

**Prevalence and Risk factors for Placental Malaria in
Nnewi, South east Nigeria**

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ABSTRACT

BACKGROUND

Placental malaria is one of the complications of malaria in pregnancy and is associated with poor pregnancy outcome. Demonstration of its prevalence and risk factors will help in modifying the measures in the prevention of malaria in pregnancy and strengthens the effective pre-existing preventive measures in our community. More so, there is paucity of studies of placental malaria in our environment using histology which is the gold standard.

AIM:

To determine the prevalence and the risk factors for placental malaria

STUDY DESIGN AND SETTING:

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

28 MATERIALS AND METHODS:

29 A cross section of pregnant women who delivered in the labour ward was recruited for
30 the study. The following data were obtained from those who met the inclusion criteria:
31 age, parity, gestational age, the booking status, educational level and husband's
32 occupation. The history of Intermittent preventive therapy (IPT), Insecticide treated net
33 (ITN), HIV status, Blood group and Haemoglobin genotype were also obtained.
34 Descriptive analysis of the results was done using the statistical package for social
35 sciences (SPSS) version 16. Univariate analysis and logistic regression were used and
36 the results expressed in Odd ratios (OR) and Confidence interval (C.I). P-value <0.05
37 was taken as significant level.

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40 RESULT:

41 Out of 200 placentae analyzed, 141 were positive for malaria, giving a prevalence of
42 70.5%. The commonest form of parasitisation was chronic infection (68.09%) followed
43 by past infection (19.14%) and acute infection (12.77%). Using univariate analysis, the
44 potential risk factors were young age, primigravidity, unbooked status, non use of IPT,
45 non use of ITN, low social class, HIV positivity and haemoglobin genotype AA.
46 However when these were subjected to logistic regression, the independent risk factors
47 identified were young age, primigravidity, unbooked status, non use of IPT, and non
48 use of ITN.

49 CONCLUSION:

50 This study highlighted high prevalence of placental malaria in our environment. The
51 independent risk factors noted were young age, primigravidity, unbooked status, non
52 use of IPT and non use of ITN.

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54 **Key words: Placental malaria, Histology, Prevalence and Risk factors.**

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61 INTRODUCTION

62 Malaria remains one of the highest contributors to the precarious maternal mortality figures in
63 sub-Saharan African. Africa accounts for 90% of the world's burden of malaria and pregnant
64 women and their under fives form the bulk of its worst victims in endemic areas¹. Approximately
65 30 million women are threatened by malaria in pregnancy annually with about 10, 000 maternal
66 mortalities attributed to the disease each year and about 200, 000 newborn deaths annually^{2, 3}.

67 The major adverse effects of malaria in pregnancy are maternal anaemia and placental malaria.
68 *Plasmodium falciparum* infected erythrocytes frequently sequester in to the intervillous spaces of
69 the placenta causing release of pro-inflammatory mediators which cause pathological
70 alterations^{4,5}. Placenta is highly vascularised organ and a favored site for parasite sequestration
71 and development. This causes damage to the placental vessels and a compromise on the
72 perfusion⁶. Placental malaria has been associated with a significant reduction in the birth weight
73 especially in primigravidae^{7,8}

74 In most studies, the prevalence of malaria is more using placental malaria in making diagnosis⁹;
75 and placental infection may be detected in the absence of peripheral parasitemia and may persist
76 after initiation of antimalaria treatment¹⁰. Light microscope is relatively simple and less technical
77 but it has inferior detection rate than placental histology which is the gold standard for diagnosis
78 of placental malaria^{7,10}. It is important to note that most studies in Africa were done with
79 placental blood smear^{11,12} instead of using placental histology which is the gold standard. Using
80 placental histology may change the result.

81 Nnewi is in malaria endemic region ; there is also limited resources and poor knowledge of
82 different preventive measure of malaria in Nnewi. Because of these, it becomes imperative to
83 identify the risk factors of placental malaria using the most reliable test (histology). This will
84 help us channel our limited resources judiciously in preventing placental malaria in pregnancy.
85 This study will also help us to evaluate the effectiveness of the preventive measures that are in
86 place.

87 **MATERIALS AND METHODS**

88 This is a cross sectional study carried out at Nnamdi Azikiwe university teaching hospital,
89 Nnewi in Anambra state. The hospital has antenatal booking rate of about 1000 pregnant women
90 per year and with the delivery rate of about 1200 per year. The ethical committee of the hospital
91 approved the study.

92 Systematic random sampling was used in selecting the patients for the study. The patients
93 were selected from among the women who presented to the delivery suite in labour, or for
94 elective caesarean section or induction of labour.

95 After due explanation and obtaining informed consent from them, the following data were
96 obtained from them: age, parity, gestational age, educational level and the husband's occupation.

History of the use of Sulphadoxine-pyremethamine (SP) for malaria prophylaxis in the index pregnancy was obtained. HIV status, the blood group and genotype were obtained. At delivery, placenta tissue was taken for histology .Full thickness placental blocks of around 2–3 cm were taken from the placenta, kept in neutral buffer formalin for histopathology examinations. The samples were taken to a histopathology laboratory where they were processed. The Placental tissues were dehydrated by immersing them in graded concentrations of alcohol till the absolute concentration is reached. Clearing and rinsing was done using Zylene. The tissues were embedded using paraffin wax and section done with microtome set at 6 microns. The sections were fixed on a clean slide. Staining was done using Haematoxylin and Eosin. The slides were read under microscope with magnification $\times 400$. Placental malaria infections were characterized based on the classification of Bulmer description and previously used by Ishag Adams et al⁶ as follows:

- Uninfected -----No parasites or pigment
- Acute infection Parasites in intervillous spaces
- Chronic infection.....Parasites in maternal erythrocytes and pigment in
fibrin or cells within fibrin
- Past infection.....No parasites and pigment confined to fibrin or cells
within fibrin.

Placenta malaria was considered positive when there is parasite and/or pigment (active and past infection respectively) on histology result and negative when both were absent in the histology report

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128 **DATA ANALYSIS AND PRESENTATION**

129 Data were analyzed using SPSS. Version 16. Univariate analysis was used to identify the risk
130 factors for placental malaria and those that were significant were subjected to logistic regression
131 analysis by entering the placental malaria as dependent variable and the significant factors
132 identified in the univariate analysis as the covariates. Those factors that still remain significant
133 after adjusting for the confounding factors were regarded as the independent risk factors for
134 placental malaria. Odd ratios, adjusted odd ration and 95% confidence interval were generated.
135 P-value of < 0.05 was considered significant.

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139 **RESULTS**

140 During the study period of 6 months, between 1st of August, 2012 and 31st January, 2013,
141 pregnant women who met the criteria of the study and consented, were recruited. The
142 women studied had age range between 17 and 37years with mean age of 27.59 ± 5.98 years
143 while their parities ranged from 0 to 6, with mean parity of 2.13 ± 1.36 . The mean
144 gestational age at delivery was 38.19 ± 1.55 weeks and ranged between 34 and 41 weeks.
145 The mean birth weight of the babies was 3.22 ± 0.53 kg, with range of 2.00 to 4.20 kg. These
146 are shown in table I.

147 **Tables I: The characteristics of the participants**

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

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150 Out of two hundred (200) placentae studied by histology, 141(70.5%) showed an
 151 evidence of malaria parasitisation, giving a prevalence of 70.5%. Of the 141 malaria
 152 positive placentae, 96(68.09%) had chronic infection (active on past infection),
 153 18(12.77%) showed evidence of acute infection while 27(19.14%) showed past
 154 infection as shown in table II.

155 **Table II: The Pattern of Placental Malaria.**

Pattern of Placental Malaria	Frequency(N=200) (%)
1. Chronic infections	96 (68.09)
2. Past infections	27 (19.14)
3. Acute Infections	18 (12.77)
Total	141 (100.00)

Table III shows the potential risk factors for placental malaria, using univariate analysis. The teenagers (OR=4.58, C.I=1.08 – 42.7) and primigravidae (OR=3.81, C.I=1.38 – 13.05) are potential risk factors for placenta malaria. The utilization of ANC (OR=0.23, C.I=0.10 – 0.44), use of IPT (OR=0.18, C.I=0.07 – 0.40) and use of ITN (OR=0.09, C.I=0.02 – 0.32) reduced the risk of placental malaria. HIV positive patients had 2-fold increase risk of having placental malaria (OR=2.000, C.I=1 – 4.10). Blood group O reduced the risk of placental malaria by 0.87 but not statistical significant (OR=0.87, C.I =0.45 – 1.67) while haemoglobin genotype AS significantly reduced the risk of placental malaria by 0.28 (OR=0.28, C.I=0.08 – 0.99). Women in low social class had 5-fold risk of placental malaria (OR=5.15, C.I =2.15 – 13.61) while those in upper social class had 0.39-fold risk (OR=0.39, C.I= 0.12 – 1.2, p-value 0.147), although the difference is not statistical significant.

Table III : The potential risk factors for placental malaria using Univariate analysis

Variables	PM+ (%)	PM- (%)	OR	C.I	P- value
1.Age					
<20yrs	20 (90.90)	2(9.10)	4.58	1.08 – 42.7	0.026
≥20yrs	121 (68.00)	57(32.00)			
2. Primp	40(88.89)	5(11.11)	3.81	1.38 – 13.05	0.005
Non-Primp	105(67.74)	50(32.26)			
3. Low S.C	68(89.50)	8(10.50)	5.15	2.15 – 13.61	<0.001
Mid S.C	66(62.30)	40(37.70)	1.00		
Upper S.C	7(38.90)	11(61.10)	0.39	0.12- 1.20	0.062
4. ANC	70(59.30)	48(40.70)	0.23	0.10 – 0.44	<0.001
No ANC	71(86.60)	11(13.40)			
5. IPT	70(58.30)	50(41.70)	0.18	0.07 – 0.40	<0.001
No IPT	71(88.80)	9(11.20)			
6. ITN	4(22.20)	14(77.80)	0.09	0.02 – 0.32	<0.001

NO ITN	137(75.30)	45(24.70)			
7. HIV+	63(78.80)	17(21.20)	2.00	1.00 – 4.10	0.037
HIV-	78(65.00)	42(35.00)			
8. Blood O	74(69.20)	33(30.80)	0.87	0.45 – 1.67	0.656
Non Blood O	67(72)	26(28)			
9. Genotype AS.	6(42.90)	8(57.10)	0.28	0.08 – 0.99	0.019
AA.	135(72.60)	51(27.40)			

* PM = Placental Malaria, OR=Odd Ratio, C.I=Confidence Interval, SC= Social Class

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172 Table IV shows the significant (independent) risk factors for placental malaria using
173 logistic regression analysis. After adjusting for confounding factors, young age and
174 Primigravidity still increased the risk of placental malaria by 5.5 and 2.1 folds (
175 Adjusted OR=5.478, C.I=1.026 – 29.25 and Adjusted OR=2.105, C.I=1.387 – 3.152)
176 respectively. Also, ANC, use of IPT, and ITN in pregnancy were found to be
177 significantly protective with the adjusted odd ratios of 0.284, 0.243 and 0.096 (P-
178 values <0.001) respectively. After adjustment for confounding factor in the logistic
179 regression analysis, Social class, HIV status, and Haemoglobin genotype were found
180 not to be independent risk factors for placental malaria (P-values >0.05).

181 **Table IV : The independent risk factors for placental malaria using Logistic**
182 **regression analysis**

Variables	Crude Odd ratio	Adjusted Odd ratio	Confidence interval	P-value
Age	4.58	5.478	1.026 – 29.25	0.047*
Parity	3.81	2.105	1.387 – 3.153	0.049*
ANC	0.23	0.284	0.146 – 0.554	0.001*
Low Class	5.15	1.028	0.000 – 1.338	0.999

Mid Class	1.00	1.000		
Upper class	0.39	0.469	0.169 – 1.306	0.147
IPT Usage	0.18	0.243	0.112 - 0.530	<0.001*
Net Usage	0.09	0.096	0.026 – 0.356	< 0.001*
HIV Status	2.00	1.771	0.839 – 3.740	0.134
Genotype AS	0.28	2.424	0.637 – 9.216	0.194
* Significant values				

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187 Discussion.

188 This study documented a placental malaria prevalence of 70.5% using placental
189 histology of 200 women, who delivered at Nnamdi Azikiwe University Teaching
190 Hospital, Nnewi over a period of six months between 1st August, 2012 and January,
191 2013. This figure is higher than, although in the same comparable range with that
192 documented at Awka, in the same state with Nnewi, by Dennis et al¹³ who reported a
193 prevalence of 64% in 2005. This is also higher than that reported in Owerri in Imo state
194 (29%), in 2005¹⁴. This figure is also higher than that reported in other studies done in
195 Calabar¹⁵, South west ^{11,16,17} and Northern Nigeria ¹⁸, Cameroun ¹⁹ and in malaria
196 unstable regions e.g. Ethiopia²⁰ and Sudan⁶. The high prevalence in this study may be
197 due to the fact that Nnewi is located in a rain forest zone where the malaria vector
198 thrives. Another reason may be that, this study was done during rainy season and there
199 was unprecedented overflow of River Niger that displaced many people during this
200 period of study. This overflow might have acted as a breeding “heaven” for the vector.

Using univariate analysis, the following were identified as the potential risk factors for placental malaria: young age, primigravidity, low social economic status, unbooked status, non use of IPT, non use of ITN, HIV positivity and genotype AA. However, when these were subjected to multivariate analysis using logistic regression (after adjusting for confounding factors), the independent (significant) risk factors identified were young age, primigravidity, unbooked status, non use of IPT, non use of ITN while haemoglobin genotype, HIV status and social economic class ceased to be risk factors.

Primigravidity, identified as a risk factor for placental malaria in this study is in keeping with other studies ^{2,7,} , but at variance with some studies in malaria non endemic areas like Sudan and Zaire ^{8,21}. This difference between malaria endemic region and malaria unstable region may be that in malaria unstable region all pregnant women are equally susceptible to malaria irrespective of parity. The difference in the prevalence of placental malaria between primigravidae and multigravidae in malaria endemic region is because parity increases the development of blocking antibodies to Chondrion sulphate A and this offers some protection against placental malaria in multigravidae. The unique susceptibility of primigravidae to placental malaria can be explained by lack of immune experience to the parasite subpopulation.

The age factor is interwoven with other factors, as the age increases; the higher the chances of higher parity and higher education for the woman, but this study after adjusting for these confounding factors, age still remained an independent risk factor. This is in agreement with the finding by Olugbenga et al ¹¹ but at variance with the work of Jeanne et al ²¹ in New Guinea where only primigravidity was identified as the independent risk factor while age was not. This finding of age as a risk factor for placental malaria can be explained by the fact that as age increases, there is increase in acquired immunity against malaria parasitemia; this may reduce the parasitemia density and by extension reduces the risk of placental malaria. This is to say that age has no direct effect on placental malaria, but indirectly affect the placental malaria by reducing blood parasitemia which is a risk factor for placental malaria. In malaria endemic region, the younger women are still in the process of acquired natural

immunity, hence at more risk of placental malaria than the adults who have acquired immunity.

Socioeconomic status of women determines the pregnancy outcome. There is interplay of social class with other factors, as women of lower class live in areas with poor drainage system, where the malaria vector thrives, and they are unlikely to attend ANC, use IPT and ITN. This study identified low social class as a potential risk factor for placental malaria but when subjected to logistic regression it failed to be an independent risk factor. This means that if a woman in low socioeconomic state can attend ANC, use IPT, and ITN that she is likely to be at the same reduced risk of placental malaria as her counterpart in the higher socioeconomic status. Pregnant women who booked for antenatal care had 0.23 reduced risk of placental malaria than those that did not book for antenatal care (OR=0.23, C.I=0.10 – 0.44). This still remain significant even when confounding factors were taken care of in logistic regression. During the antenatal clinic, women are equipped with information about pregnancy and how to prevent malaria via the use of ITN. They were also given IPT.

This study identified non utilization of ANC, IPT and ITN as independent risk factors for placental malaria. There is poor utilization of ITN among the pregnant women in this study, as only 18 (9%) of 200 women studied utilized ITN. This finding is in support of the finding by Enato et al²² in Benin City. IPT for malaria is widely utilized in current obstetric practice. Its use is based on the assumption that in malaria endemic region asymptomatic women have a level of parasitemia in them. The aim therefore is to clear this burden of parasites at predetermined times during the pregnancy. Evidence has shown that use of IPT clearly reduces the prevalence and severity of malaria in pregnancy including placental malaria.

HIV infection in pregnancy impairs the ability of pregnant women to control plasmodium infection²³. In this study, HIV infection was identified as a potential risk factor for placental malaria, but not as an independent risk factor for placental malaria. This may be because our hospital is one of the treatment sites for PMTCT, where almost all the HIV patients are booked, received antiretroviral drugs, encouraged to use ITN and were given 3 doses of IPT. These measures may have contributed in the HIV

status not being identified as an independent risk factor in this study. Therefore, pregnant women living with HIV who receive antiretroviral drug, attend ANC, use ITN and receive IPT are likely to be at the same risk as HIV negative women.

Unlike the finding in Sudan, where women with blood group O were found to have the highest risk of placental malaria ²⁴; there is no statistical difference between those with blood group O and other blood groups in this study. Haemoglobin genotype AA was identified as a potential risk factor for placental malaria but when subjected to multivariate analysis; it was found not to be an independent risk factor.

Conclusion;

This study has highlighted high prevalence of placental malaria in Nnewi. The independent risk factors identified in this study were young age, primigravidity, unbooked status, non use of IPT and non use of ITN. Low socioeconomic status, HIV positivity and haemoglobin genotype AA were demonstrated to be potential risk factors for placental malaria in univariate analysis but failed to be independent risk factors when the confounding factors were eliminated using multivariate analysis.

Recommendation:

The high prevalence and the independent risk factors for placental malaria highlighted in this study have shown measures to reduce the prevalence of placental malaria in our pregnant women. This is very important as the diagnosis of placental malaria can only be made after delivery when the harm has been done.

Our women should be empowered through quality education as this will enable them access ANC where they can receive IPT. During Antenatal care, they should be educated on the need for ITN, clearing of bushes and drainage of stagnant waters in their environment. Also to be supported is the routine counseling and testing for HIV in pregnancy and those who tested positive should start antiretroviral drugs and 3 doses of IPT should be given to them. This has been shown to reduce the incidence of placental malaria in the HIV infected women. ITN should be made available in the ANC at a reduced cost and if possible free of charge.

Researches should be encouraged from time to time to review the prevalence and the risk factors of placental malaria. This will enable strengthening of the protective measures, discard the non-working measures and possibly introduce other workable measures. After these researches, the result should be made known to the policy makers and health workers who will implement the findings. As malaria is a global concern, all hands should be on deck; it involves the partnership amongst the pregnant women, the community, the health workers, the government and NGO.

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