International Scholars Journals

International Journal of Medicine and Medical Sciences ISSN 2167-0404 Vol. 5 (8), pp. 247-257, September, 2015. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Prevalence of the co-infection of Malaria and HIV virus in a case-cohort study of women in Anambra, Nigeria

***Ogbonnaya I. K, Majimete Ochuko and Onokpasa D. A**

Department of Microbiology, Faculty of Medical Sciences, Olabisi Onabanjo University, Ago Iwoye, Nigeria.

Accepted 24 August, 2015

The daily increasing incidence of malaria and human immunodeficiency virus (HIV) co-infection and associated poor maternal and obstetrical outcomes among pregnant women in the malarious zone of Anambra east, southeast Nigeria, and the paucity of laboratory-based data on the deadly duo, necessitated this study on the prevalence of the co-infection in a case-cohort study of 450 women (15 to 45 years) from whom placental and peripheral blood samples were collected. Screening for HIV antibodies was by the DETERMINE and GENIE–II, confirmed at 95% confidence interval. Pregnancy screening was by human chorionic gonadotropin (HCG) one step pregnancy test strip. Placental malaria was determine by Pooled-biopsy; peripheral malaria, from maternal venous blood. Giemsa stain of thin and thick blood smears were assayed; results were confirmed by dipstick rapid test. Anaemia estimation was by packed cell volume. Population attributable fraction-associated co-infection was 62% (P-value, 0.019). Acute malaria (+++) was highest among dually infected multigravidas (53%) (P-value, 0.0672), and multiple infections highest in second trimester (mean parasite density, 3,471.1 ± 101.0 parasites/µl), with preponderance in the 25 to 29 age bracket (4,720.51 ± 110.3 parasites/µl). Gravidity-associated co-infection was prevalent among the multigravidas, with mean parasite density of 19,224.1 ± 136.0 parasites/µl. Placental malaria was a significant risk factor for mortality and morbidity (P < 0.01), and maternal anaemia which is the single most important adverse pregnancy outcome. Significance of abortion, pre-term delivery and low infant birth weights as serious adverse maternal outcomes were further established in the study.

Key words: Human immunodeficiency virus (HIV), placental malaria, gravidity-associated malaria, pregnancy outcomes.

INTRODUCTION

Malaria remains an important public health concern, particularly in the malarious zones with regular transmission. Young children, pregnant women, and non-immune visitors to malarious areas are at greatest risk of severe or fatal illness (Desai et al., 2007; The Global fund 2009). Malaria in pregnancy is associated with poor maternal, obstetrical and infant outcomes. Pregnant women in both high and low transmission areas fall victim of this scourge. However, in low transmission areas, primigravidas and multigravidas are both at increased

*Corresponding author. E-mail: ik.gbona@oouagoiwoye.edu.ng

risk, while in high transmission areas, primigravidas are the most affected, with more frequent bouts of severe malaria and associated febrile illnesses and adverse outcomes. Malaria commonly involves the placenta, where microscopic examination shows large intervillous accumulations of parasitized erythrocytes together with monocytes containing ingested pigment (Rennie and Roberton, 1999).

The increased high risk of malaria infection in pregnancy could be attributed to the ability of the *Plasmodium falciparum*, the major culprit, to adhere to the trophoblastic villi, extravillous trophoblasts, and syncytial bridges via its intracellular adhesion molecule-1, CD36, the chondroitin sulfate A (CSA) and hyaluronic acid which facilitate parasite attachment to placental cells, thereby interfering with oxygen and nutrient transport to the foetus and subsequently causing general hemorrhaging which contributes to the complications experienced by both mother and child (Steketee, 1996). CSA, to which the parasite binds, is present in pregnant women but not accessible to the parasite in non-pregnant tissue-beds. The putative ligand expressed by the parasite is PfCSA-L and this has been found to be antigenetically conserved among global cases of maternal malaria. Consequently, primigravidas (who have not had initial immunization or exposure to the CSA-binding parasite) are more highly susceptible to malaria infection. *P. falciparum* can be found in the cord blood of up to 16% of infants born to infected mothers (Diagne et al., 2000). Heavily parasitized placenta leads to neonatal malaria via vertical transmission (Rennie and Roberton, 1999). However, protection is observed to occur following successive pregnancies.

Pregnancy similarly elicits immunologic tolerance; a pregnant woman instinctively welcomes the implantation of the foetal allograft in her uterus (Suguitan et al., 2003). Type 2 cytokines and TGF-b appear in the human placenta, to enhance implantation and inhibit inflammatory responses (Oeuvray et al., 2000). Infections which require a Type 1 response for protection such as malaria, tuberculosis, toxoplasmosis, and leishmaniasis, which are more common in the developing countries are more severe during pregnancy; consequently, pregnancy allows Type 1 responses known to confer resistance to these infections. However, exposure to malaria elicits TNF-a, IFN-g, and IL-2 in the placenta, and these cytokine changes are associated with poor pregnancy outcomes (Adrian et al., 2000; Fried et al., 1998). Primigravidas are therefore highly susceptible to malaria infection, which culminates in high maternal and foetal mortality and morbidity and associated anaemia and low birth weight infants.

HIV infection intensifies the effect of malaria among pregnant women and infants, creating significant impairment and alteration in both cellular, humoral immunity and resistance to *P. falciparum* infection. Acute malaria infection on the other hand increases viral load, and enhances transmission of HIV and more rapid disease progression with substantial public health implications (Ned et al., 2005). Malaria infection is more frequent and more severe in HIV-positive pregnant women in malaria-endemic settings. Multigravidae with HIV infection are similar to primigravidae without HIV infection in terms of susceptibility to, and negative consequences of malaria infection. Therefore, in the presence of HIV infection, the risk associated with placental malaria appears to be independent of the number of pregnancies. Pregnant women infected with both malaria and HIV are also at higher risk of developing anaemia, delivering a low birth weight infants, and delivering prematurely. Similarly, higher viral load increases the likelihood of maternal-to-child transmission (MTCT) of HIV (Briand et al., 2009; Inion et al., 2003).

Malaria infection during pregnancy increases risks of MTCT in the intrauterine and intrapartum as well as during the breastfeeding period, presumably by increasing HIV viral load. The prevalence of, and adverse consequences of malaria and HIV co-infection on mother-to-child transmission of HIV with subsequent high infant mortality and morbidity, including maternal anaemia, low birth-weight infants, preterm deliveries and/or abortions were therefore examined among pregnant women in some locations of Anambra East Local Government area of Anambra State, with the view to create the epidemic awareness and strengthen capacity building and governmental control strategies in these neglected and infrastructure-deprived areas of south eastern Nigeria.

MATERIALS AND METHODS

Study design

This study focused on establishing the prevalence of pregnancy-associated malaria and HIV co-infection in the malaria endemic rural communities of Nigeria, with case study of Anambra East, and raises the question on population prevalence rates of the dual infection, as well as the adverse outcomes or impact of, and association of the dual epidemic on pregnancy among the local populace. Relevance of this question was explored by investigation of the effect of HIV and malaria parasitemia through a case-cohort study of pregnant women from whom placental and peripheral blood samples were collected and subsequently analyzed.

Study population

This is an economically disadvantaged area, where income per capita is below minimal wage of ₦500. Mortality and morbidity due to malaria and HIV co-infection is high, though not statistically estimated, nor epidemiologically investigated. However, the rate of pregnancy per year in the area is high; more than 3,000 women become pregnant each year, most of these deliver at home. Less than 30% of the pregnant women attend antenatal care visits during

their pregnancy terms, and there is neither intermittent presumptive treatment for malaria, insecticide-treated nets programs to specifically target the pregnant women, nor routine HIV testing. The significance of this study therefore centres on the following:

1. Poverty and related sexual promiscuity and attendant sexually transmitted infections.
2. Further spread of HIV infection as a result of increasing incidence of malaria co-infection in HIV-infected individuals.
3. Increased incidence of mortality and morbidity and loss of labour force and furthering of economic repression and associated poverty.
4. Unavailability of governmental attention in terms of infrastructure including hospitals, access roads and job opportunities.
5. Lack of surveillance programmes on improvement of living standards, and HIV/AIDS education.
6. Absence of antenatal care facilities or programmes on mother and child welfare or other pregnancy related education.
7. Call for establishment and strengthening of programmes for the prevention of malaria in the area

Data collection

Four hundred and fifty (450) women between the ages of 15 to 45 years from some local communities of Anambra East Local Government area were studied over a period of 24 months (November 3, 2009 to November 12, 2011). Due to the unreliability and unavailability of accurate procedure of gestational age in this area, women were enrolled based on the date of last menstrual period. Participants were administered a structured questionnaire with details of demographic, behavioral, pregnancy and other health related information. Informed consent was obtained from participants and promise of confidentiality duly declared. Women agreeing to voluntary counseling and testing (VCT) were screened for the HIV-1/2 antibodies in addition to receiving pre- and post-test counseling. Monthly visits were made, and at each visit, a finger-prick blood sample was collected from each woman for both thin and thick blood films for malaria parasites and hemoglobin level estimation, respectively. Maternal anemia was defined as having a packed cell volume (or haematocrit) less than 25%. However, those that presented with symptoms of acute malaria ranging from intermittent fever, headache, malaise, nausea and vomiting with joint pains were encouraged to make more regular visits. Patients were placed on monthly sulfadoxine-pyrimethamine (sulfadoxine, 1,500 mg and pyrimethamine, 75 mg), and daily multivitamin supplement consisting of 200 mg ferrous sulfate, and 5 mg folic acid until 33 weeks gestation. However, those that had intermittent fibril illnesses with PCV less than 30% were placed on 200 mg ferrous sulfate multivitamin twice daily and further advised to improve their feeding and/or diet with fresh fruits and vegetables. Those with positive malaria were then given repeat doses of sulfadoxine-pyrimethamine.

HIV screening

All the participants were screened for HIV antibodies. A finger-prick blood sample was collected from participants using sterile blood lancets after pre-test counseling and informed consent. Screening for HIV-1/2 antibodies was carried out according to manufacturers' instructions using two rapid *in vitro* test kits; the determine HIV-1 and HIV-2 (Abbot Laboratories Japan), an immunochromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2 and GENIE–II (Sanofi, Pasteur, France), which also detects HIV-1 and HIV. A 95% (95% confidence interval: 93.2 to 97.8%) between the two tests was established in the identification of HIV positive samples; HIV infection was thus defined as a positive result on the two rapid tests. HIV positive participants were categorized using the World Health Organization (WHO) guidelines on HIV staging into asymptomatic (stage-1) and symptomatic (stage-2) (WHO, 2007). Excluded from study were those in the stage-3 and 4 categories. The HIV positive women were then given post HIV counseling, assured of the safety of their babies, placed on highly active antiretroviral therapy (HAART), which was regularly supplied to them free of charge and encouraged to live normal lives with improved diet and nutritional supplement. They were further referred to nearby private hospitals for regular surveillance and privacy. The HIV status of each patient was not disclosed to any other to ensure none withdrawal out of shame or societal ostracization.

Pregnancy screening

Following oral interviews on their marital status, socio-economic and sexual behaviours, participants were screened for pregnancy by urine testing for human chorionic gonadotropin using the HCG one step pregnancy test strip (ACON Laboratories Inc, USA), a rapid chromatographic immunoassay for the qualitative detection of HCG in urine for early detection of pregnancy. It utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of HCG in urine. It has a sensitivity of 25 ml U/ml. The test strip was gently dipped into an early morning urine for 10 to 15 s to allow capillary movement of urine and subsequent reaction with the coloured conjugate, after which the strip was observed for appearance of coloured line on the test line region of the membrane. Two hundred and three (203) women: 112 primigravidas and 91 multigravidas, who tested positive to the pregnancy test were then screened for malaria parasitaemia, and thus considered eligible for the follow-up exercises.

Screening for malaria parasite

Sample collection for placental malaria

The pooled-biopsy method described by Suguitan et al. (2003) was adopted for investigation of placental malaria. A 5 × 5 × 5 (cm) piece of the placenta was excised from each placenta, allowing maternal intervillous blood to accumulate at the site. A 5 ml sample of heparinized maternal venous blood was then collected and used for both HIV screening and the placental malaria parasite examination.

Sample for analysis for peripheral and placental parasitemia

Maternal venous blood (peripheral) samples were collected from all pregnant women (with or without symptoms suggestive of malaria) as previously reported. Symptomatic malarial cases were defined as the presence of asexual forms of *Plasmodium* species on a blood smear, and associated with fever, headache, chills, and/or joint pain) for malaria parasite screening at enrollment and delivery. Screening was carried out following delivery using the "Gold Standard" by the microscopic examination of giemsa stained blood smears. Thin and thick blood films were prepared immediately in duplicate upon blood collection on the same slide. Thick films were

used to identify malaria parasites and determine parasite density, while thin smears were used for species identification. For thick films, 12 µl of blood was spread over a diameter of 15 mm, while 2 µl of blood was used for thin films. The slides were made in duplicates and labeled appropriately. The thin film was fixed in absolute methanol for 1 to 2 s and air dried. Slides were properly coded, and the blood films stained after 24 to 48 h with 3% giemsa stain solution at pH 7.2 and examined microscopically under the X 100 oil immersion microscope. Confirmation was then done using the dipstick rapid chromatographic immunoassay that detects *Plasmodium falciparum*-specific proteins and pan-*Plasmodium* aldolase antigens up to two weeks after the infection has cleared. This was carried our according to manufacturer's instructions. Two distinct coloured bands on the test strip were confirmatory for the presence of malaria parasite. Negative result was indicated by one line in the control region, and an additional line in the test region (T).

Placental blood smears were examined for the presence of both parasites and pigment. Presence of pigment within macrophages indicated parasite death; stages of infection were indicated by presence of parasites, or both; absence of infection was indicated by no parasites, no pigment; early infection, by presence of parasites, but no pigment; late infection, by presence of both parasites and pigment; cleared infection by presence of pigment without parasites.

Parasite counts

Malaria parasites for placental and peripheral parasite densities were quantified against 200 white blood cells (WBC) on giemsa-stained thick films. Slides were considered positive if the parasite load was > 200 parasites per 200 WBC; and negative following a review of 200 high-power fields. However, if parasites were identified from the smears, 100 to 200 fields of the thin smear were further examined. Quality control was ensured by further examination of 10% randomly selected slides. Parasitemia was defined as the presence of parasites in thick blood smears. Severity of malaria in women with placental malaria was defined as: mild parasitemia = < 20,000 parasites/µl; severe parasitemia = ≥ 20,000 parasites/µl. Malaria parasitemia at delivery was defined as peripheral and/or placental parasitemia following delivery.

Population attributable fraction (PAF)

Estimation of the impact and overall malaria-HIV prevalence using the population attributable fraction (PAF)

For more accurate analysis and better understanding of the magnitude of the HIV-malaria co-infection and the burden of the epidemic in the surveyed population, the population attributable fraction (PAF), which is an estimate of the proportion of overall malaria prevalence in the surveyed population attributable to HIV infection was evaluated. This was indicated as follows: G-All = all gravidae; G-1 = primigravidae; G-2= multigravidae; and then calculated as 100 × [p (RR − 1) / (1 + p (RR − 1)]. Where p is the HIV prevalence and RR, the overall risk ratio for the malaria parasitemia associated with HIV infection.

Pregnancy outcome

In establishing the impact of malaria and HIV infection in pregnancy, associated adverse outcomes such as preterm deliveries and/or spontaneous abortion, low birth weight and maternal anaemia resulting to infant mortality were evaluated.

Birth weight, preterm deliveries and abortions

Infant weights and placentas (after births) were measured immediately following delivery using the bathroom scale. Infants were considered as having low birth weights at weights less than 2,500 g.

Preterm deliveries and/or spontaneous abortion

Cases of abortion were noted, while preterm delivery was measured within the 1st three days of delivery using the new ballard score for preterm delivery.

Maternal anaemia

Estimation of packed cell volume (PCV) for investigation of anaemia: Impact of *P. falciparum* infections on anaemia, considered a direct marker of maternal and foetal morbidity and mortality in the locality was assessed by estimation of the haemoglobin or packed cell volume (PCV). Heparinized haematocrit tubes were filled with maternal peripheral blood. A constant packing of the red blood cells was achieved with a centrifugation speed of 1200 × g for 3 min. The PCV (%) was measured and reported as a ratio of the whole blood volume.

Levels of anaemia: Anaemia was in this study categorized as: (1) Severe: PCV = 14 to 23%; (2) Mild: PCV = 22 to 29% and (3) Moderate: PCV = 24 to 25%. A woman was considered to be anaemic if the PCV was less than 25%.

Ethical consent

Approval for the study was obtained from consenting volunteers to whom the purpose of the study was explained following voluntary testing and counseling (VTC). Those that gave informed consent were therefore enrolled in the study prior to questionnaire distribution and laboratory studies. The approval was on the agreement that patient anonymity must be maintained, good laboratory practice/quality control ensured, and that every finding would be treated with utmost confidentiality and for the purpose of this research only. All work was performed according to the international guidelines for human experimentation in clinical research according to the Declaration of Helsinki (World Medical Association and Council for International Organizations of Medical Sciences (CIOMS).

Statistical analysis

Available data from questionnaire responses and laboratory results were entered into EpiData software, version 3.02 (EpiData), and the analysis done using the statistical package for social sciences (SPSS), version 18.0. The student's T-test and the percentile ratios were used for the normally distributed data, while the Pearson chi-square test with Fisher's exact test with odds ratios (ORs) and 95% confidence intervals (CIs) was used in making comparisons

Table 1. Baselline profile of participants.

Number of participants	Pregnancy positive volunteers (n=203)	%
Mean age (years)±20		
Maternal age group		
15-19	24	11
20-24	46	22
25-29	52	25
30-34	44	21
35-39	30	13
40>	7	3
Total	203	-
Marital status		
Single	72	35
Married	85	41
Divorced	46	22
Total	203	
Gestational levels		
1st Trimester	47	23
2nd Trimester	61	30
3rd Trimester	95	47
Total	203	
Gravidity		
Primigravidae	91	45
Multigravidae	112	55
Total	203	

between categorical data. The relationship between peripheral and placental malaria was compared using the Wilcoxon signed rank test. Estimation of the impact and overall malaria-HIV prevalence was carried out using the population attributable fraction (PAF); while multivariable log-Binomial regression models were used to determine adjusted ORs for risk factors for anaemia, including low birth weight, preterm deliveries/abortions. The Cox proportional regression models were then used to assess the relationship between anaemia and other variables after adjusting for the following important covariates: related factors, parity levels, gravidity and maternal age.

RESULT

Baseline profile

Baseline characteristics of the study population, which serves useful tool in the assessment of their health status and the investigated risk factors, are here presented. Descriptive characteristics of the population , including maternal age, marital status, gravidity, and gestational levels are shown in Table 1. Among the 450 women of child bearing age enrolled in the study, 203 (15 to < 45 years; mean age 20 years) were considered eligible on the basis of their positive pregnancy results. Seventy-two (35%) of the participants were single, 85 (41%) married, and 46 (22%) were divorced; gestational level: 1st trimester (n = 47); 2nd trimester (n = 61); 3rd trimester (n = 95); gravidity: multigravidae (n = 112); primigravidae (n = 91).

Prevalence of maternal malaria and HIV and co-infection

In consideration of the overall impact of HIV on the burden of maternal malaria (basically among the primigravidae and multigravidae), the magnitude of the epidemic was in this study expressed as the proportional increase in pregnancy- associated malaria in the HIV infected population, here referred to as the population attributable fraction (PAF), which is a function of the HIV-associated increased risk of acquiring malaria in pregnancy; also observed to rise with subsequent increase in HIV prevalence.

PAF-associated malaria

Of the 91 (45%) primigravidas examined, the PAF-associated malaria and HIV infection was 57 (62%); 13 had only malaria and no HIV (single infection); while 9 were singly infected with HIV. Similarly, the PAF-associated malaria and HIV infection among the 112 multigravidas was 88 (79%); 21 (19%) had single malaria infection while 13 (12%) were singly infected with HIV. The pregnancy-attributable malaria due to HIV co-infection was therefore observed to rise in parallel with HIV prevalence. Overall parasite burden among the dually infected was 71% (Table 2).

Acute malaria in pregnancy-associated coinfection

Acute malaria, here defined as high levels of parasi-taemia (+++), with clinically defined signs and symptoms was nevertheless prevalent in 96 of the dually infected; with highest prevalence among the dually infected multigravidas, 51 (53%), compared with 45 (47%) among the dually infected primigravidas (Table 3).

Gravidity-associated febrile Illnesses

Febrile illnesses and malaria symptoms of fever,

Table 2. PAF associated HIV and malaria co-infections among dually infected patients.

Gravidity	Conditions investigated		
	Malaria and HIV	Malaria singly	HIV singly
Overall	145 (71.4%)	34 (16.7%)	22 (10.8%)
Parity			
Primigravidas (n=91)	57 (62%)	13 (14%)	9 (10%)
Multigravidas (n=112)	88 (79%)	21 (19%)	13 (12%)
X	6.426	0.718	0.153
P- value	0.019	0.453	0.822

Table 3. Acute malaria in pregnancy-associated co-infection.

Acute malaria parasitaemia	Number examined with Mal/HIV	Number infected	Prevalence (%)
Overall	145	96	66.2
Parity			
Primigravidae	57	45	47
Multigravidae	88	51	53
X		0.309	
P –value		0.672	

Table 4. Gravidity-associated febrile illnesses among dually infected volunteers.

Gravidity	No.	%
Primigravidae	91/203	44
Dually Infected with malaria and HIV	57/91	62
Symptomatic for malaria (febrile illness)	41/57	72
Asymptomatic	16//57	28
Multigravidae	112/203	55
Infected with malaria and HIV	88/112	78
Symptomatic for malaria (febrile illness)	63/88	71
Asymptomatic	25/88	28

headaches, sweating, tiredness, abdominal pain, diarrhea, loss of appetite, nausea and vomiting were observed in 41 (72%) of the dually infected Primigravidas; 16 (28%) were asymptomatic, while 63 (71%) of the multigravidas were symptomatic for malaria febrile illnesses while 25 (28%) were asymptomatic (Table 4). Investigation of the association of gestational levels with malaria/HIV co-infection indicated that the preponderance of the multiple infections is higher in the second trimester of pregnancy: out of the 61 (42%) persons screened, 46 (75%) were dually infected, with malaria parasite density (MPD) of 3,471.1 ± 101.0 parasites/µl; 35 (57%) had febrile illness, symptomatic for malaria parasitaemia, while 11 (18%) were asymptomatic. However, there was no statistical difference in the gestational parasitaemia of 1st and 3rd trimester of pregnancy of the study population (1,501.0 ± 215.0 and 1,696.0 ± 117.1 parasites/µl), hence not a significant risk factor for the dual infections ($P < 0.05$) (Table 5).

Table 5. Gestation-related parasitaemia among dually infected volunteers.

Trimester parasitemia level	No infected	%	Mean parasite density ± SEM (parasites/µl)
1st			
No Screened	47/145	32	
Infected with malaria and HIV	32/47	68	1,501.0±215.0[b]
Symptomatic	24/47	51	
Asymptomatic	8/47	17	
2nd			
No Screened	61/145	42	
Infected with malaria and HIV	46/61	75	3,471.1±101.0[a]
Symptomatic	35/61	57	
Asymptomatic	11/61	18	
3rd			
No Screened	95/145	65	
Infected with malaria and HIV	67/95	71	1,696.0±117.1[b]
Symptomatic	45/95	47	
Asymptomatic	22/95	23	

Table 6. Age-associated mean parasite density of multiple infection.

Participants characteristics		Number infected (n =145)	%	Mean parasite density±SD (parasite/µl)
Number of participants	**203**			
Mean age (years)	**20**			
Maternal age group				
15-19	24	13	54	1650.1±214.0[d]
20-24	46	34	77	2065.7±320.3[b]
25-29	52	49	94	4720.51±110.3[a]
30-34	44	30	68	1950.08±100.0[c]
35-39	30	17	57	1602.2±211.0[d]
40>	7	2	22	820.0±216.2[e]
X		31.597		
P value		>0.0001		

Homogeneous subsets of values are represented by superscript of the same letter.

Maternal age as risk factor

In consideration of maternal age as constituting an increased risk of morbidity and mortality for both mother and neonate, age as risk factor was therefore evaluated at varying age brackets. The age related parasite density (mean and SD) of the dually infected pregnant women was highest among those aged 25 to 29 years: out of the 52 women screened, 49 (94%) had multiple infections, with mean parasite density (MPD, and ± SD of 4,720.51 ± 110.3 parasites/ µl), followed by those aged 20 to 24 years: multiple infectivity, 73%; MPD, 2,065.7 ± 320.1 parasites/µl. However, there was no statistical difference in both the percentage multiple infection as well as the mean parasite density between the age groups; 15 to 19 (54%, MPD, 1,650.1 ± 214.0) and those aged 35 to 39 years (54% percentage multiple infection and 1,602.2 ± 211.0 parasites/µl), respectively. Nonetheless, using univariate analysis, multiple infections occurred irrespective of age; hence, age-associated risk factor in multiple infections was not statistically significant ($P < 0.01$) (Table 6 and Figure 1).

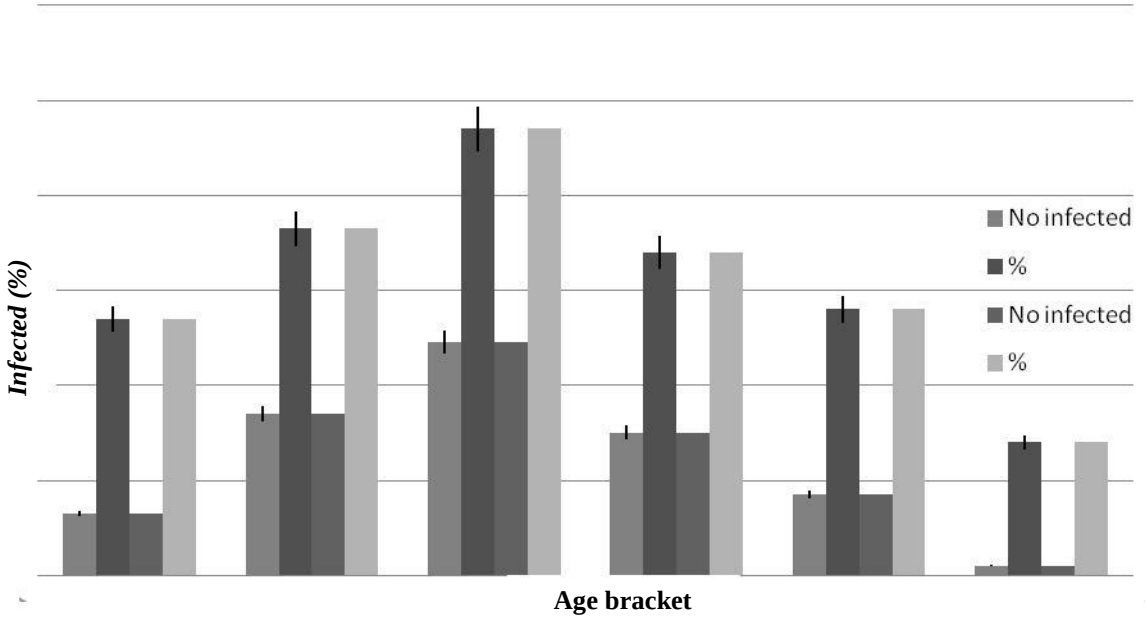

Figure 1. Maternal age and associated co-infection.

To establish the association of gravidity/parity as an important risk factor for the HIV/malaria co-infection, the primigravidae and multigravidae co-infectivity were compared in relation to their mean parasite density. From available results, highest prevalence of the multiple co-infections, 88 (96%), occurred among the multigravidas with mean parasite density ± standard error of mean (SEM) of 19,224.1 ± 136.0 parasites/µl, compared with the primigravidas, 57 (51%), mean parasite density ± SEM of 18,689.0 ± 620.1 parasites/µl. The difference was however not statistically significant ($P < 0.01$), indicative that pregnancy-associated co-infection with both malaria and HIV occurs irrespective of gravidity, thereby invalidating gravidity as a significant risk factor for the multiple HIV/malaria infection. There was significant difference in the prevalence of co-infection among pregnant women but the difference in mean parasite density was not significant.

Peripheral versus placental malaria

Since placental malaria (PM) is one of the major features of malaria during pregnancy and has been globally used as a standard indicator to characterize malaria infection in epidemiologic investigations, a comparison of the placental and peripheral blood smear positive cases was evaluated to establish the level of significance and implications of the available results. Malaria parasites were detected in both placental and peripheral blood smears of the study population. In spite of the paucity of placental parasites however, more than 48% parasitized erythrocytes were detected. Among all the malaria positive cases obtained, 104 (58%) were placental malaria while 75 (42%) were peripheral blood malaria positive cases. There was therefore a significant positive correlation between parasitaemia levels determined by placental blood ($P < 0.01$), consequently, establishing placental malarias a significant risk factor for mortality and morbidity in pregnancy.

Adverse pregnancy outcomes

Following the observation that HIV and malaria co-infections have serious adverse complications, the associated pregnancy outcomes of the deadly duo were assessed to establish their relationship with maternal anemia and associated hypoxia and perinatal mortality, low birth weight, abortion/preterm deliveries.

Anaemia as risk factor

Result of the study reports malaria as the commonest medical condition associated with pregnancy especially among those dually infected with HIV and malaria. The incidence of maternal anaemia, herein defined as PCV < 25%, was high among the dually infected women irrespective of their age and gestational levels. Overall

Table 7. Pregnancy associated risk factors: burden of HIV/malaria co-infection.

Parameter	No.	%
No of dually Infected with malaria and HIV	145	71
Anaemia	137	94
Severe or Acute anaemia (PCV ≤ 18%)	96	71
Sub-acute or Moderate anaemia (PCV = 19-26%)	30	21
Mild anaemia (PCV = 27-29%)	11	8
Gravidity associated anaemia		
Primigravidas	71	52
Multigravidas	66	48
Delivery outcome		
Abortion	103	
Primigravidae	71	69
Multigravidae	32	31
Low birth weights	69	
Primigravidae	44	64
Multigravidae	25	36
Pre-term delivery	40	
Primigravidae	23	58
Multigravidae	17	42

prevalence of anaemia among the dually infected was 137 (94%) (Table 7).

Severity of anaemia

Severe or acute anaemia, with PCV range of ≤ 18% occurred in 96 (71%) of participants; sub-acute or moderate anaemia, PCV level of 19 to 26%, in 21%; while mild anaemia, PCV range of 27 to 29%, was observed in 11(8%) participants. There was no significant statistical association between anaemia among the primigravidas (48.8%) and the multigravidas (52%) as well as the different gestational levels; 28% in the 1st trimester, 31% in the 2nd trimester and 40% in the 3rd trimester, respectively. The prevalence occurred irrespective of gravidity (Table 7).

Precipitating factors to the high incidence of anaemia

In addition to pregnancy-associated malaria parasitaemia and HIV-associated immunosuppression with resultant high rates of haemorrahge and consequently inadequate hematopoiesis among the surveyed pregnant women, other reported precipitating factors to the high incidence of anaemia (from questionnaire responses of this study) included poverty (51%), older maternal age (15%), poor nutrition and prenatal care (34%).

Pregnancy-related adverse birth outcomes

The prevalence of and impact of pregnancy-relate adverse birth outcomes: low birth-weight infants, and pre-term deliveries and/or abortions among the dually infected gravid women, studied to ascertain their association with the deadly duo, malaria parasitaemia and HIV infection is here presented. Available results indicated the prevalence of several adverse birth outcomes among the co-infected, with highest prevalence in the primigravidas. The study observed a total of 103 cases of abortions out of the 145 multiply infected persons screened: 71 (69%) among the primigravidas and 32 (31%) in the multigravidas, respectively. Mean infant birth weights were very low; the percentage of low birth weight (LBW) babies were significantly higher among the primigravidas, 44 (64%), compared with the multigravidas 25 (36%). Pre-term delivery (PTD) was similarly higher among the primigravidas 23 (58%) than the multigravidas 17 (42%) (Table 7). Using multivariable log-Binomial regression models, a significant relationship was established between the multiple infections and increased risk of anaemia, abortion, low birth-weight and

pre-term delivery (adjusted OR, 2.0; 95% CI, 1.1 to 2.6; P < 0.001). Juxtaposing the high incidence of anaemia and the established adverse birth outcomes, the study therefore indicates a direct relationship between these risk factors and the multiple co-infections, malaria and HIV. While establishing anaemia as a significant risk factor of the multiple infections (from reported high prevalence rates), its association with or impact on other risk factors and birth outcomes is also here underscored, further implicating anaemia as a single most important adverse pregnancy outcome.

DISCUSSION

The study examined the relationship of HIV and malaria in mother to child transmission (MTCT), and their combined effects on pregnancy outcomes. The observed preponderance of, and risk of young single women of child bearing age (mean age, 20 years) to pregnancy; reasons, consequences and the health implications which questions their economic status, social and or/sexual life style were evaluated. Though high incidence of pregnancy was observed among both the married and single women, however, the reported single parent pregnancy (out of wedlock pregnancy) was reckoned and has remarkable societal problem with serious economic and public health implications. This questionable phenomenon could be attributable to prevailing lifestyle in the locality, which is synonymous with sexual promiscuity, a common occurrence in economic repressed areas. The observed effect of poverty among the natives in the rural settings is consistent with reports from Dibua (2010), suggesting that the underlying cause of promiscuity and associated exposure to sexually transmitted diseases including HIV/AIDS is a combined effect of poverty, lack of job opportunities and improved life condition as well as adverse socio-cultural norms, ignorance and illiteracy, parental neglect, low self-esteem, influence of media, widowhood, divorce or separation.

This study observed endemicity of malaria, as well as a high prevalence of both HIV and malaria confection (a superimposed condition): HIV infection almost doubling the risk of malaria parasitemia and clinical malaria episode; overall parasite density of 71%; expressed as population attributable risk (PAF), an indication of a direct or synergistic interaction between the deadly duo; one increasing the susceptibility of the other and thus accelerating the progression of the HIV disease, AIDS. The increasing incidence and magnitude are multi-faceted, and could thus be traced to an overlap between the geographic and transmission intensity in the studied rural malarious zone where malaria and HIV advocacy have neither attention nor impact. Previous reports had indicated that areas of such overlap are characterized by heavy infestation of chloroquine-resistant *P. falciparum* (TerKuile et al., 2004). Result of this study thus suggests that individuals living in areas with high *P. falciparum* parasitemia therefore have a higher risk of being infected with HIV, and that severe malaria in HIV-co-infected patients presents with higher parasite burden as previously reported elsewhere in Africa (Whitworth et al., 2000). This research further showed that pregnant women living with HIV/AIDS in the rural and peri-urban malarious areas of Nigeria (irrespective of their gravidity levels) have higher incidence of both placental and peripheral malaria with higher parasite densities and associated higher parasite burden, in conformity with reports on the risk of HIV-associated malaria, attributable to modifications in systemic and placental immunologic factors following gross impairment of the gravidity-dependent acquired immunity to malaria resulting from HIV infection and associated high viral load (UNAIDS/WHO, 2007; vanEjik et al., 2003).

Previous reports on the cellular consequences of the co-infections on pregnancy, attributed the increased susceptibility to excessive destruction of the IL-12-mediated IFN-γ pathway due to HIV infection: intervillous blood mononuclear cells (IVBMCs) which produce interferon-γ (IFN-γ), usually accords protection against placental malaria in the absence of HIV (Moore et al., 1999); nevertheless, this protection is lost in HIV-infected pregnant women with *P. falciparum* malaria, in response to malarial antigen stimulations, result of severe destruction of the interleukin-12 (IL-12); (IL-18-mediated IFN-γ pathway is however not affected), with resultant low CD4 counts, thus indicating a shift in the cytokine responses with HIV progresses (Chaisavaneeyakorn et al., 2002).

The observed prevalence of placental malaria among the dually infected women is in this study, considered as a major determinant of congenital malaria, with serious consequences for mother to child transmission, suggestive of trans-placental transmission of *P. falciparum* in neonates during malaria-exposed pregnancies as indicated by previous reports (Uneke et al., 2007; Mwapasa et al., 2004). Mounting evidence also suggests that malaria increases HIV viral load in pregnant women and increases CCR5 expression on intervillous maternal and fetal villous macrophages, which may increase risk for fetal infection, posing an increased risk for mother-to-child transfer of HIV-1, and buttressing that high density of population-associated *P. falciparum* malaria is intrinsically linked to the geographical location of the area, (riverrine area); characterized by numerous water-bodies and/or waterlogs, several of which are stagnant and grossly contaminated with human and domestic wastes, which thus provide favourable breeding grounds for infestation by *Anopheles* mosquitoes , the implicated

species involved in *P. falciparum* parasitaemia. Next to this dilemma is the lack of health education and/or governmental intervention programmes in the area.

The study further observed prevalence of placenta-associated anaemia and other adverse pregnancy outcomes: birth weight, (ILBW babies), and preterm deliveries (PTD) and/or abortions, and aptly indicates that placental malaria significantly increased the prevalence of adverse obstetric outcomes in the multiply infected group irrespective of age or gravidity, underscoring the severity of malaria infection in HIV-positive pregnant women particularly in malaria-endemic setting; further implicating pregnancy-associated malaria as enhanced risk factor for MTCT in the intrauterine and intra-partum periods through lateral transfer of HIV infection by increased HIV viral load, as well as establishing that malaria, anaemia and HIV infections are the indirect causes of poor maternal health outcomes and serious public health problem in the local settings and poor resource areas of Nigeria.

This study reported an approximate 40 cases of preterm delivery and 103 cases of abortions (basically spontaneous), reflecting a breakdown in normal mechanisms responsible for maintenance of uterine quiescence, attributed to formation of an early gap junction and abnormal rise in oxytocin receptor concentration as earlier reported (Nahlen et al., 1996). Though these abnormalities may be reckoned as common adverse pregnancy outcomes, nevertheless, the overtly amplified condition suggests a subtle interplay between the already compromised placental integrity due to the combined effects of malaria parasitaemia and high viral load associated with HIV disease progression, resulting in further down-regulation and/or dysfunction of the uterine musculature, as well as dys-balance of the oxytocin and prostaglandin receptors which could have triggered the observed anomalous physiologic and pathological changes in the placenta. Therefore, the study generally observed and concluded that, dual infections with HIV and malaria have serious complications for both mother and child as a result of the increased susceptibility of pregnant women infected with HIV to malaria; preponderance of clinical malaria, expressed as fever or a history of fever in the presence of microscopically detected malaria parasitemia among dually infected pregnant women, culminates in high maternal and foetal mortality and morbidity and associated anaemia, preterm delivery/spontaneous abortions and low birth weight infants; acute malaria triggers increased HIV replication (high viral load), and subsequently, increases transmission of HIV and more rapid disease progression to advanced disease, AIDS, with significant public health implications; higher viral load resulting from increased HIV replication and malaria parasitemia, in addition to placental inadequacies, increases the likelihood of MTCT of HIV in the intrauterine and intrapartum periods. Increased maternal anemia resulting from multiple interactions of HIV and malaria infections is a complication for and serious risk factor for other adverse pregnancy outcomes including preterm deliveries and spontaneous abortions.

REFERENCES

Briand V, Badaut C, Cot M (2009). Placental malaria, maternal HIV infection and infant morbidity. Ann. Trop. Paediatr. 29:71-83.

Chaisavaneeyakorn S, Moore JM, Otieno J, Chaiyaroj SC, Perkins DJ, Shi YP, Nahlen BL, Lal AA, Udhayakumar V (2002). Immunity to Placental Malaria. III. Impairment of Interleukin (IL)-12, not IL-18, and Interferon-Inducible Protein-10 Responses in the Placental Intervillous Blood of Human Immunodeficiency Virus/Malaria-coinfected Women. J. Infect. Dis. 185:127–131.

Desai M, Kuile FO, Nosten F, McGready R, Asamoa K, Brabin B, Newman RD (2007). Epidemiology and burden of malaria in pregnancy. Lancet Infect. Dis. 7:93-104.

Diagne N, Rogier C, Sokhna CS *(*2000*)* Increased susceptibility to malaria during the early postpartum period. New Engl. J. Med. 343, 598–603.

Dibua UME (2010). Socio-Economic and Socio-Cultural Predisposing Risk Factors to HIV/AIDS: Case Study of Some Locations in Eastern Nigeria.Int. J. Trop. Med. ISSN:1540-2681.

Fried M, Muga RO, Misore AO, Duffy PE (1998). Malaria elicits type 1 cytokines in the human placenta: IFN-gamma and TNF-alpha associated with pregnancy outcomes. J. Immunol. 160:2523-2530.

Inion I, Mwanyumba F, Gaillard P, Chohan V, Verhofstede C, Claeys P, Mandaliya K, van Marck E, Temmerman M (2003). Placental malaria and perinatal transmission of human immunodeficiency virus type 1. J. Infect. Dis.188:1675–1678.

Moore JM, Nahlen BL, Misore A, Lal AA, Udhayakumar V (1999*)*. Immunity to placental malaria. I. Elevated production of interferon-gamma by placental blood mononuclear cells is associated with protection in an area with high transmission of malaria. J. Infect. Dis. 179:*1218*–1225. Mwapasa V, Rogerson SJ, Molyneux ME, Abrams ET, Kamwendo DD, Lema VM, Tadesse E, Chaluluka E, Wilson PE, Meshnick SR (2004). The effect of *Plasmodium falciparum* malaria on peripheral and placental HIV-1 RNA concentrations in pregnant Malawian women. AIDS 18:1051–1059.

Nahlen BL, Parise ME, Ayisi J, Oloo AJ, Schultz LJ, Steketee RW (1996). HIV-1 infection impairs the efficacy of sulfadoxine-pyrimethamine in prevention of placental malaria infection in western Kenya [abstract

Tu.B.413]. Program and abstracts: XI International

Ned RM, Price AE, Crawford SB, Ayisi JG, Van Eijk, AM, Otieno, Nahlen BL, Steketee RW, Shi YP, Lanar DE, Udhayakumar V (2005). Effect of Placental Malaria and HIV Infection on the Antibody Responses to *Plasmodium falciparum* in Infants. J. Infect. Dis. 198 (11): 1609-1619.

Oeuvray C, Theisen M, Rogier C, Trape JF, Jepsen S, Druilhe P (2000). Cytophilic immunoglobulin responses to Plasmodium falciparum glutamate-rich protein are correlated with protection against clinical malaria in Dielmo, Senegal. Infect. Immun. 68:2617–2620.

Rennie JM, Roberton NR (1999). Textbook of Neonatology (3rd Edition). Edinburgh: Church Hill Living Stone, Pp. 1165-1166.

Steketee RW, Wirima JJ, Slutsker L, Heymann DL, Breman JG (1996). The problem of malaria and malaria control in pregnancy in sub-Saharan Africa. Am. J.Trop. Med .Hyg. 55:2–7.

Suguitan AL Jr, Leke RG, Fouda G, Zhou A, Thuita L, Metenou S, Fogako J, Megnekou R, Taylor DW (2003). Changes in the levels of chemokines and cytokines in the placentas of women with *Plasmodium falciparum* malaria. J. Infect. Dis. 188:1074–1082.

TerKuile FO, Parise ME, Verhoeff FH, Udhayakumar V, Newman RD, van Eijk AM, Rogerson SJ, Steketee RW (2004). 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. 71:41-54.

The Global Fund Web site, authors. Malaria. (2009). http://www.theglobalfund.org/documents/publications/diseasereport/di sease_reportmalaria_en.pdf

UNAIDS/WHO (2007). *AIDS epidemic update*. Geneva http://www.unaids.org/en/KnowledgeCentre/HIVData/EpiUpdate/EpiU Uneke CJ, Sunday-Adeoye I, Iyare FE, Ugwuja EI, Duhlinska DD (2007). Impact of maternal Plasmodium falciparum malaria and haematological parameters on pregnancy and its outcome in Southeastern Nigeria. J. Vect. Bor. Dis. 44:285–290.

van Eijk AM, Ayisi JG, ter Kuile FO, Misore AO, Otieno JA, Rosen DH, Kager PA, Steketee RW, Nahlen BL (2003). HIV increases the risk of malaria in women of all gravidities in Kisumu, Kenya. AIDS. 17:595– 603.

Whitworth J, Morgan D, Quigley M, Smith A, Mayanja B, Eotu H, Omoding N, Okongo M, Malamba S, Ojwiya A (2000). Effect of HIV-1 and increasing immunosuppression on malaria parasitaemia and clinical episodes in adults in rural Uganda: a cohort study. Lancet 356:1051–1056.

World Health Organization (2007). Report of the technical expert group meeting on intermittent preventive treatment in pregnancy (IPTp). Geneva.