Original Research Article

PLACENTA AND CORD BLOOD MALARIA IN MOTHERS AND NEONATES ATTENDING FEDERAL UNIVERSITY TEACHING HOSPITAL, OWERRI, SOUTH EAST NIGERIA

ABSTRACT

Introduction

In Malaria endemic countries, gestational and cord blood malaria prevalence are highly variable. A comprehensive study to determine the prevalence of placental and cord malaria in this part of the country has not been undertaken. Thus, the need to determine fever cases associated with *Plasmodium falciparum* infection among children aged 1-72months attending a Secondary Health facility in Imo State, Nigeria.

Methodology

A hospital based cross sectional descriptive study carried out between the months of July 2021 and June, 2022 in some public and private hospitals in Owerri, south eastern Nigeria. Ethical approval was obtained from both Health institutions while written consent was obtained from patients before carrying out the study. Malaria transmission is stable with a high seasonal transmission from July to October. Placental and umbilical cord blood was collected in an EDTA from mothers who consented and their neonates at delivery. The presence of *Plasmodium* were assessed microscopically by WHO Certified Malaria Microscopists. Malaria parasite density was grouped as 1-500parasites / µl, 501-5,000parasites/µl, 5,001–10,000 parasites/µl, and >10,000 parasites/µl respectively.

Result

Placental and congenital malaria prevalence by microscopy was 21.3% vs. 8.2%. The primigravid had the highest infection rate of 33.0%. Considering the relationship between infection prevalence and parity of pregnancy, there was significant difference P=.001. 4.2% of 119 neonate and 13.6% of 88 neonates from multigravid and primigravid mothers respectively examined had cord malaria. There was significant difference P=.002 comparing cord malaria infection prevalence and parity of pregnancy of matched mothers. The relationship between parasite malaria density and parity of pregnancy both in placental and cord malaria were not significant. Age group 20-25 years (45%) had highest Prevalence while age groups 26-30 years recorded prevalence of 33.3% for the primigravid and multigravid group, respectively (P<0.05). The Geomean range of 220 (3-8,250) vs. 23(2-6,412) parasite/µl of blood were recorded in primipare vrs multipare group.The result of this study showed moderate placental malaria infection and low prevalence cord malaria by microscopy. The presence of malaria parasites in cord blood at delivery and non in maternal placental blood was also demonstrated.

Conclusion

Antenatal exposure to malaria parasites may have profound effects on the fetus therefore prevention of malaria infection during pregnancy which may reduce the incidence of adverse perinatal outcomes should be strongly advocated.

Keywords: *Plasmodium falciparum*, placenta, umbilical cord blood, parity, malaria parasite density

Comment [BS1]: Not sure if this study has not been undertaken. You need to specific the exact place instead of generalized

Comment [BS2]: Rephrase sentence

Comment [BS3]: Remove descriptive... Cross sectional study is appropriate

Comment [BS4]: Xo many incomplete sentences

Comment [BS5]: Incomplete sentences

1. INTRODUCTION

The prevalence of gestational malaria is highly variable in sub-Saharan Africa regions. Reports of prevalence ranging from 5% in Ghana [1] 28% in Uganda [2], 28.2% to 42.7% in Burkina Faso [3, 4] and in Nigeria 68.3% [5], 69.6% [5], 70.5% [6] have been documented. Early peripheral infection during pregnancy may be a particularly important risk factor for placental infection, due to lower immune protection at the beginning of pregnancy. Nevertheless, susceptibility may be correlated to high exposure to malaria, and repeated episodes of parasitemia, as well as the interplay between several other factors. Pregnant women are more susceptible to malaria infection than their nonpregnant counterparts in malaria endemic areas [7, 8]. Susceptibility diminishes with successive pregnancies, and this pattern is most prominent in high transmission areas where primigravidae are significantly more susceptible to *Plasmodium falciparum* infection and disease than multigravidae [7, 8]. This parity-dependent epidemiological signature distinguishes *P. falciparum* from several other infectious agents that can afflict pregnant women. Although in low transmission areas, women of all parties have increased susceptibility to malaria, infection rates may still be highest in primigravidae [9].

Consequent to various evidence of the relative failure of many traditional antimalarial drugs, particularly chloroquine, the WHO has put forward new guidelines for combating and preventing malaria during pregnancy [10, 11]. The guidelines recommend that women living in high transmission areas of Africa receive intermittent preventive treatment (IPT) with an effective antimalarial agent such as sulfadoxine-pyrimethamine (SP) at scheduled antenatal visits, and all pregnant women in targeted areas should undergo at least two sessions of IPT after first fetal movements (i.e., between 20 to 35 weeks) [11]. WHO recommends IPTp with sulfadoxine-pyrimethamine (IPTp-SP) in all areas with moderate to high malaria transmission in Africa. As of October 2012, WHO recommends that this preventive treatment be given to all pregnant women at antenatal care visit starting as early as possible in the second trimester, Each IPTp-SP dose should be given at least 1 month apart. WHO recommends at least 3 doses during each pregnancy [11].

Gestational malaria is responsible for a high maternal and infantile morbidity; a high susceptibility to malaria infection during the first months of life is one of the serious consequences of Malaria vertical transmission on the newborn [16, 17] and also an early susceptibility to other infections [18, 20]. This susceptibility to infection is due to neonatal T cells imbalance and proinflammatory and anti-inflammatory immune responses after their sensitization by *Plasmodium falciparum in utero* [21, 22, 23] Exposure of a fetus to malaria may prime immune responses or induce immune tolerance that may subsequently affect susceptibility to infection and disease during infancy [19].

In this present study, we compared malaria infection during female delivery in maternal placental blood and neonatal cord blood. We went ahead to also quantify malaria parasites in paired maternal placental-blood, and cord-blood samples obtained from women and their neonates living in a malaria-endemic area of Nigeria. This study was carried out by the use of microscopy, which is also sensitive, specific when performed by a competent microscopist.

Comment [BS6]: Just use a range and cites the 6 articles

Comment [BS7]: What do you mean by traditional antimalarial drugs; Chloroquine has been eradicate as the malaria treatment

Comment [BS8]: IS this the most recent treatment plan

Comment [BS9]: Reword the sentence

Comment [BS10]: Sentence reconstruction

2. MATERIALS AND METHODS:

Enrolment of Study Participants

The study was conducted in Imo state, Nigeria, specifically at the Federal medical center, Owerri, a government tertiary health facility. Two hundred and seven (207) pregnant women who attended their antenatal clinics (ANCs) in the hospital (booked) who gave written consent to participate in the study were recruited. These women completed a questionnaire to determine their demographic and maternal characteristics (age, gravidity, and gestational age, number of antenatal visits, SP dosage, and history of fever attack during pregnancy). Some of the Exclusion criteria were (a) Expectant mothers with evidence of chronic illness and complicated pregnancy (hypertension, preeclampsia, diabetes) (b) multiple pregnancies. Codes were given to the study participants and their newborn immediately after delivery and all forms personal identifiers removed to ensure confidentiality.

Comment [BS11]: It might good idea to have the table of the list of the variables collected

Comment [BS12]: Explain why are these excluded from the data collected

Collection of samples:

Blood samples were collected from cords of live, singleton, full term neonates delivered in the hospital and also from their matched mother's placenta. Multiple aspirations were made on the maternal half of the placenta, just below halfway between the maternal and fetal surfaces using a 19-gauge needle attached to a 2ml syringe. From the aspirates (blood), duplicate thick and thin films were made on clean microscopic slides. Cord blood was collected by cannulation of the umbilical vein after inversion of the placenta and cleaning of the umbilical cord with 70% alcohol to avoid maternal blood contamination and incised at ~15cm from its attachment to the placenta with a fresh blade.

Laboratory Methods

Thin and thick smears were prepared for each participant following standard procedures for malaria microscopy. Examination of 3% Geimsa stained blood film was done using x100 objective. Two competent microscopists read each slide, and when there was discordance in reading, a third microscopist reread the slide and served as a tie breaker. Essentially, the discordance level for the acceptance of any two parasite counts was set at less than 20%.

Ethical Consideration

Approvals to conduct this study were obtained from the Ethical committee of Federal medical center owerri, Imo state and the protocol was conducted in accordance with Good Clinical Practice (GCP), Good Clinical Laboratory Practices (GCLPs)

Data Analysis

The data generated from the study were analyzed using EPIINFO 2002 statistical software (CDC, Atlanta, GA, USA). Tests for associations and differences were done by chi-square analysis, Fischer Exact test, and analysis of variance was done as appropriate. Value less than 0.05 was taken as significant.

Comment [BS13]: Need to explain why this type of analysis chi square was used. Also, explain the data e.g. categorical, numerical etc.

3. RESULTS

Table 1: Profile of Study Participants

Character	Test
Number of participants	207
Age (years)	23.3±18.4
Mean ±SD	43 (20.8%)
20-25	63 (30.4%)
26-30	62 (30.0%)
31-35	26 (12.6%)
36-40	13 (6.3%)
41-45	
Microscopy(placenta)	
Positive	44 (21.3%)
Negative	163(78.7%)
Primigravidae	29 (33.0%)
multigravidae	15 (12.6%)
Microscopy(cord)	
Positive	17 (8.2%)
Negative	190 (98.1%)
Primigravidae (neonate)	12 (13.6%)
Multigravidae (neonate)	05 (4.2%)
Placental Parasitaemia Geomean (range)	
Primigravidae	220(3-8,250) (p/µL)
Multigravidae	23(2-6,412) (p/µL)
1-500	14 (31.8%)
501-5,000	25 (56.8%)
5001-10,000	5 (11.3%)
>10,000	0 (00%)

Study showed placenta Infection prevalence of 21.3%, the primipare had the highest infection rate of 33.0%. Considering the relationship between infection prevalence and parity of pregnancy, there was significant difference *P*=.001. This study also showed overall cord malaria of 8.2% while 4.2% of 119 neonate and 13.6% of 88 neonates from multipare and primipare mothers respectively examined had cord malaria. There was significant difference *P*=.02 comparing cord malaria infection prevalence and parity of pregnancy of matched mothers (Table 1)

Comment [BS14]: Need to write about chi square

Comment [BS15]: The table 1 does not shows or indicate significant. This is just a descriptive table

Table 2: Overall prevalence of placental and umbilical cord malaria

Parity	No Examined	No infected		No uninfected	
		Placenta	Cord	Placenta	Cord
Primipare	88	29(33.0)	12(13.6)	59(67.1)	76(86.4)
Multipare	119	15(12.6)	5(4.2)	104(87.4)	114(95.8)
Total	207	44(21.3)	17(8.2)	163(78.7)	190(91.8)

Considering the relationship between parity of pregnancy and placental /cord parasite malaria density as shown in table 5, there was no significant difference. Few of the women recorded high parasite density count (5001-10,000 parasite/µL) as seen in 3 (three) out of 29 primigravid and 1(one) out of 15 multigravid . No neonatal cord was seen with high parasite density.

Table 3: Relationship between parity of pregnancy and placental /cord parasite malaria density.

Comment [BS16]: This table is not clear is this a cross tabulation?

Density (p/µL)	Primipare Freq. (%)		multipare Freq.(%)	
	placenta	Cord	Placenta	Cord
1-500 (low)	9(31.0)	8(66.7)	5(33.3)	3(60.0)
501-5000 (mid)	17(58.6)	4(33.3)	8(53.3)	2(40.0)
5001-10,000 (high)	3(10.3)	0 (0.0)	2(13.3)	0 (0.0)
>10,000 (very high)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Considering placental malaria prevalence by age, 26-30 had higher infection in multigravidae while 20-25 years had highest prevalence in the primigravidae

Table 4: Age related distribution of placenta malaria

Comment [BS17]: All of these tables are descriptive. Which one is actually showing the chi square analysis. Also, move all the descriptive tables up

Age (years)	Primipare		Multipare	
	No examined	No.infected	No examined	No.infected
20-25	20	9(45.0)	23	2(13.3)
26-30	29	10(34.5)	34	5(33.3)
31-35	22	7 (31.8)	40	4 (26.7)
36-40	11	2 (18.1)	15	3(20.0)
41-45	6	1(16.7)	7	1(6.7)
TOTAL	88	29(33.0)	119	15(12.6)

There was no significant difference as regards antimalaria /SP usage between the different parity groups. *P*=0.70(Table 4) while there was a significant difference in the use of ITNs by these pregnant mothers. Study showed highest infection rate among mothers with no formal education (26.7%) while least infection was recorded among mothers who had only primary (19.4%) education (Table 5)

Table 5: Academic status/ rate of placental malaria infection

Academic status	Total	Uninfected	Infected
Non formal Education	15	11(73.5)	4(26.7)
Primary education	62	50(80.6)	12(19.4)
Secondary education	89	70(78.7)	19(21.3)
Tertiary education	41	32(78.0)	9(22.0)
Total	207	163(78.7)	44(21.3)

Table 6: ITN Usage compliance rate among pregnant mothers

ITN USAGE	Total	Primigravid	Multigavid
Daily	33(15.9)	18(20.4)	15(12.6)
Once a week	58(28.0)	22(25.0)	36(30.3)
Not at all	122(58.9)	48(54.5)	68(57.1)
Total	207	88	119

Key:
ITN: Insecticide Treated Nets

Table 7: Antimalaria drug usage by pregnant mothers

SP/anti malaria intake	No Examined	%Primipare	%Multipare
(2-4 times in 9months	81(39.1)	42(47.7)	39(32.7)
Once in 9 months	94(45.4)	35(39.8)	59(49.6)
None	32 (15.5)	11(12.5)	21(17.6)
Total	207	88	119

4. DISCUSSION

This study showed placental and cord malaria prevalence rate of 21.3% and 8.2% respectively. Similar studies conducted in parts of South-eastern Nigeria on placental malaria showed higher prevalence of 69.6% and 70.5% [5, 6], which they attributed majorly to un-booked maternal status, nonuse of both IPT and insecticide treated nets (ITN).

Comment [BS18]: Citation

This study is in consonance with Previous Studies that have demonstrated the relationship between placental malaria and parity and have reported the prevalence to be higher in primigravidae than multigravidae, and in these studies, results are controlled for age [24, 25, 26, 27].

The Geo mean of placental parasite density in this study was 851 (6-9830) parasite/ µl .while the primigravidae recorded a Geo mean of 420(20- 8,942) parasite/ µl, multigravidae had a Geo mean of 431(6- 6,200) parasite/ µl. It is also important to note that both the primigravid and multigravids recorded almost same frequency of distribution parasite density. This study is therefore in contrast with Nnajietal 2006 who reported that the primigravidae had a higher mean parasite density (2,155/micro l) when compared with the multigravidae (1,950/micro l). Parity In this study had no effect on placental malaria parasite density however there was significant difference in cord-blood parasitaemia obtained from newborns of women with placental malaria, compared with parasitaemia of cord-blood samples obtained from newborns of women without placental malaria

The exact reason why primigravidae are more susceptible to placental malaria and suffer from its consequences more than multigravidae is still of great concern and research interest. This notwithstanding , in an attempt to understand the reason for the primigravide susceptibility, one study explained that pregnancy is associated with a decrease in immunity, which is more pronounced in primigravidae than in multigravidae and may be associated with age [28] 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 [29, 30]. Thus, the placenta may select for the CSA-binding *P. falciparum* phenotype, putting primigravidae 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 [31]. Another possible explanation for this parity-related susceptibility is given by the findings of Duffy and Fried [32], who showed that multigravid mothers develop malaria antibodies that block adhesion of parasites to CSA receptors in the placentae in subsequent pregnancies

In this study, the daily use of malaria preventive measure such as ITNs was low (15 .9%) while 58.9% of pregnant mothers never slept under an LLINs during their gestational period . it was observed also that only 39% of the women had SP administration for IPTp in accordance with WHO recommendation [11] and this suggest a low compliant rate.

Furthermore, considering placental malaria prevalence by age in this study, 20-25 years having highest prevalence in the primigravidae is in agreement with a report that suggested the role age-associated immunity may have played in limiting *P. falciparum* to low parasite densities in areas of high and stable transmission [10]. the low Geo mean parasite density may be explained by some favorable characteristics of the study site/ population but the reason for the shift of Placental Malaria prevalaence in multigravidae from the widely canvassed <20 years to 26-30 years is yet to be understood it may be attributed but not limited to low compliant to intake of administered SP for IPTp among the group ,further investigations is advocated for especially as regards to hormonal and immunologic response [10].

Comment [BS19]: Remove capitalization

Comment [BS20]: Reword citation. Reword sentence

Comment [BS21]: Incomplete sentences

Limitations encountered in this present study were seen during determination of placental and cord malaria primarily by blood-smear microscopy and not by use of placental biopsy for PM or RTQ-PCR which is more sensitive. There are studies that are in concomitant with the present findings that suggest that with the limitation encountered above, it could account for the low parasitaemia.Other methods other than microscopy i.e. polymerase chain reaction (PCR) assays has been seen to be sensitive and further has identified falciparum malaria parasites in 10%–32% of cord-blood samples obtained from individuals in areas where malaria is endemic [12, 13, 14, 15].This has therefore suggested that the presence of malaria parasites in cord blood occurs with greater frequency than previously appreciated.

More so, if there is failure to examine placental biopsy specimen, it therefore leads to permitted determination of only acute placental malaria and not determination of either the severity of placental malaria or whether changes had occurred. This study therefore suggests and advocates for further studies on the use of more sensitive and specific approach to determine the prevalence of placental, cord malaria and possible outcomes of the frequency of administration of malaria chemoprophylaxis during pregnancy.

Comment [BS22]: What is your conclusion.

Availability of data and material (There is transparency of all data)

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Comment [BS23]: Citation too old. Use 10 year reference

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Comment [BS28]: Citation too old. Use 10 year reference