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CLINICAL RESEARCH

Prevalence of Placental Malaria among Pregnant Women in Imo State University Teaching Hospital (Imsuth), Orlu, Imo State, Nigeria

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ABSTRACT

Malaria in pregnancy is a major public health problem in endemic areas of sub-Saharan Africa and has important consequences on birth outcome. The study followed a prospective cohort of pregnant women who completed ante natal care at IMSUTH between February 2014 and June 2017. After delivery of the placenta, the maternal surface of the placenta was incised with scalpel and placental blood were collected with a syringe into EDTA bottle within 1hour of delivery. The specimens were labelled and then sent to haematology laboratory where thick and thin films were prepared. Three full thickness placental blocks measuring 2 cm × 2 cm × 1 cm were taken from the placenta. The biopsy specimens were stored in 20ml of formalin and sent to histology laboratory for processing. A total of 936 pregnant women were enrolled in the study. The findings revealed placental malaria prevalence of 20.1% which was higher in primigravida (43.4%) and younger age group 16 years - 25 years (26.4%). There was significant relationship between placental malaria prevalence among pregnant women in IMSUTH, their gravidity status (χ^2 = 123.09; P <0.05) and their maternal age group (χ^2 = 29.30; P <0.05). Placental malaria had a significant effect on perinatal mortality (χ^2 = 142.93; P <0.05). There should be regular environmental sanitation early antenatal booking for effective monitoring and prompt treatment of malaria in pregnancy.

KEYWORDS

Malaria; Pregnancy; Haematology

1. INTRODUCTION

Placental malaria which is one of the major features of malaria during pregnancy has been widely used as a standard indicator to characterize malaria infection in epidemiologic investigations [1]. Generally, placental malaria was associated with increased risk of maternal anemia, HIV infection, and maternal mortality, with younger women and primigravida more likely to be affected [2].

A variety of adverse perinatal outcomes, including low birth weight, preterm delivery, intrauterine growth retardation, reduced fetal anthropometric parameters, fetal anemia, congenital malaria, increased mother-to-child HIV transmission, and perinatal mortality, were associated with placental malaria [3].

Malaria infection in pregnancy is a major risk factor for maternal and child health, and substantially increases the risk of miscarriage, stillbirth and low birth weight [4]. In sub-Saharan Africa alone, approximately 25 million pregnant women are at risk of *Plasmodium falciparum* infection every year.

Plasmodium falciparum infections of the placenta remain a major medical challenge among pregnant women in Sub-Saharan Africa. A number of factors influence the prevalence of placental malaria in pregnant women, including maternal age, gravidity, use of prophylaxis, nutrition, host genetics, and level of anti-parasite immunity, as well as parasite genetics and transmission rates [5].

Placental malaria poses a great challenge in malaria control strategies in that it may occur in asymptomatic parasitaemic as well as aparasitaemic pregnant women [6]. The placenta of infected pregnant women may be full of parasitized erythrocytes, with parasite densities sometimes in excess of 50% of the total placental erythrocyte count, the peripheral blood may remain free of parasites [7]. Consequently, interventions directed towards symptomatic parasitaemic pregnant women may leave out those who are equally at risk of anemia and low birth weight.

Recent data from Imo State indicate that total loss due to malaria in pregnancy within a six month period was estimated at 5.8 million naira. The study has confirmed that the burden of malaria in pregnant women in Imo State, Nigeria is high [8]. It has also been suggested that anemia might be associated with low birth weight [9]. However low birth weight might be due to malaria-induced pathological lesions that occur in the placenta leading to intrauterine growth retardation [10].

The effect of asymptomatic malaria as well as malaria infection of the placenta on pregnancy outcome have not been well documented in Orlu, Imo State particularly in the wake of malaria drug resistance. This study therefore tailored towards investigating the status of placental malaria among pregnant women. Data from this study would be useful in planning and implementation of malaria control strategies.

2. MATERIALS AND METHOD

The study was carried out at the Department of Paediatrics and Obstetrics unit of Imo State University Teaching Hospital (IMSUTH), Orlu, Nigeria, from February 2014 to June 2017. The IMSUTH is a tertiary centre located in Orlu, south eastern Nigeria and a centre of excellence in infectious diseases and immunology. It also serves as a referral site for south eastern states. IMSUTH is the only Tertiary Health Institution owned by Imo State

Government and the only Teaching Hospital in the state poised to train the needed medical manpower for the state and country including medical students to become doctors, doctors to become specialists, training of nurses, house officers and all interns etc.

A cohort research design was employed to recruit 936 pregnant women that came for ante natal clinic in IMSUTH from February 2014 to June 2017. The study protocol was reviewed and cleared by the Ethical Clearance Committee of Imo State University Teaching Hospital, Orlu. Informed consent was obtained from all participants hence two senior nursing officers from department of Paediatrics and Obstetrics unit and two senior laboratory technologist of Imo State University Teaching Hospital (IMSUTH), Orlu were involved on the research. All work was performed according to the guidelines for clinical research.

Data collection involved clinical assessments/examinations of placental tissue and placental blood. To register all laboratory assessments, a data collection schedule form (DCSF) was used to record the laboratory result. The DCSF questionnaire was designed as described [11-13]. After delivery of the placenta, the maternal surface of the placenta was incised with scalpel and placental blood collected with a syringe into EDTA bottle within 1hour of delivery. The specimen was labeled and then sent to hematology laboratory were thick and thin films were prepared. Three full thickness placental blocks measuring 2 cm × 2 cm × 1 cm were taken from the placenta [13]. The biopsy specimens were stored in 20 ml of formalin and sent to histology laboratory for processing. The placental biopsies were processed and embedded in paraffin wax using standard techniques [13]. Paraffin sections of 5mm thickness were stained with haematoxylin and eosin (H&E) and then examined by light microscopy. The pattern of malaria parasitization of placental tissue was graded according to Bulmer's (2003) description of grade 0 (no active infection), grade 1 (active infection), grade 2 (active-chronic infection) and grade 3 (chronic infection). Statistical analysis of generated data was carried out using SPSS for windows version 16, Software Package and percentages were calculated. Statistical comparisons and test of significance between positive and negative groups were calculated using the non-parametric Chi-square test. Differences were considered significant at $P < 0.05$.

3. RESULTS

Table 1 shows that age group 16 years - 25 years (50.5%) had highest proportions of participants than other age groups while age group 46 years - 55 years (1.5%) had the least.

As age increases, number of participants decreases. For gravidity, the greatest proportion was multigravida women (60.0%) and the least was great grand multigravida (4.1%). For level of education, all the women had formal education, those with secondary level of education were higher (67.5%). The least school attended was primary education (7.2%). For marital status, majority were married (86.0%) while separated/divorced accounted for 1.6%.

Table 2 revealed that a total of 188 (20.1%) were recorded for placental histology whereas placental blood parasitemia revealed only 165 (17.6%) thus showing 23 (12.2%) discordant infection.

Table 1: Socio-demographic characteristics of the study participants.

Variables	Category	F	%
Age Group (Years)	16 - 25	473	50.5
	26 - 35	377	40.3
	36 - 45	72	7.7
	46 - 55	14	1.5
Gravidity	Primigravida (1st Pregnancy)	258	27.6
	Multigravida (2nd to 4th Pregnancy)	562	60
	Grand Multigravida (5th to 6th Pregnancy)	78	8.3
	Great-Grand Multigravida (7th Pregnancy and Above)	38	4.1
Level of Education	No Formal Education	0	0
	Primary Education	67	7.2
	Secondary Education	632	67.5
	Tertiary Education	237	25.3
Marital Status	Single	24	2.6
	Married	805	86
	Widowed	92	9.8
	Separated/Divorced	15	1.6

Table 2: Distribution of placental malaria blood film and placental malaria histology.

Test of Placental Parasitaemia	No. Examined	Positive (%)	Negative (%)
Placental Blood	936	165 (17.6)	771 (82.4)
Histology	936	188 (20.1)	748 (79.9)

Table 3 revealed a placental malaria prevalence of 20.1% using placental histology among 936 women who delivered at IMSUTH, Orlu. Out of 188 women that had placental malaria, primigravida women (43.4%) were mostly affected followed by multigravida (12.3%), grand multigravida (6.4%) and great grand multigravida (5.3%). The most common pattern of placental parasitaemia in these women was active malaria infection (grade 1, 62.2%), followed by active-chronic infection (grade 2, 33.5%) and then chronic malaria infection (grade 3, 4.3%). There is no significant difference among different gravidities and placental malaria pattern of infection ($\chi^2 = 5.73$; $P > 0.05$) but there is significant difference between gravidity and placental malaria prevalence ($\chi^2 = 123$; $P < 0.05$).

Table 3: Distribution of placental malaria pattern according to gravidity.

Gravidity	No Examined	Placental Malaria infection (%)	Active Infection (%)	Active-chronic Infection (%)	Chronic Infection (%)
Primigravida	258	112 (43.4)	69 (61.6)	38 (33.9)	5 (4.5)
Multigravida	562	69 (12.3)	43 (62.3)	24 (34.8)	2 (2.9)
Grand Multigravida	78	5 (6.4)	4 (80.0)	0 (0)	1 (20.0)
Great Grand Multigravida	38	2 (5.3)	1 (50.0)	1 (50.0)	0 (0)
Total	**936**	**188 (20.1)**	**117 (62.2)**	**63 (33.5)**	**8 (4.3)**

$\chi^2 = 5.73$; $P > 0.05$ $\chi^2 = 123$; $P < 0.05$

Table 4 revealed that out of 188 women that had placental malaria, maternal age of 16 years - 25 years (26.4%) were mostly affected followed by 26 years - 35 years (15.6%), 46 years - 55 years (7.1%) and 36 years - 45

years (4.2%). The most common pattern of placental parasitaemia in these women was active malaria infection (grade 1) which recorded 62.2%, followed by active-chronic infection (grade 2), 33.5%; and then chronic malaria infection (grade 3), 4.3%. There is no significant relationship among different maternal age groups and placental malaria pattern of infection ($\chi^2 = 3.84$; $P < 0.05$) but there is significant difference between maternal age groups and placental malaria prevalence ($\chi^2 = 29.33$; $P < 0.05$).

Table 4: Distribution of placental malaria pattern according to maternal age.

Age Group (Years)	No Examined	Placental Malaria Infection (%)	Active Infection (%)	Active-Chronic Infection (%)	Chronic Infection (%)
16 - 25	473	125 (26.4)	81 (64.8)	38 (30.4)	6 (4.8)
26 - 35	377	59 (15.6)	34 (57.6)	23 (39)	2 (3.4)
36 - 45	72	3(4.2)	2 (66.7)	1 (33.3)	0 (0)
46 - 55	14	1 (7.1)	0 (0)	1 (100)	0 (0)
Total	**936**	**188 (20.1)**	**117 (62.2)**	**63 (33.5)**	**8 (4.3)**

$\chi^2 = 3.84$; $P > 0.05$ $\chi^2 = 29.33$; $P < 0.05$

4. DISCUSSION

The study revealed a histological evidence of placental infection of 188(20.1%) but placental blood investigation revealed only 165(17.6%) thus showing 23(12.2%) discordant infection. The observed sensitivity of placental histology is in accordance with report of which emphasize the gross underestimation of placental malaria infection by placental blood microscopy in pregnant women living in endemic areas [14]. Also, studies in various parts of sub-Saharan Africa by Mockenhaupt et al. (2006) [15] revealed that placental malaria prevalence ranging from 9.5% to 37.1% were obtained by placental blood smear microscopy, 35% to 75.5% by placental histological examination, 41% to 43.1% by HRP2, and 51% to 59% by PCR. It is possible that peripheral parasitaemia may remain below the levels of microscopic detection while parasites may be harboured by the placenta and evade circulation. The study further revealed placental malaria pattern/degree/category of active infection being higher (117,62.2%) than active-chronic infection (63,33.5%) and chronic infection (8,4.3%).

The study further revealed a very high prevalence (43.4%) of placental malaria in primigravida than in other gravidities. 12.3% of multigravida had placental malaria followed by 6.4% for grand multigravida and 5.3% for great grand multigravida. The study also revealed that there is significant relationship between placental malaria prevalence and gravidity status. This findings correlates with many studies which established that relationship establish between placental malaria and parity. Many recent studies have found the prevalence to be higher in primigravida than multigravida [5,16-18]. These observations are consistent with the findings of earlier studies in malaria-endemic regions, where, among several factors, parity independently influenced the placental malaria prevalence rate, with primigravida having a two- to four-fold increased risk of placental malaria compared to multigravida [19-21]. In Gambia, it was observed that the severest form of placental parasitization occurred in a higher proportion of the primigravida than in the multigravida [17].

The exact reason why primigravida are more susceptible to placental malaria and suffer from its consequences more than multigravida is yet to be fully elucidated. However, a common explanation is that pregnancy is

associated with a decrease in immunity, which is more pronounced in primigravida than in multigravida and may be associated with age [22-24]. Immunological studies have shown that this increase in susceptibility could be related to the property of parasitized erythrocytes to adhere to chondroitin sulfate A (CSA) expressed by the syncytiotrophoblast of the placenta [25,26]. Thus, the placenta may select for the CSA-binding *P. falciparum* phenotype, putting primigravida with no previous exposure to this parasite form at increased risk for developing placental malaria. The decreasing susceptibility to pregnancy-associated malaria with increasing parity is reflected in the acquisition of antibodies specific to parasites' variant antigens expressed on the surface of infected erythrocytes [27]. Another possible explanation for this parity-related susceptibility is given by the findings of Duffy & Fried (2006) [28], which showed that multigravida mothers develop malaria antibodies that block adhesion of parasites to CSA receptors in the placentae in subsequent pregnancies. More studies using both immunological and molecular biological tools are urgently needed to properly elucidate this parity-related susceptibility to placental malaria. However, since pregnant women in malarious areas produce antibodies that specifically recognize CSA-binding *P. falciparum*, this could form a basis for the development of a vaccine to protect pregnant women against placental malaria.

Further analysis of this study also revealed that placental malaria was higher (26.4%) among maternal age groups of 16 years - 25 years than other maternal age groups. 15.6% of maternal age groups of 26 years - 35 years had placental malaria as against 4.2% and 7.1% for maternal age groups of 36 years - 45 years and 46 years - 55 years respectively. Also, there is significant relationship between placental malaria prevalence and maternal age groups. The difference is because younger maternal age (particularly adolescence) carries a higher risk of infection and adverse effects [29]. A number of studies conducted in Sub-Saharan Africa have reported a significant association between maternal age and malaria infection during pregnancy [4,30-32]. Under conditions of low-to-moderate transmission, pregnancy-specific immunity is slow to develop, and age-related immunity may influence malaria prevalence in childbearing years [31].

Studies have shown that young women of child-bearing age may be more susceptible than older women to malaria because they are still in the process of acquiring natural immunity to malaria [33-35]. In Cameroon, age was a major risk factor for placental malaria, with younger first-time mothers more likely to have placental malaria [5]. Similarly, in Zaire, mothers with malarious placentae were younger (mean age 24) than mothers with non-malarious placentae (mean age 29) [36]. It was suggested that development of pregnancy-associated immunity, i.e., production of antibodies that inhibit the adherence of placental parasites to chondroitin sulfate A (CSA), may be very important in women less than 25 years of age who have lower levels of acquired immunity (through less frequency of exposure to the bites of *P. falciparum* infected mosquitoes) than in older women who may have obtained adequate immunity following repeated exposures and thus are less dependent on anticytoadherent antibodies [5]. However, it is important to state that in malarious areas, pregnancy-associated and age-dependent immunity to placental malaria may be influenced by host or environmental factors.

These results indicate that placental malaria is still unacceptably high in the Sub-Saharan Africa and calls for the intensified efforts in malaria control in pregnancy. The importance of effective control measures cannot be overstated because apart from the adverse perinatal outcomes associated with placental malaria, some studies also suggest that children born to mothers with placental malaria are at a high risk of acquiring larger numbers

of *P. falciparum* infections in the first 2 years of life compared to infants born to women without placental malaria [37]. Furthermore, it was reported that placental malaria infection diminished the development of antibody responses to malarial epitopes in the first year of life, the age at which most of the severe malaria-associated morbidity occurs in areas of holoendemicity [38].

5. CONCLUSION

This study indicated that placental malaria is still high among the study population and calls for the intensified efforts in malaria control in pregnancy.

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