

Placental Malaria and Its Associations with Pregnancy Outcome

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Authors' contributions

This work was carried out in collaboration among all authors. Authors VEO and NJO designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors ZCO, CFO, LO and AOO wrote the protocol and managed the literature search while authors LSA and VEO managed the analysis of the study.

All authors read and approved the final manuscript

ABSTRACT

BACKGROUND

Placental malaria constitutes a risk for both the pregnant women and their unborn child hence there is need to demonstrate the adverse associations between placental malaria and pregnancy outcome.

AIM:

To demonstrate the adverse maternal and fetal associations with placental malaria

STUDY DESIGN AND SETTING:

This is a cross sectional study carried out at Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, South East, Nigeria between 1ST August, 2012 and 31ST January, 2013.

RESULT:

Two hundred placentae were analyzed, 141 (70.5%) were positive for malaria, Placental malaria was associated with lower maternal pcv (30.67 ± 1.60 vs 32.51 ± 2.82 , p-value < 0.001), preterm delivery (OR=4.99, C.I=1.15 – 45.08, p-value=0.020), and low birth weight (OR = 4.71, C.I=1.08 – 42.71, p-value=0.027) but not associated with maternal anaemia (OR=2.27, C.I= 0.90-6.47, p-value=0.063) and perinatal deaths (OR=Infinity, C.I =0.38 – infinity, p-value = 0.143).

34 **CONCLUSION:**

35 Placental malaria was associated with low birth weight, lower pcv and preterm delivery but
36 associated neither with maternal anaemia nor perinatal deaths.

37 Keywords: placental malaria, associations and pregnancy outcome.

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39 **INTRODUCTION**

40 Malaria is one of the world's most deadly diseases which caused approximately 627,000
41 deaths in the year 2012, with more than 90% of these deaths occurring sub-Sahara Africa [1].
42 In addition to the human cost, malaria also carries a huge economic burden within the region
43 as it is estimated that malaria costs African countries more than US\$12 billion every year in
44 direct losses and as much as 40% of Africa's health budgets are spent on malaria each
45 year[2]. Pregnant women and the under-5 children are mostly affected by malaria infection
46 and each year in sub-Saharan Africa, approximately 30 million pregnant women are at risk of
47 contracting malaria and suffering its adverse consequences [3]

48 Placenta is highly vascularised organ and a favored site for parasite sequestration and
49 development. Plasmodium falciparum infected erythrocytes frequently sequester in to the
50 intervillous spaces of the placenta causing release of pro-inflammatory mediators which
51 cause pathological alterations [4,5]. This considerable abnormality in intervillous spaces may
52 jeopardize the nutritional function of the placenta, resulting in poor fetal outcome [6]. By this
53 mechanism, it adversely affects the unborn child and even in the neonatal life.

54 Nnewi is in the malaria endemic region, demonstration of the adverse fetal and maternal
55 associations with placental malaria will go a long way in the understanding of the burden of
56 malaria in our pregnant women and the unborn child. It is important to note that most studies
57 in Africa were done with placental blood smear [7,8] instead of using placental histology
58 which is the gold standard. Using placental histology may change the result as it
59 demonstrates both active and past infections unlike blood smears which detect only active
60 infections.

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63 **MATERIALS AND METHODS**

64 **STUDY DESIGN/ SETTING:**

65 This is a cross sectional study carried out at Nnamdi Azikiwe university teaching hospital,
66 Nnewi in Anambra state. The hospital has antenatal booking rate of about 1000 pregnant women
67 per year and with the delivery rate of about 1200 per year.

68 STUDY POPULATION.

69 Systematic random sampling was used in selecting the patients for the study. The patients were
70 selected from among the women who presented to the delivery suite of NAUTH, Nnewi in
71 labour. They were asked for consent and those who consented were interviewed and examined;
72 and eligibility criteria applied to screen the patients. All the patients were managed according to
73 departmental protocol. **The INCLUSION CRITERIA** were -The pregnant women with sure
74 date (sure LMP or first half of pregnancy scan for dating) and Single fetus with gestational age
75 of at least 28 weeks at the time of delivery. The **EXCLUSION CRITERIA** were : Antepartum
76 hemorrhage, Diabetes mellitus, Pre-eclampsia/ Eclampsia, Multiple gestations, And Sickle cell
77 disease. The sample size was determined the formula [9], $N=Z^2 \cdot P \cdot (1-P)/E^2$

78 N = minimum sample size required for the study; Z =the standard normal deviation, usually 1.96
79 at 95% confidence level; P =prevalence rate 14.7 % in this case, prevalence of placental malaria
80 in Calabar; E =precision rate (5%), degree of accuracy. Therefore $N= (1.96)^2 \times 0.147 \cdot (1 -$
81 $0.147)/0.05^2 = 193$ parturient women (but 200 were used).

82 SAMPLE COLLECTION AND ANALYSIS

83 After due explanation and obtaining informed consent from the pregnant women that met up
84 with the above mentioned criteria, the following data were obtained from them: age, parity
85 gestational age and phone number. Before delivery, blood samples were collected for pcv
86 estimation. At delivery, placenta tissue was taken for histology. The new born was weighed
87 within 12 hours of delivery. All these data were recorded in a proforma.

88 Full thickness placental blocks of around 2–3 cm were taken from the placenta, kept in neutral
89 buffer formalin for histopathology examinations. The Placental tissues were dehydrated by
90 immersing them in graded concentrations of alcohol till the absolute concentration is reached.
91 Clearing and rinsing was done using Zylene. The tissues were embedded using paraffin wax and
92 section done with microtome set at 6 microns. The sections were fixed on a clean slide. Staining
93 was done using Haematoxylin and Eosin. The slides were read under microscope with
94 magnification $\times 400$. Placenta malaria was considered positive when there is parasite and/or
95 pigment (active and past infection respectively) on histology result and negative when both are
96 absent in the histology report.

97 Birth weight of less than 2.5kg was considered low birth weight and PCV of less than 30% as
98 anemia.

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100 4.4 DATA ANALYSIS AND PRESENTATION.

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102 Data were analyzed using spss version 16. Results presented as percentages for categorical
103 variables and as mean $\pm$ sd for continuous variables. The mean values were compared with

student-t-test. Odds ratios and 95% confidence intervals were generated. P-value of < 0.05 was considered significant.

RESULTS

Out of two hundred (200) placentae studied, 141(70.5%) were infected. The women had age range between 17 and 37yrs with mean age of 27.59±5.98 while their parities ranged from 0 to 6, with mean parity of 2.13±1.36. The mean gestational age at delivery was 38.19±1.55weeks and ranged between 34 and 41weeks. The mean birth weight of the babies was 3.22±0.53kg, with range of 2 to 4.2kg. These are demonstrated in table I.

Tables I: The range and the mean of the data collected.

Variables	Range	Mean ± Standard Deviation
1. Age. (years)	17 – 37	27.59 ± 5.98
2. Parity	0 – 6	2.13 ± 1.36
3. Gestational Age (weeks)	34 – 41	38.19 ± 1.55
4. Birth weight (kg)	2.00 – 4.20	3.22 ± 0.53

The table 11 below showed that 20% of the studied population was anaemic while 11.5% had preterm delivery. Low birth weight and perinatal death occurred in 11% and 2.5% of the studied population respectively.

Table 11: The demographic features of the subjects

Variables	NO. (%)
Primigravidae	45(22.5)
Maternal Anaemia	40 (20)

Preterm birth	23(11.5)
Low birth weight	22(11)
Perinatal death	5(2.5)

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123 The mean age of mothers whose placentae were infected and those whose placentae were not
 124 infected were 27.04 ± 6.07 and 28.90 ± 5.60 (P-value 0.045) respectively. The mothers with
 125 placental malaria were of lower parity (1.82 ± 1.11 vs 2.86 ± 1.62 , P-value < 0.001) than those

Table III : Comparison of the placental malaria positive and placental malaria negative women

Variables	PM+ve (Mean)	PM-ve (Mean)	P-value
1. Age	27.04 ± 6.07	28.90 ± 5.60	0.045*
2. Gestational Age	37.96 ± 1.60	38.72 ± 1.28	0.001*
3. PCV	30.76 ± 1.60	32.51 ± 2.28	$< 0.001^*$
4. Mean Birth weight (kg)	3.17 ± 0.521	3.35 ± 0.534	0.025*
5. Parity	1.82 ± 1.11	2.86 ± 1.62	$< 0.001^*$
PM+ve = placental malaria positive and PM-ve = placental malaria negative			
*= significant values			

126 without placenta malaria. The mean gestational age at delivery of babies whose placentae
 127 were infected was lower than the mean of those whose placentae were not infected ($37.96 \pm$
 128 1.60 VS 38.72 ± 1.28 , P-value 0.001) and the mean packed cell volume of mothers who had
 129 placental malaria had significantly lower value than their counterparts whose placentae were
 130 negative to malaria parasite (30.76 ± 1.60 vs. 32.51 ± 2.28 , p-value < 0.001) as shown in Table
 131 III.

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Table IV, shows the associations of placental malaria with pregnancy outcome. Women with placental malaria had 2.27 folds increase risk of anaemia (OR= 2.27, C.I = 0.90 – 6.47, P value= 0.063) but this is not statistically significant. Also the table demonstrated that mothers whose placentae were infected had 5 folds increase risk of delivery preterm babies (OR= 4.99, C.I= 1.15 – 45.08). Placental malaria has been demonstrated to be significantly associated with low birth weight (OR=4.71, C.I 1.08-42.72, P-value 0.026). There is no significant difference in the occurrence of perinatal death between those with placental malaria and those without placental malaria (OR= infinity, C.I =0.38 – infinity, p-value =0.144). But it is important to note that all the five perinatal deaths recorded in the study, had placental malaria.

TABLE IV: THE ASSOCIATIONS OF PLACENTAL MALARIA

Variables	PM+ve (%)	PM-ve(%)	OR	C.I	P-value
1. Mat. Anaemia	33 (82.5)	7 (17.5)	2.27	0.90 – 6.47	0.063
No Anaemia	108 (67.5)	52 (32.5)			
2. Preterm	21 (91.3)	2 (8.7)	4.99	1.15- 45.08	0.020
Term	120 (67.8)	57 (32.3)			
3. Low Birth wt	20 (90.9)	2(9.1)	4.71	1.08 – 42.71	0.027
No Low Birth wt	121 (68.0)	57(32.0)			
4. Perinatal death	5 (100.0)	0(0.0)	Infinity.	0.38 – infinity	0.143
No Perinatal death	136 (69.7)	59 (30.3)			

Discussions

This study documented a placental malaria prevalence of 70.5% using placental histology of 200 women, who delivered at Nnamdi Azikiwe University Teaching Hospital, Nnewi over a period of six months between August 1st, 20012 and 31st January, 2013. This figure is in the same range with that reported by Ezebialu et al [10] in 2010.

In malaria endemic regions like Nigeria, anaemia is a common complication of malaria in pregnancy and is even worse in primigravida. In this study, Mothers with placental malaria were associated with 2.27 folds increase risk of anaemia than those that do not have placental malaria. (OR= 2.27, C.I= 0.90 – 6.47, P-value =0.063), however it is not statistically significant. This is similar to the finding from Maiduguri, northern Nigeria [11] and the work at Zaire by Anagnos et al [12] , who noted that there is no association between placental malaria and maternal anaemia. This differs with the finding in Cameroun by Ernest et al [13] who demonstrated that maternal anaemia and placental malaria were associated in all gravidity and age group. It is also interesting to note that the mean pcv value for those women whose placentae were positive for malaria parasite is significantly lower than their counterpart whose placentae were negative for malaria parasite (30.76 ±1.60 vs 32.51 ± 2.82 P-value <.001). The difference in pcv between the two groups is about 2%. This difference in

PCV between the 2 groups was also noted in the work by Allen et al [14] in New Guinea. The reason for the variation is not clear but it may be related to the multifactorial etiology of anaemia in pregnancy. It may be that most women take haematinics in pregnancy even when they are not booked and this may mask the association between the maternal anaemia and placental malaria; that PCV may drop but does not drop to reach the anemic level.

Placental malaria is known to be associated with preterm delivery and low birth weight [15]. This study demonstrated that placental malaria increased the risk of preterm delivery by 5 folds and the mean gestational age at delivery in the babies whose placentae were positive for malaria was lower than their counterpart whose placentae were negative for malaria (37.96 ± 1.60 vs 38.72 ± 1.28 p-value 0.001). This is in keeping with the work of Menendex et al [15].

In this study, the mothers whose placentae were positive for malaria infection had about 5 folds increase risk of delivering low birth weight babies than those whose placentae were negative to malaria (OR=4.71 C.I= 1.08-42.71). The mean birth weight of former was found to be 180g less than the later group and the finding is statistically significant. This is in keeping with the findings of other works [13,16,17]. This finding cut across both the malaria endemic regions and malaria unstable regions, the only exception is the work by Anagos et al[12] in Zaire where the difference in birth weight between the two groups was 100g which is not statistically significant. But the Zaire study did not exclude mothers with chronic medical diseases and this may account for this difference with other studies above. This low birth weight can result either from preterm delivery or intrauterine growth restriction or both. The intrauterine growth restriction result from sequestration of malaria parasites at the placental bed (placental malaria) causing reduced nutrition supply to the fetus. The placenta is highly vascularised and a favored 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.

This study demonstrated that there is no statistically significant relation between placental malaria and perinatal death, but it is interesting to note that all the five perinatal deaths recorded in the study had placental malaria. This finding is at variance with finding by Newsman et al[18] who reported a seven folds increased risk of stillbirth in association with placental malaria in an area with unstable malaria transmission in Ethiopia.

Conclusion;

This study has highlighted high prevalence of placental malaria in Nnewi. The study also demonstrated that placental malaria was associated with low birth weight, preterm delivery and lower maternal pcv but associated with neither maternal anaemia nor perinatal deaths.

The limitations of the study are as follows:

1. The study could not demonstrate that these associations were the effects of placental malaria, as it could not exclude all the possible causes of these adverse effects

2. Only early neonatal death was studied, but late neonatal death can occur as a consequence of the placental malaria.

CONSENT AND ETHICAL APPROVAL

All authors hereby declared that this research was approved by the ethical committee of our Institutions and that an informed consent was obtained from the parturient.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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