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Original Research Article

Placental malaria parasitaemia and foeto-maternal outcome in a tertiary hospital in North Central Nigeria

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ABSTRACT

Background: Placental malaria parasitaemia; a well-known complication of malaria in pregnancy has been associated with poor foetomaternal outcome. There are some conflicting reports on the effects of placenta malaria parasitaemia on pregnancy outcome, hence the need for this study. This study was therefore carried out to determine the effect of placental malaria parasitaemia on foetomaternal outcomes among parturients at Federal University Teaching Hospital (FUTH), Lafia, northcentral Nigeria (formally Dalhatu Araf Specialist Hospital, Lafia).
Methods: This cross-sectional study was done by consecutive recruitment of 200 parturients who met the eligibility criteria after obtaining an informed consent. Maternal, cord and placental blood were examined for malaria parasites using thick and thin blood film. Data obtained was analysed using software (IBM SPSS for windows® version 23.0, SPSS Inc.; Chicago, USA). Chi-square test was used for degree of association between placental malaria infestations and foetomaternal outcome, a p value of <0.05 at 95% confidence interval was considered statistically significant. Logistic regression analysis was used to determine factors predictive of adverse pregnancy outcomes.
Results: There was statistically significant association between placental malaria parasitaemia and low birth weight (x^2=20.30, p=0.000), congenital malaria (x^2=147.5, p=0.000), congenital anaemia (x^2=65.1, p=0.000) and maternal anaemia (x^2=44, p=0.000).
Conclusions: There was statistically significant association between placental malaria parasitaemia and low birth weight (x^2=20.30, p=0.000), congenital malaria (x^2=147.5, p=0.000), congenital anaemia (x^2=65.1, p=0.000) and maternal anaemia (x^2=44, p=0.000).

Keywords: Placental malaria parasitaemia, Foeto-maternal outcome, Malaria in pregnancy

INTRODUCTION

Globally, the highest burden of malaria infection is in the Low and middle-income countries[1]. Of the 228 million cases reported by WHO in 2018, 93% occurred in Africa.[2] Pregnant women and children under the ages of five years are most vulnerable to malaria infection in our region.[1,2] As the number of malaria-endemic regions decline, studies show that immunity to malaria in previously endemic areas is waning, resulting in increased adverse pregnancy outcomes among infected women.[3]

About 63% of pregnant women in endemic regions have been found to have placental malaria regardless of whether they show symptoms of malaria infection or not.[4] The pathophysiology of adverse obstetrics and neonatal outcomes of placental malaria occurs when the parasite-infected red blood cells sequester in the intervillous spaces of the placenta.[3] The parasitised erythrocytes have the ability to adhere to chondroitin sulphate A on the syncytiotrophoblast.[5] This leads to the accumulation of these infected erythrocytes in the intervillous space and subsequent infiltration of maternal monocytes/macrophages causing massive chronic

intervillositis especially in severe placental malaria.[6,7] Placental malaria is thought to be the mechanism through which malaria exerts its poor obstetrics outcomes. These negative effects occur through the disruption of the maternofoetal nutrient exchange and the inflammatory response in the placenta. Recent studies of pregnant women with and without placental malaria demonstrated that placental malaria had a significant association with low birth weight, preterm birth, still birth, small for gestational age and higher rates of maternal anaemia.[1,8-11]

Recent data from the United Nations Children Fund estimates that 13% of all babies born in sub-Saharan Africa are low birth weight; and malaria during pregnancy is thought to be an important contributor, accounting for up to 900,000 LBW neonates every year.[10] Although, malaria may affect weight through malaria induced anaemia, it may also reduce birth weight via the effects of placental infection.[3]The low birth weight associated with placental malaria is a significant global public health concern. Neonates and infants with low birth weight have increased risk of death in the first year of life, with 200,000 infant deaths a year as a result of maternal malaria.[12,13] Additionally, these infants are at increased risk for poor developmental outcomes through childhood and beyond.[14] studies have consistently associated placental malaria with low birth weight as babies born to mothers with placental malaria have significant reduction in birth weight compared to those without placental malaria.[1,3,15-19]

Over 200,000 stillbirths in sub-Saharan Africa are attributed to malarial infection, making malaria one of the most preventable infectious causes of stillbirth worldwide.[20,21]Acute or chronic placental malaria infection is associated with a 2-fold increase in stillbirth risk and is thought to be the link between malarial infection and stillbirth.[3]

The prevalence of foetal anaemia defined as cord blood haemoglobin level less than 12.5 g/dl, is reportedly very high in sub-Saharan Africa.[22] The aetiology of foetal anaemia is complex and multi-factorial; placental malaria could play either major or minor role depending on the local epidemiological situation. It has been suggested that exposure of the foetus to the malaria antigens due to damage of placental barrier may make the new born more susceptible to immunologically mediated haemolysis or to dyserythropoiesis.[23]

There are conflicting reports on the effect of placental malaria on preterm labour.[23,24] Although the precise effect of malaria parasitised placentae on preterm delivery is uncertain, malaria-infected placentas frequently carry antibodies, cytokines, and macrophages, which are indicative of an active immune response, and this immune response may stimulate early labor.[24]

The biological processes that mediate intrauterine growth restriction (IUGR) as a result of placental malaria remain uncertain, given that they can be studied only after the placenta has been delivered. However, the IUGR effect appears to relate to nutrient transport to the foetus.[3]A high density of parasites and chronic parasite infection in the placental blood and the associated cellular immune response may result in consumption of glucose and oxygen that would have gone to the foetus. Histopathological studies of infected placentas have found thickening of the cytotrophoblastic membranes, which may interfere with nutrient transport.[3]Although IUGR is more common than preterm delivery with chronic placental infection, chronic infection of the placenta (with pigment and parasites) may be associated with LBW, through both prematurity and IUGR.[25]

Malaria in pregnancy may result in foetal exposure if parasites are transmitted across the placenta and could result in congenital malaria. Trans placental transmission of *P.falciparum* has been well described and the reported frequency of this event in babies born in malaria exposed pregnancies has ranged from 1.1% to 37%.[26-28] Recent review of studies in sub-Saharan Africa has indicated that congenital malaria is more common than previously thought.[29] It is defined as presence of asexual stages of *P.falciparum* in cord blood smear at delivery or peripheral blood smear of the neonate in the first seven days of life irrespective of clinical symptoms.[27]

The maternal anaemia associated with placental malaria tend to be severe, with haemoglobin of less than 7g/dl.[3] Maternal anaemia is one of the major complications of malaria in pregnancy in sub-Saharan Africa.[2]Anaemia is a commonly encountered complication of *falciparum* malaria and most pregnant women with persistent parasitaemia will have some degree of anaemia upon evaluation. The mechanisms by which malaria causes anaemia include haemolysis of parasitised red blood cells that occurs in intravascular compartment and by sequestration in the microcirculation mainly in the spleen, specific/nonspecific immune responses which shortens red cell survival, and causes defective erythropoiesis and premature destruction of the red cells during maturation in the bone marrow. If anaemia is very severe, other cardiovascular complications may occur. In addition to its effects, anaemia also increases the risk of low birth weight.[3]

Severe anaemia during pregnancy not only increases the risk of low-birth-weight infants but also increases the risk of death from postpartum haemorrhage; a common obstetric complication independent of malaria.[30,31] This again highlights the important and devastating impact of placental malaria on pregnant women.

The aim of the study was to determine the effect of placental malaria parasitaemia on foetomaternal outcomes among parturients at Federal University Teaching Hospital (FUTH), Lafia, northcentral Nigeria (formally Dalhatu Araf Specialist Hospital, Lafia).

METHODS

Study area

The study was conducted at the Department of Obstetrics and Gynaecology of FUTH Lafia, Nasarawa state. The hospital is the only tertiary health institution located in the state capital, receiving patients from other parts of the state and parts of neighbouring states of Kaduna, Benue Taraba and Plateau.

Study population

The study population comprised all consenting pregnant women who presented to labour ward for delivery and were eligible for the study.

Study design

This was a cross-sectional study

Sampling technique

Consecutive sampling technique was carried out.

Selection criteria

All pregnant women who presented to the delivery suite (for delivery) and consented to be part of the study excluding the following: women with chronic medical conditions, vaginal bleeding, alcohol/tobacco intake, multiple gestation, and congenital foetal malformations.

Sample size determination

The sample size for this study calculated using Fisher's formula below

$$N = \frac{Z^2 P\,(1-P)}{d^2} = \frac{(1.96)^2[0.137\,(1-0.137)}{(0.05)^2} = 182$$

Where,

N is minimum sample size; Z is proportion of normal distribution corresponding to the required (5%) significance level (which is1.96); P is prevalence of placental malaria parasitaemia (which is 13.7% or 0.137 from a previous study.[10] d is Degree of accuracy/ precision expected (0.05).

The sample size is therefore 182. Giving allowance for a 10% attrition rate, the minimum sample size for the study is 200 participants.

Data collection

The procedure was explained to the subjects and a written consent was obtained from each one of them. Data was collected from each subject using a well-designed questionnaire administered by the researcher with the aid of research assistants and the information was entered into the pro forma. The questionnaire contained information that includes maternal age, marital status, educational status, occupation, gravidity, antenatal care, malaria preventive measures. Birth weight of babies, maternal blood, cord blood and placental blood microscopy for malaria parasite detection as well as parasite density.

Two millilitres (ml) of blood was collected from the ante cubital vein, using disposable syringe, was placed in a specimen bottle containing ethylene diamine tetra-acetic acid (EDTA) and sent to the laboratory. In the laboratory, the packed cell volume (PCV) was evaluated by centrifuging blood sample in a capillary tube using a haematocrit centrifuge and the PCV was read on a micro haematocrit reader. Blood film for malaria parasites was also done.

The portion of the cord attached to the placental was cleaned and 2 ml of cord blood was collected from this portion into heparinised (EDTA) bottle for estimation of baby's packed cell volume and blood film for malaria parasites.

Placental blood collection

The aspiration method of placental blood collection was employed.[28] This was done immediately after delivery of the placenta. The placenta (with its maternal surface facing up) was placed on a smooth surface. The maternal placental surface was pierced (to a depth of about 0.5 cm) with a sterile disposable syringe on a 14-gauge hypodermic needle. Two millilitres of placental blood was aspirated and put into an EDTA bottle. The specimen was labelled to correspond with the code on the questionnaire (for easy identification) and was sent to the laboratory for processing (smearing, staining and microscopic examination).

Weighing of neonates

Immediately after delivery and necessary neonatal resuscitation, neonates were cleaned and weighed with an electronic baby weighing scale to the nearest 0.1 g.

Laboratory procedure

Preparation and staining of the thick blood smear: Two drops of placental blood sample were placed on a slide using a small rubber pipette. Using the edge of a second slide, the drops of blood were joined and spread to make an even thick smear and was allowed to air-dry. The slide was immersed in the staining trough, containing 10% of freshly prepared Giemsa solution at pH 7.2 and contact maintained for 10 minutes. The slide was then removed and allowed to dry.

Preparation and staining of the thin blood smear: One drop of blood (maternal, placental and cord blood) was placed

on a clean slide using a rubber pipette. The edge of a second slide was held steadily at an angle of 45º to the first was used to spread the drop of blood to create the thin blood film. It was stained as above.

Microscopic examination of slides for malaria parasite and determination of parasite density: The stained placental blood smears were examined under × 100 oil immersion lens of a light microscope. Malaria diagnosis was made based on identification of asexual stages of Plasmodium species on the thick blood smear while the thin blood smear was used for species identification. Parasite density was determined by counting the number of parasites per high power field in the thick blood smear.

The same procedure was repeated for maternal blood and cord blood samples.

Data analysis

Socio-demographic characteristics of the mothers and information from the structured questionnaire was entered and statistical analysis was done using statistical software (IBM SPSS for windows® version 23.0, SPSS Inc.; Chicago, USA). Quantitative data such as age, birth weight, gestational age and placenta weight was presented using mean and standard deviation. Chi-square test was used for degree of association between placental malaria infestations (primary outcome and independent variable) and maternal anaemia, low birth weight, congenital malaria, and congenital/maternal anaemia (secondary outcome and dependent variables), a p-value of <0.05 at 95% confidence interval was considered statistically significant. Logistic regression analysis was used to determine factors predictive of pregnancy outcomes such as low birth weight, congenital malaria, preterm delivery, congenital anaemia, stillbirth and maternal anaemia.

RESULTS

The study was conducted over a period of 10 weeks, between 13th of July and 20th of September 2023. There were 450 deliveries out of which 200 pregnant women who met the inclusion criteria for the study and consented were recruited.

Table 1 shows the socio-demographic characteristics of subjects. The ages of the 200 women ranged from 17-41 years with mean age of (Mean±SD) 28.0±6.1 yrs. Majority 82 (41.0%) had attained secondary education,77 (38.5%) had tertiary education, few women 21 (10.5%) had primary education while 20 (10.0%) of the women had no formal education. The study further revealed that greater percentage of the women 97.5% were married and only 2.5% were single. Most 103 (51.5%) of the subjects were unemployed while 93 (46.5%) were employed. Majority of the women 151 (75.5%) reside in the city while 49 (24.5%) lived in rural areas.

Most of the women 70 (35.0%) were para 3 and above, 51 (25.5%) were para 2 while 42 (21.0%) were para 0. The assessment of IPT usage and doses showed that, of the total number of mothers in this study 54 (27.0%) did not take intermittent preventive treatment for malaria (IPT), 94 (47.0%) had 3 doses and above, 22 (11.0%) had 2 doses while 30 (15.0%) had one dose.

Table 2 shows prevalence of malaria infection at delivery. Placental blood malaria parasitaemia, maternal peripheral blood malaria parasitaemia and Cord blood malaria parasitaemia were 35 (17.5%), 37 (18.5%) and 27 (13.5%) respectively. *Plasmodium Falciparum* was the only malaria specie detected in all the blood films.

Tables 3 and 4 show foetal outcome. The association between placental blood malaria parasitaemia at delivery and infant birth weight showed 48.1% of babies that tested positive to placental malaria had low birth weight as opposed to 12.7% that tested positive to placental malaria but had normal weight. The mean birthweight of babies with placental malaria is 2492.3g and those without placental malaria is 3183.7 g. Low birthweight is significantly associated with placental malaria on both bivariate and multiple logistic regression analysis (X^2=20.30, p<0.005, OR=6.373).

The relationship between placental blood malaria parasitaemia and congenital malaria showed; all babies (100%) that have congenital malaria tested positive to placental malaria and only 4.6% of babies tested positive to placental malaria but had no congenital malaria. This shows statistically significant association between placental malaria parasitaemia and congenital malaria with a p value of (X^2=147.15, p<0.005) on both bivariate and multiple logistic regression analysis.

The association between placental blood malaria parasitaemia and congenital anaemia. This assessment revealed 62.2% of babies that tested positive to placental malaria had congenital anaemia while only 5.9% of babies tested positive to placental malaria but have normal haemoglobin concentration. Congenital anaemia was significantly associated with placenta malaria parasitaemia (X^2 =65.1, p value<0.005).

Total stillbirth in this study was 8 (4%) and 2 (25%) tested positive to placental malaria. There was no statistically significant association between placental malaria and stillbirth with p value (p>0.05)

Majority of the babies in this study 177 (88.5%) were born at term ≥37 weeks while only few 23 (11.5%) were born preterm, 6 (26.1%) of babies that were preterm had placenta malaria parasitaemia. This therefore, showed that placenta malaria parasitaemia had no significant effect on prematurity with a p value (p=0.249).

The assessment of birth asphyxia and placenta malaria parasitaemia revealed no statistically significant

relationship. Presence of placental malaria and Neonatal anthropometry showed statistically significant association between placental malaria and head circumference with a p value ($p<0.005$). However, there was no association with neonatal length. Low placental weight (placental weight less than or equal to 330 g) was seen in eight of the mothers at delivery. There was no statistically significant association between placental malaria and low placental weight.

Table 5 shows maternal outcome of placental malaria. The association between placental blood malaria parasitaemia and maternal anaemia showed that 63.0% that had placental malaria also had maternal anaemia as opposed to 10.4% of women that tested positive to placental malaria but had no anaemia. The risk of anaemia was significantly associated with placenta blood parasitaemia (X^2 =44.0, p=0.00).

Table 1: Sociodemographic characteristics of subjects.

Characteristics	Frequency	Percent (%)
Mean age (years)	28.0	
Age (years)		
17-22	42	21.0
23-28	68	34.0
29-34	50	25.0
35-41	40	20.0
Total	200	100
Mothers level of education		
None	20	10.0
Primary	21	10.5
Secondary	82	41.0
Tertiary	77	38.5
Total	200	100
Marital status		
Married	195	97.5
Single	5	2.5
Total	200	100
Employment status		
Unemployed	103	51.5
Employed	93	46.5
Total	200	100
Place of residence		
Rural	49	24.5
Urban	151	75.5
Total	200	100
Parity		
0	42	21.0
1	51	25.5
2	37	18.5
3+	70	35.0
Total	200	100
Mean parity	2.34	
IPT Dosage		
None	54	27.0
1dose	30	15.0
2 doses	22	11.0
3+ doses	94	47.0
Total	200	100

Table 2: Prevalence of malaria infection at delivery.

Characteristics	Frequency (n= 200)	Percent (%)
Placental blood malaria parasitaemia	35	17.5
Maternal peripheral blood malaria parasitaemia	37	18.5
Cord blood malaria parasitaemia	27	13.5

Table 3: Foetal outcome of placental malaria.

Characteristics	Placental malaria		X^2	P value
	Yes (%)	No (%)		
Low birth weight (kg)				
Yes (<2.5)	13 (48.1)	14 (51.9)	20.30	0.000
No (≥2.5)	22 (12.7)	151 (87.3)		
Congenital malaria				
Yes	27 (100)	0 (0.0)	147.15	0.000
No	8 (4.6)	165 (95.4)		
Congenital anaemia (g/dl)				
Yes (<12.5)	25 (62.5)	15 (37.5)	65.1	0.000
No (>12.5)	8 (5.9)	128 (94.1)		
Birth asphyxia: APGAR				
Yes<5	0 (0.0)	3 (100)	2.92*	0.404
No>5	33 (40.5)	156 (59.5)		
Stillbirth				
Yes	2 (25.0)	6 (75.0)	0.325*	0.569
No	33 (17.2)	159 (82.8)		
Gestational age at delivery (weeks)				
Preterm<37	6 (26.1)	17 (73.9)	1.327	0.249
Term≥37	29 (16.4)	148 (83.6)		
Neonatal length (cm)				
<45	6 (25.0)	18 (75.0)	1.063	0.303
>45	29 (16.5)	14 (51.9)		
Head circumference (cm)				
<33	21 (36.2)	151 (87.3)	19.8	0.000
>33	14 (9.9)	0 (0.0)		
Placental weight (g)				
<330	2 (25.0)	165 (95.4)	0.325*	0.569
>330	33 (17.2)	15 (37.5)		

Note: Chi square (x), Fischer's exact test (*)

Table 4: Logistic regression analysis of foetal outcomes that are associated with placental malaria.

Foetal outcome	Odds ratio	95% CI	P value
Low birth weight	6.373	2.65-15.3	0.000
Congenital malaria	3.332	0.212-9.998	0.000
Congenital anaemia	1.00	0.014-3.283	0.000

DISCUSSION

Placental malaria parasitaemia is associated with adverse foetomaternal outcomes in this study. Mothers whose placentae were positive for malaria infection had 6 folds increased risk of delivering low birth weight babies than those whose placentas were negative to malaria (OR=6.373 C.I 2.65-15.3, p=0,000). The mean birth weight of babies whose mothers tested positive to placental malaria was 691g less than those that tested negative to placental malaria. This finding is statistically significant and is in keeping with findings from other studies.[10,16-21,34,35] This low birth weight can result either from preterm delivery or intrauterine growth restriction or both. The intrauterine growth restriction results from sequestration of malaria parasites at the placenta bed causing reduced nutrition supply to the foetus. The placenta is highly vascularised and a favourite site for parasite sequestration and development. This leads to damage to the placental vessels associated with thickening of the basement membranes and necrosis and ultimately compromised placental perfusion.

The prevalence of congenital malaria in this study is 13.5%; this is much lower than the prevalence of 50.5% reported by Bassey and collegues.[32] Presence of placental malaria is strongly associated and also an independent risk factor for congenital malaria. Babies born to mothers with placental malaria have a 3- fold risk of having congenital malaria (X^2=147.15, OR=3.332 p=0.000). In this study all positive cases of cord blood smear also had positive placental blood smear. It was also noted that none of the babies with positive cord smear had clinical manifestation of malaria at birth, this is consistent with findings in Port Harcourt.[32] This may be due to the effect of maternal IgG antibodies transferred to the foetus. These antibodies may

delay or modify the onset of clinical manifestation of congenital malaria.[33]It is therefore important that babies with positive cord blood should be followed up closely as some cases of congenital malaria have been reported several weeks after birth.[33]

The rate of congenital anaemia of 20% in this study is similar to that found in two separate studies in Malawi with prevalence rates of 23.4% and 23.3% respectively.[33,34] Placental malaria is significantly associated with congenital anaemia (p value=0.000). It has been suggested that exposure to malaria antigens due to damage of the placenta barrier, may increase newborn susceptibility to immunologically mediated haemolysis or to dyserythropoiesis leading to anaemia.[35]

The prevalence of maternal anaemia in this study is 13.5%, and placental parasitaemia was found to be significantly associated with lower maternal packed cell volume (PCV). This was not unexpected as the association of malaria in pregnancy and lower maternal haematocrit has been recognised and reported by previous workers.[16,17,32] The anaemia in this study was mostly mild and non-needed blood transfusion. Malaria parasitaemia is a well-documented cause of anaemia in pregnancy, the causes of anaemia in pregnancy are multifactorial and could be as a result of poor nutrition, poverty, sickle cell disease, helminthiasis etc.[36] Poor nutrition and poverty were unlikely causes of anaemia in the study population as most of the women that took part in this study had tertiary level of education and were of middle or upper socioeconomic class. Efforts were made in this study to exclude other causes of anaemia in pregnancy as women with sickle cell anaemia, multiple pregnancy and retroviral disease were excluded from this study. Despite this effort, it was still difficult to attribute this prevalence of anaemia solely to the effect of malaria parasitaemia as other causes such as helminthiasis were not excluded in this study.

The average gestational age at delivery in this study was 38.8 weeks with prematurity rate of 11.5%. This prematurity rate of 11.5% was lower than findings in the Gambian population with prematurity rate of 20.8%, there was no significant association between placental malaria and preterm delivery in the study in Port Harcourt.[19,32] Similarly, this study did not find any significant relationship between placental malaria and prematurity. However, other reports across Sub-Saharan Africa have shown that placental malaria was significantly associated with prematurity.[18,37] With these contrasting findings, it suggests that other risk factors could be responsible for the preterm delivery.

Our findings did not establish any relationship between placenta malaria parasitaemia and birth asphyxia (p=0.404). The total stillbirth in this study was 8 (4%) out of which two tested positive to placenta malaria. There is no statistically significant association between placenta malaria and still birth. This is in contrast to studies in Gambia and Ethiopia who found 2 folds and 7 folds increase in still birth rate with placental malaria respectively.[18,38]

The finding that *Plasmodium falciparum* was the only parasite species detected in all the placental blood films in this study confirms previous reports that placental malaria is an exclusive feature of *Plasmodium Falciparum* infection in pregnancy[39] and that *Plasmodium falciparum* is the most prevalent specie in Nigeria accounting for about 98% of malaria cases in the country.[40]

CONCLUSION

Placental malaria parasitaemia is associated with adverse foeto-maternal outcome of congenital anaemia, congenital malaria, low birth weight and maternal anaemia. Concerted effort should be put in place to reduce the incidence of malaria thereby preventing these untoward effects.

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