree of parasitemia was graded thus: (1–999/μL) as mild or +, (1000–9999/μL), moderate or ++, and >10000/μL severe or +++. A negative result was recorded after a thorough examination of 100 fields without any parasite.

Furthermore, participants' sociodemographic characteristics including age, marital, educational, and employment status, parity and gestational age were obtained using structured questionnaires administered by trained interviewers. Exclusion criteria included HIV-positive or HIV-negative women who did not receive at least 3 doses or at least 2 doses of IPT-SP respectively during pregnancy, women who did not sleep under insecticide-treated bed nets, and HIV-positive women who were not on ART. The main outcome measures included the prevalence of placental malaria in the HIV-positive and HIV-negative women.

The study was designed to detect 40% point difference in placental malaria between the 2 groups, with 5% level of significance and 80% power. Using 29.9% prevalence of placental malaria,[23] a minimum sample size of 101 was determined. To account for attrition, 10% of the sample size was added, and this resulted in 111 participants for each group. The study was approved by the Institutional Review Board of the UNTH, Enugu, Nigeria. Statistical analysis was both descriptive and inferential at 95% confidence level using Statistical Package for Social Sciences (SPSS) computer software version 16 (SPSS Inc., Chicago, IL, USA). Data were summarized using frequency tables, means and standard deviations, and ranges. Fisher's Exact and Pearson's Chi-square tests were used to test for associations between categorical variables, and Student's *t*-test for continuous variables. $P < 0.05$ was considered statistically significant.

Occupation was defined as any job done to help earn a living, such as trading or farming, while employment was defined as a "paid job" for which a wage (salary) is paid at specific intervals, for example, monthly.[24]

Results

A total of 222 pregnant women met with the eligibility criteria for the study; however, 18 women (8.1%) did not give birth in our hospital and were lost to follow-up. Thus, 204 women (91.9%) delivered in our hospital and completed the study, comprising 102 participants in each group. The baseline characteristics of the two groups are similar [Table 1].

Table 1: Sociodemographic characteristics of the study participants

Variable	Sub-group variable	HIV positive *n* (%)	HIV negative *n* (%)
Age (in years)	<20	6 (5.9)	4 (3.9)
	20-29	50 (49.0)	51 (50.0)
	30-39	40 (39.2)	42 (41.2)
	40-49	6 (5.9)	5 (4.9)
Marital status	Single	6 (5.9)	4 (3.9)
	Married	96 (94.1)	98 (96.1)
Educational level	No formal education	2 (2.0)	3 (2.9)
	Primary education	18 (17.6)	16 (15.7)
	Secondary education	32 (31.4)	31 (30.4)
	Tertiary education	50 (49.0)	52 (51.0)
Employment status	Employed	41 (40.2)	40 (39.2)
	Unemployed	61 (59.8)	62 (60.8)
Gestational age (weeks)	<37	16 (15.7)	13 (12.7)
	37-40	56 (54.9)	59 (57.8)
	>40	30 (29.4)	30 (29.4)
Parity	0	16 (15.7)	19 (18.6)
	1	46 (45.1)	42 (41.2)
	2-4	24 (23.5)	26 (25.5)
	≥5	16 (15.7)	15 (14.7)

HIV=Human immunodeficiency virus

Table 2: Associations between degrees of placental malaria in human immunodeficiency virus-positive women and human immunodeficiency virus negative women

Degree of placental malaria	HIV positive *n* (%)	HIV negative *n* (%)
Mild	24 (34.3)	27 (75)
Moderate	32 (45.7)	8 (22.2)
Severe	14 (20.0)	1 (2.8)
Total	70 (100)	36 (100)

$\chi^2=16.65$; df=2; $P=<0.001$. df=Degrees of freedom; HIV=Human immunodeficiency virus

The mean age of all the participants was 26.2 ± 4.8 years (range: 16–42). The age range of 20–29 years constituted the majority in both groups (HIV-positive; 47.2% [$n = 50$] and HIV-negative; 52.8% [$n = 56$]). Majority of the women in both groups were Christians, married, unemployed, had tertiary education, multiparous, and at a gestational age of between 37 and 42 weeks at the time of delivery. Details of the sociodemographic characteristics of the participants are as shown in Table 1.

Overall, 106 women in the groups combined, had positive placental malaria, giving a prevalence of 52.0%. The prevalence of placental malaria was significantly higher in HIV-positive women than in HIV-negative women (68.6% [$n = 70$] vs. 35.3% [$n = 36$]; odds ratio [OR]: 4.0; 95% confidence interval [CI]: 2.2–7.2; $P < 0.001$). Not only had the HIV-positive women had more placental malaria, but also the distribution of severity was also significantly skewed in the direction of higher severity ($P < 0.001$) [Table 2].

The employment status was the only sociodemographic factor significantly associated with the development of placental malaria in HIV-positive women (OR: 21.60; 95% CI: 7.1–66.2; $P < 0.001$). Details are as shown in Table 3.

Overall, the mean PCV of all the study participants was 32.1 (3.6) %. The mean PCV of the HIV-positive women was significantly lower than that of the HIV-negative women (31.1 [3.6] vs. 33.2 [3.6]; $t = 4.1$; $P < 0.001$). The overall prevalence of anemia in both groups (PCV <33%) was 60.8% ($n = 124$). The prevalence of anemia was significantly higher among HIV-positive women than HIV-negative women (66.7% [$n = 68$] vs. 44.1% [$n = 45$]; OR: 2.5; 95% CI: 1.4–4.5; $P = 0.001$).

Table 3: Association between positive placental malaria among 102 human immunodeficiency virus-positive women and certain maternal characteristics

Factors	Malaria parasitemia frequency (%)		OR (95% CI)	P
	Yes	No		
Age (in years)				
<35	59 (67.8)	28 (32.2)	0.77 (0.22-2.62)	0.67
>35	11 (73.3)	4 (26.7)		
Marital status				
Single	6 (100)	0 (0)	6.55 (0.36-120)	0.21
Married	64 (66.7)	32 (33.3)		
Educational level				
≤Tertiary education	37 (71)	15 (29)	1.27 (0.55-2.94)	0.58
Tertiary education	33 (66)	17 (34)		
Employment status				
Unemployed	56 (91.8)	5 (8.2)	21.60 (7.1-66.2)	<0.001
Employed	14 (34.1)	27 (65.9)		
Gravidity				
Primigravidae	12 (75)	4 (25)	1.45 (0.43-4.90)	0.55
Multigravidae	58 (67.4)	28 (32.6)		
Gestation (weeks)				
<37	10 (62.5)	6 (37.5)	0.72 (0.24-2.20)	0.57
>37	60 (69.8)	26 (30.2)		

OR=Odds ratio; CI=Confidence interval; HIV=Human immunodeficiency virus

Discussion

This study demonstrated a significantly higher prevalence of placental malaria among HIV-positive pregnant women than HIV-negative pregnant women. This finding suggests that HIV-positive women may have significant alterations in cellular and humoral immunity that favor higher placental parasite sequestration and multiplication even if they are on ART. Placental malaria is no doubt associated with increased risk of maternal anemia, intrauterine growth restriction, preterm birth, and low birth weight.[17] Thus, the HIV-positive women with positive placental malaria are more likely to be predisposed to these adverse maternal and fetal outcomes than their placental malaria negative counterparts.

The prevalence of 68.6% obtained in this study among HIV-positive pregnant women is exceedingly higher than 19% obtained in a similar study from Uganda in 2009.[25] However, it is important to note that while the HIV-positive pregnant women in this study received anti-malarial prophylaxis using IPT with (IPT-SP), those in the Uganda study received daily trimethoprim-sulfamethoxazole (TMP-SMX). The very high prevalence obtained in this study despite the use of IPT-SP suggests that this strategy appears not to be effective for the prevention of placental malaria in the study population. This finding corroborates the findings of a similar recent study from the study area that IPT-SP does not seem to protect pregnant women from malaria parasitemia in the study population.[22] Thus, a study on the efficacy of alternatives to IPT-SP such as TMP-SMX in HIV-positive pregnant women in the study population is necessary.

The observations that HIV-positive pregnant women have lower mean PCV and higher prevalence of anemia is in keeping with the previous report from Abakaliki, Nigeria in 2007.[18] Nevertheless, the negative effect of ART on hemoglobin level[21] may be contributory to this observed lower prevalence among the HIV-positive women. There is thus clear need to strengthen malaria and anemia prevention and intervention in HIV-positive pregnant women in view of the adverse effects of malaria and anemia in pregnancy.

The current strategies of routine folic acid and iron supplementation (for anemia prevention) in combination with antimalarial appear to be suboptimal considering the 66.7% prevalence of anemia obtained in this study among HIV-positive women who were on these strategies. Since there is a significant association between the

nutritional status of individuals and the hemoglobin level as demonstrated in a previous study,[26] additional nutritional support of HIV-positive pregnant women may improve their hemoglobin levels. This is also in view of the fact that HIV-positive women in resource-poor countries are significantly more malnourished than their HIV-negative counterparts, as shown in a recent study.[27] Since many of the pregnancies in HIV-positive women are unintended, preventing unwanted pregnancies in HIV-positive women by way of provision of adequate and appropriate contraception may help in preventing malaria infection among them. This is in addition to scaling up the free distribution of ITNs and encouraging our women to comply accordingly with the usage of the nets.

The finding that employment status of the women was the only factor significantly associated with placental malaria shows that any strategies to reduce the prevalence of placental malaria in the study population should take cognizance of improving the employment opportunities of the HIV-positive women. This observation of lower prevalence of placenta malaria among the employed may be related to the expected effect of income on their quality of life, and hence resistance to infections including malaria.

The limitations of this study include the fact that the effective utilization of ITNs (which is part of the inclusion criteria in this study) cannot be guaranteed because usage was not checked. Similarly, the re-treatment process of the ITNs on regular basis which are user dependent cannot be guaranteed. The possible impact of variations in environmental factors such as place of residence and vector control measures may affect the results obtained in this study. The very small frequencies and wide confidence intervals obtained in some of the outcome measures of interest in the study suggest that a larger sample size would have improved the study's precision and external validity. Training of interviewers and other quality control strategies noted in the study's methods ruled out substantial measurement bias. Despite these limitations, this study is very relevant because it targeted a population of pregnant women (HIV positive) which are very vulnerable to malaria infections and hence the findings of this study may help the policy makers in designing strategies aimed at controlling placental malaria in HIV-positive women in our population.

Conclusion

The prevalence of placental malaria and maternal anemia is very high among HIV-positive pregnant women in Enugu, Nigeria. Scaling up free distribution of insecticide treated nets in the short term and employment opportunities of HIV-positive women, in the long run, may reduce the prevalence of placental malaria in the population.

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Conflicts of interest
There are no conflicts of interest.

References

1. Abu-Raddad LJ, Patnaik P, Kublin JG. Dual infection with HIV and malaria fuels the spread of both diseases in sub-Saharan Africa. Science 2006;314:1603-6.
2. UNAIDS. Global HIV/AIDS Response: Epidemic Update and Health. Available from: http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2011/20111130_ua_report_en.pdf. [Last accessed on 2013 Feb 11].
3. Brahmbhatt H, Kigozi G, Wabwire-Mangen F, Serwadda D, Sewankambo N, Lutalo T, *et al.* The effects of placental malaria on mother-to-child HIV transmission in Rakai, Uganda. AIDS 2003;17:2539-41.
4. Kourtis AP, Lee FK, Abrams EJ, Jamieson DJ, Bulterys M. Mother-to-child transmission of HIV-1: Timing and implications for prevention. Lancet Infect Dis 2006;6:726-32.
5. De Cock KM, Fowler MG, Mercier E, de Vincenzi I, Saba J, Hoff E, *et al.* Prevention of mother-to-child HIV transmission in resource-poor countries: Translating research into policy and practice. JAMA 2000;283:1175-82.
6. National HIV & AIDS and Reproductive Health Survey (NARHS Plus II, 2012). Federal Republic of Nigeria: Federal Ministry of Health, Abuja, Nigeria; November, 2013. Available from: http://www.nascp.gov.ng/demo/wp-content/uploads/2014/02/NARHS-Plus-2012-Final-18112013.pdf. [Last accessed on 2014 Jun 27].
7. Villamor E, Msamanga G, Aboud S, Urassa W, Hunter DJ, Fawzi WW. Adverse perinatal outcomes of HIV-1-infected women in relation to malaria parasitemia in maternal and umbilical cord blood. Am J Trop Med Hyg 2005;73:694-7.
8. French N, Gilks CF. Royal Society of Tropical Medicine and Hygiene meeting at Manson House, London, 18 March 1999. Fresh from the field: Some controversies in tropical medicine and hygiene. HIV and malaria, do they interact? Trans R Soc Trop Med Hyg 2000;94:233-7.
9. Msamanga GI, Taha TE, Young AM, Brown ER, Hoffman IF, Read JS, *et al.* Placental malaria and mother-to-child transmission of human immunodeficiency virus-1. Am J Trop Med Hyg 2009;80:508-15.
10. ter Kuile FO, Parise ME, Verhoeff FH, Udhayakumar V, Newman RD, van Eijk AM, *et al.* The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-Saharan Africa. Am J Trop Med Hyg 2004;71 2 Suppl: 41-54.
11. Inion I, Mwanyumba F, Gaillard P, Chohan V, Verhofstede C, Claeys P, *et al.* Placental malaria and perinatal transmission of human immunodeficiency virus type 1. J Infect Dis 2003;188:1675-8.
12. van Eijk AM, Ayisi JG, ter Kuile FO, Misore AO, Otieno JA, Rosen DH, *et al.* HIV increases the risk of malaria in women of all gravidities in Kisumu, Kenya. AIDS 2003;17:595-603.
13. Ticconi C, Mapfumo M, Dorrucci M, Naha N, Tarira E, Pietropolli A, *et al.* Effect of maternal HIV and malaria infection on pregnancy and perinatal outcome in Zimbabwe. J Acquir Immune Defic Syndr 2003;34:289-94.
14. Ayisi JG, van Eijk AM, ter Kuile FO, Kolczak MS, Otieno JA, Misore AO, *et al.* The effect of dual infection with HIV and malaria on pregnancy outcome in western Kenya. AIDS 2003;17:585-94.
15. Ladner J, Leroy V, Simonon A, Karita E, Bogaerts J, De Clercq A, *et al.* HIV infection, malaria, and pregnancy: A prospective cohort study in Kigali, Rwanda. Am J Trop Med Hyg 2002;66:56-60.
16. World Health Organization. A strategic framework for malaria prevention and control during pregnancy in the African Region AFR/MAL/04/09. Geneva: WHO; 2004. http://afro.who.int/malaria/publications/malaria_in_pregnancy_09/2004 [Last accessed on 2016 Mar 10].
17. Matteelli A, Caligaris S, Castelli F, Carosi G. The placenta and malaria. Ann Trop Med Parasitol 1997;91:803-10.
18. Uneke CJ. Impact of placental *Plasmodium falciparum* malaria on pregnancy and perinatal outcome in sub-Saharan Africa: II: Effects of placental malaria on perinatal outcome; malaria and HIV. Yale J Biol Med 2007;80:95-103.
19. Mount AM, Mwapasa V, Elliott SR, Beeson JG, Tadesse E, Lema VM, *et al.* Impairment of humoral immunity to *Plasmodium falciparum* malaria in pregnancy by HIV infection. Lancet 2004;363:1860-7.

20. Ned RM, Moore JM, Chaisavaneeyakorn S, Udhayakumar V. Modulation of immune responses during HIV-malaria co-infection in pregnancy. Trends Parasitol 2005;21:284-91.
21. Federal Ministry of Health. Nigeria National Guidelines on prevention of Mother-To-Child Transmission (PMTCT) of HIV; 2007. Available from: http://www.ilo.org/wcmsp5/groups/public/---ed_protect/---protrav/---ilo_aids/documents/legaldocument/wcms_125389.pdf. [Last accessed on 2013 Feb 13].
22. Umeh UA, Obi SN, Onah HE, Ugwu EO, Ajah LO, Umeh CR, *et al.* The impact of intermittent preventive treatment with sulfadoxine-pyrimethamine on the prevalence of malaria parasitaemia in pregnancy. Trop Doct 2012;42:133-5.
23. Ukaga CN, Nwoke BE, Udujih OS, Udujih OG, Ohaeri AA, Anosike JC, *et al.* Placental malaria in Owerri, Imo State, South-Eastern Nigeria. Tanzan Health Res Bull 2007;9:180-5.
24. Ugwu EO, Dim CC, Uzochukwu BS, Iloghalu EI, Ugwu AO. Malaria and anaemia in pregnancy: A cross-sectional study of pregnant women in rural communities of Southeastern Nigeria. Int Health 2014;6:130-7.
25. Newman PM, Wanzira H, Tumwine G, Arinaitwe E, Waldman S, Achan J, *et al.* Placental malaria among HIV-infected and uninfected women receiving anti-folates in a high transmission area of Uganda. Malar J 2009;8:254.
26. Silva CL, Lima-Costa MF, Firmo JO, Peixoto SV. Hemoglobin level in older adults and the association with nutritional status and use of health services: The Bambuí project. Cad Saude Publica 2012;28:2085-94.
27. Obi SN, Ifebunandu NA, Onyebuchi AK. Nutritional status of HIV positive individuals on free HAART treatment in a developing nation. J Infect Dis 2010;4:745-9.