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Effect of Placental Malaria on Perinatal Mortality 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 embarked on a prospective cohort of pregnant women who completed antenatal care at Imo State University Teaching Hospital (IMSUTH). Between February 2014 and June 2017, 936 women were enrolled in a prospective cohort study. At delivery blood smear and placental biopsy were obtained from the placenta to check for placental malaria (microscopic examination and histology respectively). Blood smear and tissue obtained from the placenta was processed using standard methods. The study revealed that 11.2% had stillbirths, 17.5% were lost before 20th week of pregnancy and 71.3% were life births. Placental malaria was significantly associated with perinatal mortality in the study population (x^2 = 142.93; P < 0.05). Placental malaria significantly affected perinatal mortality based on gravidity (x^2 = 115.15; P<0.05) and maternal age groups (x^2 = 115.32; P<0.05). Multivariate analysis in this study indicated that the odds of having a stillbirth is 25.85, i.e. over 25 times more than those who were not exposed to placental malaria. The study further revealed that the odds of having a stillbirth is 27.63, i.e. over 27 times more than those who were not exposed to placental malaria. The study suggests that any meaningful control measures in pregnancy should start as early as possible to curb the menace of miscarriages and still birth.*

I. INTRODUCTION

Malaria has been the most devastating infectious parasitic disease of human kind for centuries. In 2015, an estimated 438,000 malaria deaths around the world have been reported, of which approximately 69% (306,000) were children less than 5 years of age. Of all malaria deaths, 90% were reported from African regions and the rest were from South-East Asia region and the Eastern Mediterranean region (World Health Organization, 2015). Malaria causes a very high risk to the pregnant woman and her fetus/new born. In malaria endemic areas, it is estimated that at least 25% of pregnant women are infected with malaria, which attribute to more than 20% of all maternal deaths. Malaria accounts for over 10,000 maternal and 200,000 neonatal deaths per year globally (Schantz-Dunn & Nour, 2009). Consequences of maternal malaria in fetus are abortion, still birth, intra uterine growth retardation (IUGR), premature delivery, and low birth weight (LBW) (Menendez, Ordi & Ismail, 2009;

Watkinson & Rushton, 2004). LBW of the infant has been suspected for poor cognitive and neurosensory development of the child (Breman, Egan & Keusch, 2011; Guyatt & Snow, 2009). WHO (2015) recommends that in areas of high malaria transmission, people should be provided with insecticide-treated mosquito nets and intermittent preventive treatment (IPT) with sulphadoxine–pyrimethamine as a part of antenatal care. Placenta, which is the interface between mother and fetus, plays important role in successful pregnancy outcome and growth of the fetus that is critically dependent on the placenta (Tarning, 2016).

Malarial infection in placenta is characterized by sequestration of *Plasmodium falciparum*-infected erythrocytes and infiltration of immune cells within the intervillous spaces of the placenta. The placenta turns black due to deposition of the malarial pigment. The parasite densities are much higher in the placenta compared to peripheral blood (Poovassery & Moore, 2006). The thickening of placental basement membrane, perivillous fibrinoid deposits, and syncytial knotting results into altered exchange system between mother and fetus. The placental insufficiency to provide nutrients to the fetus causes IUGR (Davison, Cogswell & Baskin, 2000; Ismail, Ordi & Menendez, 2010). The enhanced susceptibility to infections during pregnancy results into high parasitemia and heavy infiltration of parasite-infected RBCs (iRBC) in placental vasculature, a privilege site where the parasite can avoid maternal immune response (Saba, Sultana & Mahsud, 2008; Reeder, 2009). Furthermore, it has been observed that woman who is pregnant for the first time (primigravidae) is more susceptible to malarial infection than woman who has conceived for second or third time (multigravidae). This resistance to the malarial infection in multigravidae is due to the development of placental parasite-specific immunity in second and third pregnancies (Desai, *et* al, 2007;Rogerson, Hviid & Taylor, 2007). Contrary to malaria endemic areas, in areas with low malaria incidence, primigravidae and multigravidae are equally susceptible. The more complications of placental malaria in primigravidae are due to the absence of placental parasite-specific immunity which develops in subsequent pregnancies (Ndam & Deloron, 2007; Rogerson, Mwapasa & Meshnick, 2007).

II. MATERIALS AND METHODS

This study embarked on a prospective cohort of pregnant women who completed antenatal care at Imo State University Teaching Hospital (IMSUTH), Orlu, Nigeria. Orlu is a city in Nigeria which lies on the geographical coordinates of 5^0 47^I 0^{II} N, 7^0 2^I 0^{II} E. Orlu (Igbo: *Ọlu*) is the third largest city in Southeast NigeriaImo State with an estimated population of 220,000. It has a long history and has played a critical role as the headquarters for humanitarian relief agencies during the Nigerian civil war. 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 (laboratory investigations) of placental blood and placental biopsy. 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 by Amal, *et al* (2013); Salafia, Charles & Mass (2006); Bulmer, *et al* (2003). After delivery of both the baby and 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 specimen was labeled and then sent to hematology laboratory where thick

and thin films were prepared. In hematology laboratory, thick smear of the placental blood specimen was prepared on glass sides. The slides were allowed to dry and then stained with 3% Giemsa stain for 30minutes, rinsed with water and allowed to dry. The slides were then viewed under a microscope using oil immersion at x 100 magnification for presence of parasite (Mayor, Moro, Aguilar & Ordi,, 2012). Staining of slides and parasite counting were done by a medical laboratory scientist working in the hematology laboratory who was unaware of the results of the histology (Murray, Grasser, Magill & Miller, 2008).

Three full thickness placental blocks measuring 2cm x 2cm x 1cm were taken from the placenta (Bulmer, *et al*, 2003). The biopsy specimens were stored in 20ml of formalin and sent to histology laboratory for processing. The placental biopsies were processed and embedded in paraffin wax using standard techniques. Paraffin sections of 5mm thickness were stained with haematoxylin and eosin (H&E) and then examined by light microscopy (Bulmer, *et al*, 2003; Aguilar, Machevo & Mayor, 2012; Uneke, 2008).The pattern of malaria parasitization of placental tissue was graded according to Bulmer, *et al*, (2003); description of grade 0, no evidence of malaria parasite or pigment; grade 1 (active infection), parasites and pigments in maternal red blood cells in the intervillous spaces but no pigment in fibrin or cells within fibrin; grade 2 (active-chronic infection), parasites and pigments in maternal red blood cells and pigments in fibrin or cells within fibrin; and grade 3 (chronic infection), parasites not present but pigments confined to fibrin or cells within fibrin.

Statistical analysis of generated data was carried out using SPSS for windows version16, 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 and Multinomial Logistic Regression. Differences were considered significant at $P< 0.05$.

III. RESULTS

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	60.0
	Grand Multigravida (5th to 6th Pregnancy)	78	8.3
	Great-grand Multigravida (7th pregnancy and above)	38	4.1

Socio-demographic variables showed that age group 16-25 years (50.5%) had highest proportions of participants than other age groups while age group 46 – 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 multigravidae (4.1%).

Table 2: Comparison of placental malaria blood film and placental malaria histology

Test of Placental Parasitemia	No. examined	Positive (%)	Negative (%)
Placental blood	936	165 (17.6)	771 (82.4)
Histology	936	188 (20.1)	748 (79.9)

Out of 936 women that had their babies in IMSUTH and were examined for prevalence of placental malaria, placental histology revealed 188 (20.1%) whereas placental blood parasitemia revealed only 165 (17.6%) thus showing 23 (12.2%) discordant infection.

Table 3: Overall prevalence of perinatal mortality and live births

	Placental Malaria		
Parameter	**Positive (%)**	**Negative (%)**	**Total**
Still birth	21 (11.2)	4 (0.6)	25 (13.3)
Miscarriage	33 (17.5)	16 (2.1)	49 (26.1)
Life babies	134 (71.3)	728 (97.3)	862 (60.6)
Total	**188 (20.1)**	**748 (79.9)**	**936**

$x^2 = 142.93$; $P < 0.05$

Overall prevalence of perinatal prevalence and live births revealed that out of 188 pregnant women that had placental malaria, 21 (11.2%) were still birth, 33 (17.5%) died within 28 days of delivery, 134 (71.3%) were alive. Also, out of 748 pregnant women that do not have placental malaria, 4 (0.6%) died in utero (still birth), 16 (2.1%) died within 28 days of delivery and 728 (97.3%) survived. There were a total of 25 (13.3%) still births, 49 (26.1%) miscarriage and 862 (60.6%) life babies. There is significant effect of placental malaria on perinatal mortality ($x^2 = 142.93$; $P < 0.05$).

Table 4: Distribution of pattern of placental malaria on perinatal mortality

Pattern of placental malaria	Perinatal Mortality		Placental malaria positive life babies (%)	Total (placental malaria prevalence)
	Still birth (%)	Miscarriage (%)		
Active	6 (28 6)	10 (30.3)	101 (75.4)	117
Active-Chronic	13 (61.9)	18 (54.5)	32 (23.9)	63
Chronic	2 (9.5)	5 (15.2)	1 (0.7)	8
Total	**21 (11.1)**	**33 (17.6)**	**134 (71.3)**	**188**

$x^2 = 40.58$; $P < 0.05$

Pattern of placental malaria on perinatal mortality showed that out of 188 pregnant women that had placental malaria, 21 had still births and from them, 6 (28.6%) had active pattern of placental malaria, 13 (61.9%) had active-chronic placental malaria and 2 (9.5%) chronic pattern of placental malaria. Also, out of 33 miscarriage that were recorded as a result of placental malaria, 10 (30.3%) had active pattern of placental malaria, 18 (54.5%) and 5 (15.2%) had active-chronic and chronic pattern of placental malaria respectively. 134 (71.3%) of pregnant women that had placental malaria had life babies of which 101 (75.4%) had active pattern of placental malaria, 32 (23.9%) and 1 (0.7%) had active-chronic and chronic pattern of placental malaria respectively.

There is significant effect of the pattern of placental malaria infection on perinatal mortality (x^2 = 40.58; P < 0.05).

Table 5: Overall prevalence of perinatal mortality based on gravidity

Gravidity	Plac. Mal. +ve	Perinatal Mortality		Plac. Mal. –ve	Perinatal Mortality		Total (Perinatal mortality prevalence)
		Stillbirth (%)	Miscarriage (%)		Stillbirth (%)	Miscarriage (%)	
Primigravida	112	13 (11.6)	20 (17.9)	146	2 (1.4)	8 (5.5)	43
Multigravida	69	6 (8.7)	11 (15.9)	493	2 (0.4)	7 (1.4)	26
Grand Multigravida	5	1 (20.0)	1 (20.0)	73	0 (0.0)	1 (1.4)	3
Great Grand Multigravida	2	1 (50.0)	1 (50.0)	36	0 (0.0)	0 (0.0)	2
Total	**188**	**21**	**33**	**748**	**4**	**16**	**74**

x^2 = 115.15; P < 0.001

Perinatal mortality based on gravidity revealed that 112 primigravida were exposed to placental malaria, 13 (11.6%) had stillbirth, 20 (17.9%) had miscarriage whereas out of 146 with no placental malaria, 2 (1.4%) and 8 (5.5%) had stillbirths and miscarriage respectively. A total of 69 multigravida had placental malaria, 6 (8.7%) and 11 (15.9%) had stillbirths and miscarriage respectively whereas out of 493 that were not exposed to placental malaria 2 (0.4%) and 7 (1.4%) had stillbirths and miscarriage respectively. For grand multigravida, out of 5 that had placental malaria 1 (20%) had both stillbirths and miscarriage whereas out of 73 that were not exposed to placental malaria, none had stillbirth and only 1 (1.4%) had miscarriage. For great grand multigravida, 2 were exposed to placental malaria, 1 (50%) and 1 (50%) had stillbirth and miscarriage respectively whereas out of 36 of them that were not exposed to placental malaria none had stillbirth nor miscarriage. Placental malaria significantly affect perinatal mortality based on gravidity x^2 = 115.15; P < 0.001

Table 6: Overall prevalence of perinatal mortality based on maternal age

Maternal Age (years)	Plac. Mal. +ve	Perinatal Mortality		Plac. Mal. –ve	Perinatal Mortality		Total (Perinatal mortality prevalence)
		Stillbirth (%)	Miscarriage (%)		Stillbirth n (%)	Miscarriage (%)	
16 – 25	125	16 (12.8)	19 (15.2)	348	2 (0.6)	5 (1.4)	42
26 – 35	59	5 (8.5)	11 (18.6)	318	1 (0.3)	9 (2.8)	26
36 – 45	4	0 (0.0)	3 (75)	82	1 (1.2)	2 (2.4)	6
46 – 55	0	0 (0.0)	0 (0.0)	0	0 (0.0)	0 (0.0)	0
Total	**188**	**21**	**33**	**748**	**4**	**16**	**74**

x^2 (6) = 115.32; P < 0.001

Perinatal mortality based on age groups revealed that 125 maternal age groups of 16 – 25 years were exposed to placental malaria, 16 (12.8%) had stillbirth, 19 (15.2%) had miscarriage whereas out of 348 that were not exposed to placental malaria, 2 (0.6%) and 5 (1.4%) had stillbirths and miscarriage respectively. A total of 59 age groups of 26 – 35 years had placental malaria, 5 (8.5%) and 11 (18.6%) had stillbirths and miscarriage respectively whereas out of 318 that were not exposed to placental malaria 1 (0.3%) and 9 (2.8%) had stillbirths

and miscarriage respectively. For maternal age groups of 36 – 45 years, out of 4 that had placental malaria none had stillbirth and 3 (75%) had miscarriage whereas out of 82 that were not exposed to placental malaria, 1 (1.2%) had stillbirth and 2 (2.4%) had miscarriage. Placental malaria significantly affects perinatal mortality based on maternal age groups (x^2 = 115.32; P < 0.001).

IV. DISCUSSION

The studyon effect of placental malaria on perinatal mortality among pregnant women in Imo State University Teaching Hospital (IMSUTH), Orlu revealed that 13.3% stillbirths and 26.1% miscarriages were recorded among the study subjects. Of the 25 stillbirths, 28% were macerated, and 72% were fresh stillbirths. This finding correlates with the study done by Okoko, Ota& Yamuah (2008) who recorded 77.1% fresh stillbirths. This finding suggests the urgency of improving the management of pregnancy and labour in the study area. Also out of 188 pregnant women that had placental malaria, 11.2% had stillbirths, 17.5% were lost before 20th week of pregnancy and 71.3% were life births. This simply means that out of 188 women that had placental malaria, 54 28.7% experienced perinatal mortality but 71.3% had life babies. Although the number of perinatal deaths (54) may seem small compared to number of life babies (134) for those that were exposed to placental malaria but statistically, placental malaria was significantly associated with perinatal mortality in the study population (x^2 = 142.93; P < 0.05). This is similar to Okolo & Ibanesebhor (2012) study where placental malaria infection was associated with higher risk of perinatal mortality.

The study further revealed that out of 21 stillbirths, 28.6% had active placental malaria, 61.9% had active-chronic placental malaria and 9.5% had chronic placental malaria. Also, out of 33 neonatal deaths that were recorded as a result of placental malaria, 30.3% had active placental malaria, 54.5% and 15.2% had active-chronic and chronic placental malaria respectively. Majority (71.3%) of pregnant women that had placental malaria had life babies of which 75.4% had active placental malaria, 23.9% and 0.7% had active-chronic and chronic placental malaria respectively. Active, active-chronic and chronic infection were significantly associated with both stillbirth and miscarriage; x^2 = 40.58; P < 0.05. This finding shows that prolonged defects in maternal-fetal relationship could result in poor fetal outcome. Active infection characterized by extensive microvillious necrosis, which has been linked with high stillbirth and neonatal death rates in other studies could be correlated with this study, although study designs and classification of histological changes in these studies differed (Gschwind & Huber, 2013; Bulmer, *et al*, 2005).

Further analysis on the effect of placental malaria on perinatal mortality based on gravidity revealed that 29.5% of primigravida with placental malaria and 6.9% without placental malaria experienced perinatal mortality, 24.6% of multigravida with placental malaria and 1.8% that were not exposed to placental malaria experienced perinatal mortality. For grand multigravida, 40% who had placental malaria and 1.4% who were not exposed to placental malaria had perinatal mortality whereas all (100%) of great grand multigravida who had placental malaria lost their babies either through stillbirth or miscarriage but none of those who were not exposed to placental malaria had perinatal mortality. The finding revealed that gravidity influenced the prevalence of placental malaria in this study population. The primigravidae had a higher risk of being infected (placental parasitemia) compared to grand and great grand multigravidae. The severest outcome of placental parasitemia occurred in the great grand multigravidae. When chi-square analysis was conducted to assess the association of gravidity and placental malaria status with perinatal mortality (stillbirth and miscarriage), the study thus revealed that placental malaria infection was significantly associated with a higher risk of delivering stillbirths and miscarriage based on gravidity among this population; x^2 = 115.15; P < 0.001.

With multivariate analysis using logistic regression, placental malaria had a highly significant effect on the outcome of pregnancy (x^2 = 81.44, P < 0.001). For miscarriage; using parameter estimates, the three (3) gravidity levels on the output table were not significant and being within any of the gravidity does not significantly predict whether there will be a miscarriage or a life baby. The odds ratio tells us that as these variables increase by a unit, the changes in the odds of having a miscarriage are 1.60, 0.86 and 0.88 respectively even though they are not significant. This simply implies that if the value of placental malaria increases then

perinatal mortality rate will increase. Being exposed to placental malaria significantly predicts whether a pregnant woman would end up with a miscarriage: b = 2.18.The odds ratio further tells us that as this variable increases by a unit, the odds of having a miscarriage is 8.88, i.e. over 8 times more than those who were not exposed to placental malaria. For stillbirth analysis using parameter estimates revealed that being in any of the gravidity does not significantly predict whether a pregnancy would end up with a stillbirth. The odds of having a stillbirth are 0.49, 0.29 and 0.40 respectively. Being exposed to placental malaria significantly predicts whether pregnancy ends up in a stillbirth: b = 3.25. The odds ratio also revealed that the odds of having a stillbirth is 25.85, i.e. over 25 times more than those who were not exposed to placental malaria. These observations are consistent with the findings of previous studies in malaria-endemic regions where, among several factors, gravidity independently influenced the placental malaria prevalence rate and subsequent negative outcome (Okoko, Ota & Yamuah, 2008; McGregor, Wilson & Billewicz, 2004). It is not yet fully clear why primigravidae are more susceptible to placental malaria but great grand multigravidae suffer from its consequences more than other gravidities. The general concept of pregnancy immunosuppression does not satisfactorily explain this. One possible explanation for this parity-related susceptibility is given by the findings of Fried & Duffy (2006), which show that multigravid, grand and great grand multigravid mothers develop malaria antibodies which block adhesion of parasites to Chondroitin Sulphate Antigen (CSA) receptors in the placentae in subsequent pregnancies.

Analysis on the effect of placental malaria on perinatal mortality based on maternal age revealed that 28% of maternal age group of 16 – 25 years with placental malaria and 2% without placental malaria experienced perinatal mortality, 27.1% of maternal age group of 26 – 35 years with placental malaria and 3.1% that were not exposed to placental malaria experienced perinatal mortality. For maternal age groups of 36 – 55 years, 75% who had placental malaria and 3.6% who were not exposed to placental malaria had perinatal mortality. The finding revealed that the influenced of young maternal age on the prevalence of placental malaria in this study population was significant. The young maternal age had a higher risk of being infected (placental parasitemia) compared to older maternal age. The severest outcome of placental parasitemia occurred in the older maternal age (36 – 55 years). When chi-square analysis was conducted to assess the association of maternal age and placental malaria status with perinatal mortality (stillbirth and miscarriage), the study thus revealed that placental malaria infection was significantly associated with a higher risk of delivering stillbirths and miscarriages among this population.

With multivariate analysis using logistic regression, placental malaria had a highly significant effect on perinatal mortality (x^2 = 110.27, P < 0.001). For miscarriage using parameter estimates, being with either age group 1 or 2 does not significantly predict whether there will be a miscarriage or a life baby. The odds ratio tells us that as these variables increase by a unit, the changes in the odds of having a miscarriage are 0.33 and 0.50 respectively even though they (two age groups) are not significant. This simply implies that if the value of placental malaria increases then perinatal mortality rate will increase. Being exposed to placental malaria significantly predicts whether a pregnancy ends up with a miscarriage; thus; b = 2.60. The odds ratio tells us that as this variable increases by a unit, the odds of having a miscarriage is 13.49, i.e. over 13 times more than those who were not exposed to placental malaria. For stillbirth analysis using parameter estimates revealed that being within any of the age groups do not significantly predict whether a pregnancy would end up with a stillbirth. The odds of having a stillbirth are 0.88 and 0.57 respectively. Being exposed to placental malaria significantly predicts whether pregnancy ends up in a stillbirth: b = 3.32. The odds ratio also revealed that the odds of having a stillbirth is 27.63, i.e. over 27 times more than those who were not exposed to placental malaria.

V. RECOMMENDATION

Malaria is largely responsible for the high placental malaria with its resultant effect seen in this study population. The study suggests that any meaningful control measures in pregnancy should start as early as possible to curb the menace of miscarriages and still birth. The study suggests regular environmental sanitation to dislodge mosquitoes from their breeding places. Also early antenatal booking for effective monitoring and prompt treatment of malaria in pregnancy will contribute significantly in reducing maternal morbidity and

mortality, and its perinatal mortality. It is of necessity that routine intermittent preventive treatment of malaria is recommended for pregnant women in this area.

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