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Association between Placenta Malaria Parasites and Preeclampsia/Eclampsia among Parturient Mothers in Alex Ekwueme Federal University Teaching Hospital Abakaliki

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Abstract

In tropical countries, malaria and preeclampsia/eclampsia are common diseases of pregnancy; and placenta have been implicated in the pathophysiology of both disease processes. The two diseases have pathophysiologic similarities in the placenta such as placenta ischaemia, endothelial dysfunction and production of pro-inflammatory cytokine. Yet, there is paucity of studies on the association of these two disease processes. Determining the association between the two disease processes may help to unravel the pathogenesis of preeclampsia and also help in its prevention and patient management. **Objective:** Determined the association between placenta malaria parasitemia and preeclampsia/eclampsia among parturients at Alex Ekwueme Federal University Teaching Hospital Abakaliki. **Materials and Methods:** This was a case control study that was conducted in the Labour wards of department of Obstetrics and Gynaecology, Alex Ekwueme Federal University Teaching Hospital Abakaliki (AEFUTHA) and Mile 4 Missionary Hospital Abakaliki, a comprehensive health care centre in Abakaliki, Ebonyi state. It was conducted over a period of 6 months between 1st October 2021 and 31st March, 2022. The cases in this study were parturients that developed preeclampsia/eclampsia in the course of pregnancy, while the controls were parturient without preeclampsia/eclampsia. Interviewer-administered questionnaires were used to collect data on socio-demographic characteristics, obstetrics and medical histories. Histological examinations were conducted to isolate plasmodium falciparum parasites from placenta samples obtained from the maternal surface of the placenta. The data was processed using Epi Info software. Cate-

gorical variables were analyzed using Mc Nemar X^2 test, with a p-value of 0.05 considered statistically significant. Logistic regression models were used to estimate the odds ratios (OR) and 95% CI of the association between placenta malaria parasites and preeclampsia/eclampsia was conducted. Relative risk with 95% CI was used to determine both fetal and maternal outcomes. **Results:** The prevalence of preeclampsia during the study period was 2.9%. Placenta malaria was positive in twenty one (21) of the 67 cases of preeclampsia/eclampsia analyzed, giving a prevalence of 31.3% and in eleven (11) out of 68 controls (normotensive) patients analyzed, giving a prevalence of 16.2%. The presence of placenta malaria significantly increased the odds of developing preeclampsia/eclampsia among parturients (OR = 2.4, 95% CI = 1.0 - 5.4, P value = 0.04). Presence of placenta malaria in mothers with preeclampsia/eclampsia was associated with adverse pregnancy outcomes such as cerebrovascular accident (RR = 19.2, 95% CI = 1.1 - 341.7, P value = 0.04), DIC (RR = 10.9, 95% CI = 1.4 - 88.0, P value = 0.02), abruptio placenta (RR = 2.4, 95% CI = 1.2 - 4.8, P value = 0.01), pulmonary edema (RR = 2.7, 95% CI = 1.1 - 25.9, P value = 0.03), IUGR (RR = 2.1, 95% CI = 1.1 - 4.5, P value = 0.03) and IUFD (RR = 3.8, 95% CI = 1.3 - 11.7, P value = 0.02). Presence of placenta malaria also increased the risk of NICU admission (RR = 2.6, 95% CI = 1.1 - 6.0, P value = 0.03), Low 1st minute APGAR score (RR = 2.7, 95% CI = 1.2 - 6.1, P value = 0.02) and Low 5th minute APGAR score (RR = 3.0, 95% CI = 1.0 - 8.6, P value = 0.04) among neonates delivered by mothers with preeclampsia/eclampsia. However, presence of placenta malaria did not significantly increase maternal and perinatal mortalities. **Conclusion:** There is a higher prevalence of placenta malaria among mothers with preeclampsia/eclampsia when compared with normotensive controls and this was associated with increased risk of certain maternal and perinatal morbidities. Placental malaria was not associated with increased risk of either maternal or perinatal mortality.

Keywords

Placental Malaria, Preeclampsia/Eclampsia, Maternal, Perinatal, Morbidities and Mortality

1. Introduction

Placenta malaria and preeclampsia occur frequently in women in the tropics and are leading causes of maternal and perinatal morbidities and mortalities [1] [2]. 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 was reported [3], of which 90% were reported in African region and the rest were from South-East Asia region and the Eastern Mediterranean region [3]. Malaria threatens the health of both mother and her fetus. In areas with stable malaria infection, it is estimated that at least 25% of pregnant women are in-

fected with malaria, and it contributes more than 20% of all maternal deaths [4] [5]. Malaria accounts for over 10,000 maternal and 200,000 neonatal deaths per year globally [4] [5]. Although the pathogenesis of placental malaria is not completely understood, placental sequestration of *Plasmodium falciparum* has been shown to result in the accumulation of parasitized erythrocytes in the intervillous space, infiltration by inflammatory cells, and release of pro-inflammatory mediators, which cause pathologic alterations that could impair materno-fetal exchanges, often resulting in adverse pregnancy outcome [5] [6].

Preeclampsia is a pregnancy-specific disorder characterized by hypertension, significant proteinuria, with or without edema [6] [7]. It is multifactorial and forms an integral part of the continuum of hypertensive disorders of pregnancy [6] [7]. The end stage of preeclampsia is eclampsia, and it is defined as generalized tonic-clonic seizures, with or without raised blood pressure and proteinuria, occurring during or after pregnancy with or without other identifiable cause. Globally, preeclampsia complicates about 2% to 10% of pregnancies [6] [7], but value as high as 17% have been recorded in Nigeria [7] [8]. Placenta has been implicated in the etiology of preeclampsia and has adverse effects on both the mother and her fetus. The cause of eclampsia is usually multifactorial, including cerebral vasoconstriction, ischemia, vasogenic-edema, or other pathology. Although eclampsia occurs mostly in women with severe preeclampsia, there is no convincing test for predicting its onset [6] [7] [8].

In malaria endemic areas, at least one in four pregnant women have evidence of peripheral or placental malaria at delivery [4] [5]. Of the four species of malaria parasites, *Plasmodium falciparum* appears to be the only specie that infects human placentae. A prevalence rate of 37.8% has been recorded in the peripheral blood films of pregnant women and a prevalence rate of 59.3% has been recorded in the placental blood films with *Plasmodium falciparum* as the only species detected [9]. Malaria during pregnancy is characterized by the sequestration of infected erythrocytes (IEs) in placental inter-villous spaces binding chondroitin sulphate A (CSA), a receptor on placenta surface, and resulting in placenta malaria (PM) [4] [5] [10]. Antibodies to placenta chondroitin sulphate A help in protection against malaria infection and absence of these antibodies in primigravidae predispose them to more frequent and severe malaria infection [9]. Placenta malaria is associated with adverse pregnancy outcomes, such as miscarriages, stillbirths, preterm births (PTB), low birth weight (LBW) and congenital malaria [10] [11].

Preeclampsia/eclampsia has been described as disease of theories as the primary pathology is yet to be defined. Pathologic theories are closely intertwined and placenta tissue appears to play a central role in the pathogenesis [12], just like placenta malaria. It is also commoner among primigravidea [12]. Placental studies consistently showed aberrant uterovascular development of the placental bed. At the core of this is a complete or partial failure of trophoblastic invasion of the myometrium and the spiral arteries, resulting in muscular vasculature in the placental bed that is responsive to vasoactive substances [12]. These changes

at vascular bed result in the generation of inflammatory mediators such as fms-like tyrosine kinase 1 (sFlt-1) and tumour necrosis factor alpha [13]. These changes have adverse effect on both the mother and the fetus. Preeclampsia may result in damage to the kidneys, liver, lung, heart, or eyes, may result in HELLP syndrome and may cause a stroke or other brain injury in the mother. It may also result in miscarriages, intrauterine growth restriction, stillbirths, premature births and sudden fetal death [14].

Association between malaria and preeclampsia has been reported [5]. Basically, both diseases have been considered as diseases of the placenta [15]. Reduced placenta perfusion has been a recognized feature of both preeclampsia and malaria [2] [5] [15]. Both conditions are commoner in primigravidae and are associated with increased incidence of intrauterine growth restriction and maternal mortality [1] [11] [16]. Study has shown that women who were pregnant with female babies were at 2.55 higher risk of having placenta malaria infection, and placenta malaria parasites have been shown to be commoner among women with blood group O [17]. Also, higher rates of maternal death from eclampsia have been reported during the rainy season when malaria is more prevalent [1] [10] [18]. Nevertheless, the question as to whether the relationship is causal or casual is the object of further research [1] [18]. There are lots of inconsistencies in the findings of studies associating placenta malaria infection with preeclampsia/eclampsia [1] [2] [18]. The risk factors for preeclampsia/eclampsia have long been established but it is not completely understood whether these factors are modified or worsened by factors peculiar to Sub-Saharan African like poverty, lack of access to equipped healthcare facilities and high malaria exposure.

2. Materials and Methods

2.1. Study Design

This was a case control study that was conducted at the Labour wards of department of Obstetrics and Gynaecology of Alex Ekwueme Federal University Teaching Hospital Abakaliki (AEFUTHA) and Mile 4 Missionary Hospital Abakaliki, a comprehensive health care centre in Abakaliki, Ebonyi state.

2.2. Justification for the Study

Placenta malaria and preeclampsia/eclampsia have a lot of pathologic processes in common [1]. They are both commoner among primigravidae, both diseases are related to placenta, they both activate inflammatory responses and cause pathologic changes at the placental bed that often result in serious morbidities and mortalities for both the expectant mother and her fetus [1] [2]. In studies done by Adam *et al.* in Sudan [1] and another study done Ndao *et al.* in Senegal [2], association between placenta malaria and preeclampsia was established, but in both studies it is not known if such relationship is causal or casual. However, Brabin *et al.* in his study "Placenta Malaria and Preeclampsia through the look-

ing glass backward?" suggested that there is no significant relationship between placenta malaria and preeclampsia. All these three studies suggested further studies in order to properly ascertain these relationships.

Based on medline search and other search engines, there is paucity of literature on association of placenta malaria parasites and preeclampsia/eclampsia in Nigeria and there has not been any study on association of placenta malaria parasites and preeclampsia/eclampsia in AEFUTHA, to the best of our knowledge. This study is therefore designed to know if there is any association between placenta malaria and preeclampsia/eclampsia in Alex Ekwueme Federal University Teaching Hospital Abakaliki.

3. Aim and Objectives

3.1. Aim

To determine the degree of association between placenta malaria parasites and adverse pregnancy outcome among expectant mothers with preeclampsia/eclampsia in AEFUTHA.

3.2. Objectives

1) To determine the prevalence of placenta malaria parasites among parturients with preeclampsia/eclampsia using placenta histology.

2) To determine if the presence of placenta malaria parasites increase the odd of developing preeclampsia/eclampsia by comparing cases with control.

3) To determine if placenta malaria parasites worsen the prognosis of preeclampsia/eclampsia among parturients by comparing those that have both preeclampsia and placental malaria to those that have preeclampsia without placental malaria.

3.3. Research Questions

Are there any strong associations between placenta malaria parasites and preeclampsia/eclampsia?

Does the presence of placenta malaria parasites worsen prognosis in parturient with preeclampsia/eclampsia?

3.4. Null Hypothesis and Alternate Hypothesis

3.4.1. Null Hypothesis

Placenta malaria parasites are not associated with adverse pregnancy outcomes among parturient mothers with preeclampsia/eclampsia in AEFUTHA.

3.4.2. Alternate Hypothesis

Placenta malaria parasites are associated with adverse pregnancy outcomes among parturient mothers with preeclampsia/eclampsia in AEFUTHA.

3.4.3. Study Sample

Participants included in this study were patients with preeclampsia/eclampsia

admitted and managed at the Alex Ekwueme Federal University Teaching Hospital, Abakaliki and Mile 4 Hospital Abakaliki, who meet the inclusion criteria. Detailed history was obtained and thorough examination was carried out on all the patients. Relevant investigations, which include hemoglobin concentration, platelet count, bedside clotting time and urine analysis for proteinuria, were carried out on all the cases. Controls were asymptomatic normotensive parturient mothers who delivered in the facilities, pair-matched the cases and met the inclusion criteria.

3.4.4. Study Duration

This study lasted for a period of 6 months between 1st October, 2021 and 31st March, 2022 as extrapolated from the number of patients managed over the last one year in both hospitals.

3.4.5. Inclusion Criteria

Patients that were included in this study were pregnant women with singleton fetus, at gestational age of ≥28 weeks, with preeclampsia/eclampsia as cases, while healthy normotensive parturient mothers at gestational age ≥28 weeks served as control. On admission, both cases and controls were counseled on the aim and objectives of the study, consent for the study was obtained. In cases of severe preeclampsia or eclampsia in which the patient was disoriented and/or unconscious, accompanying relative or next of kin that followed the patient to the hospital was counseled, and consent was obtained from such individuals. Controls were pregnant woman that present in labour, had normal delivery and consented to the study. The cases of preeclampsia/eclampsia were recruited from labour ward of Department of Obstetrics and Gynaecology of AEFUTHA and Labour ward of Mile 4 Missionary Hospital, Ishieke. For each case of preeclampsia/eclampsia, a control that pair-matched the case for age, parity, residential area, booking status and social class were recruited.

3.4.6. Exclusion Criteria

1) Patients who do not consent to the study.

2) Patients who received intermittent preventive treatment (IPT) and/or treatment for malaria within the last 4 weeks.

3) Patients with multiple gestations.

4) Patients with active malaria infection.

5) Patients with gestational diabetes.

6) Sickle cell anaemic patients.

3.4.7. Sample Size Determination

The minimum sample size was determined using statistical formula for case controlled study design [19]:

$$N = r + 1 \Big/ r \left\{ \left(P^*\right)\left(1 - P^*\right)\left(Z_\beta + Z_{\alpha/2}\right)^2 \right\} \Big/ \left(P_1 - P_2\right)^2$$

N = Sample size

R = Ratio of cases to controls = 1

P_2 = Proportion of controls. Since 29.2% of pregnant women have elevated blood pressure of ≥ 140/90 mmHg in Ebonyi State [20], the proportion of normotensive women is therefore 70.8% = 0.71

P_1 = Proportion of case = $ORp_{\text{control}}/p_{\text{control}}(OR-1)+1$

O = Odd Ratio. Using OR of 4.0

$$P_1 = 4\times 0.71/0.71(4-1)+1 = 2.84/3.13 = 0.91$$

P^* = Average proportion exposed, calculated as proportion of cases + proportion of controlled cases/2 *i.e.* {0.91 + 0.71}/2 = 0.81

Z_β = Standard normal variate for power = 0.84 (at 80%)

$Z_{\alpha/2}$ = Standard normal variate for level of significance = 1.96

$P_1 - P_2$ = Effective size or difference expected based on previous studies

$$N = 2/1(0.81)(1-0.81)(0.84+1.96)^2/(0.91-0.71)^2$$

N = 60.3 ≈ 60.

This represents the number of patients per group. Ten percent of this minimum sample size was added to correct for any attritions that may occur in the course of the study. The final sample size on each arm of the study was now 66 while the total was 132.

3.5. Patients' Selection, Sample Collection and Study Procedure

3.5.1. Patients' Selection, Sampling Method and Data Collection

Consecutive consenting client sampling method was used in the study. All clients with preeclampsia/eclampsia that meet the inclusion criteria and gave consent to the study were selected till the sample size was achieved. A case was defined as a woman who had given birth at gestational age of ≥28 weeks and who, in the antenatal period or before progressing to labour, develops preeclampsia or eclampsia. Preeclampsia was diagnosed by presence of elevated blood pressure after 20 weeks gestational age, and with associated proteinuria. A blood pressure of ≥140 mmHg systolic and/or diastolic blood pressure of ≥90 mmHg arising after 20 weeks of gestation in a woman, who was normotensive before 20 weeks gestation, was taken as hypertensive range. Proteinuria was defined as excretion of ≥300 mg of protein in 24 hour urine sample or ≥2+ or more on dipstick.

There was matched pairing for cases and controls based on their age, parity, residential area, booking status and social class. The age pairing was done based on age groups < 20 years, 20 - 24 years, 25 - 29 years, 30 - 34 years, 35 - 39 years and ≥40 years. Pairing for parity was done by grouping them as P_0, P_1 - P_4, and ≥P_5. Pairing of residential area was done by using rural, semi-urban, or urban area, while booking status pairing was either booked or unbooked. The pairing for social class was done by using social class ≤1, 2 - 3 and 4 - 5.

The control that was selected for a particular case was the next parturient mother that pair-matched with the index case, that met the inclusion criteria and

gave consent to the study. Controls were parturients who were admitted and gave birth, with blood pressure of less than 140/90 mmHg and with no significant proteinuria seen or recorded during pregnancy or at the time of delivery. Two measures of blood pressure were taken using a sphygmomanometer. The first was on admission after a 10-minute rest and far from a uterine contraction; the second measure of blood pressure was taken between 30 and 60 minutes after the first measurement. Presence of proteinuria (albumin) was checked by multistix of fresh urine sample collected on admission. After obtaining an informed consent, questionnaires containing information on socio-demographic, obstetrics and medical characteristics were filled for both cases and controls. The questionnaire was designed by the chief researcher using ChatGPT app, the content generated online was redesigned to be able to address the aim and objective of the study. Also, enquiry was made on usage of bed nets and previous malaria infections in the index pregnancy.

3.5.2. Collection of Placenta Tissue and Its Analysis

These procedures were done with involvement of a pathologist, who played a supervisory role during collection and analysis of the samples. Two to three centimeters full thickness of placental block was taken from the placenta, kept in neutral buffer formalin for histopathology examinations. Sections were made on cryostat, air-dried, fixed with methanol and stained with Giemsa for 12 minutes. Placental malaria infections were characterized based on the classification of Muehlenbachs *et al.*, [21] that used the extent malaria pigment deposition within intervillous space of the placenta to a high power field of 60, to grade placenta malaria parasitisation. In null ("0") category, the pigment is absent in the intervillous space, category I malaria pigment is present in <10% of the intervillous space, category II have pigments in 10% - 40% of the intervillous space and category III have pigments in >40% of the intervillous space.

3.6. Quality Control

Certified pathologists were used for the sample analysis. Preparation and examination of slides and samples were done in Histopathology Labouratory of department of Morbid Anatomy of AEFUTHA. Random samples from both cases and controls were sent to another independent Pathologist for analysis to ensure that the results are within the normal range. The investigator was guided on the basic principle of histological examination and randomly partook in the examination of the stained specimen. Also standard tissue preparation methods were used through-out the procedure. The data were checked daily by the Principal Investigator for comprehensiveness prior to entry into the data base. Double data entries were done by two persons using the EPI-DATA programme and any missing data were checked against hospital records and other source documents.

3.7. Data Analysis

The data were processed using Epi Info software. Categorical variables were

analyzed using Mc Nemar chi square test. Univariate and multivariate analyses were performed where preeclampsia/eclampsia was dependent variables and maternal socio-demographic characteristics (age, parity and maternal blood group, past history and family history of preeclampsia) and placenta malaria were the possible influencing factors. $P \leq 0.05$ was regarded as significant. All variables with a P value ≤ 0.05 and those with sound biologic plausibility were included in the regression models and adjusted odds ratios (AOR) at 95% Confidence intervals (CI) were used. The final regression model was evaluated using Hosmer-Lemeshow goodness-of-fit test.

3.8. Ethical Consideration

Permission to carry out this research was sought and obtained from the Research and Ethical Committee of the Alex Ekwueme Federal University Teaching, Abakaliki with Reference Number FETHA/REC/VOL2/2019/168. Assent was also obtained from management of Mile 4 Hospital, Abakaliki.

3.9. Informed Consent

A signed written consent was obtained from each participant before recruitment into the study. The study objectives, procedure and full implication of participation were discussed with the participants and their consents were obtained. Participants were made to understand that declining participation had no consequences and will not prevent them from obtaining adequate care.

Confidentiality of data: All information, including history, physical examination findings and results, obtained from the participants were kept strictly confidential. Participants were assured that their identity would be kept in confidence by the investigator.

Beneficence to the participants: Histology of the placenta samples were done at no cost to the participants. Those with positive findings were referred to the pediatricians and were properly followed up and treated.

Non-maleficence to the participants: All precautions were taken to ensure that no harm was inflicted on the patients by the study.

Justice: Method of patients' selection was scientifically objective and fair to all participants.

3.10. Dissemination of Results from Study

The result from this study had been submitted to the human research ethics committee (HREC)-AEFUTHA. It has also been presented in the departmental clinical conference.

4. Results

During the study period, there were 2941 deliveries in both facilities and 84 cases of preeclampsia/eclampsia were managed. The prevalence of preeclampsia/eclampsia was therefore 2.9%. Out of the 84 preeclampsia/eclampsia cases, 70 met

the eligibility criteria and were selected for the study. This, along with 70 controls that pair-matched the cases and met the inclusion criteria were recruited for the study, making a total of 140 patients according to the flow-chat below (Figure 1).

Table 1 represents the socio-demographic characteristics of both groups of patients in the two hospitals. From this table, the P values of all the parameters tested were > 0.05 and this means that there was no statistically significant difference between both groups of study participants on these parameters.

In Table 2, the risks factors for preeclampsia/eclampsia and placenta malaria were analyzed.

Placenta malaria was positive in twenty one (21) out of the 67 cases of preeclampsia/eclampsia analyzed (31.3%) and eleven (11) out of 68 controls (normotensive) patients analyzed (16.2%). From Table 3, placenta malaria significantly increased the risk of developing preeclampsia/eclampsia among the participants.

Table 4 showed that placenta malaria is associated with adverse maternal outcome but has no effect on mode of delivery and risk of maternal mortality.

From Table 5, the P value is less than 0.05 for NICU admissions and low APGAR scores at first and fifth minutes and this implies that placenta malaria is associated with significant increase in NICU admission and low APGAR scores. However, with P value of greater than 0.05 for neonatal jaundice and perinatal mortalities, placental malaria is not associated with increased risk of neonatal jaundice and perinatal mortality.

5. Discussion

Placenta malaria and preeclampsia/eclampsia are common diseases of pregnancy in the African region, and placenta have been implicated in the pathophysiology of both disease processes. The two diseases have pathophysiologic similarities on

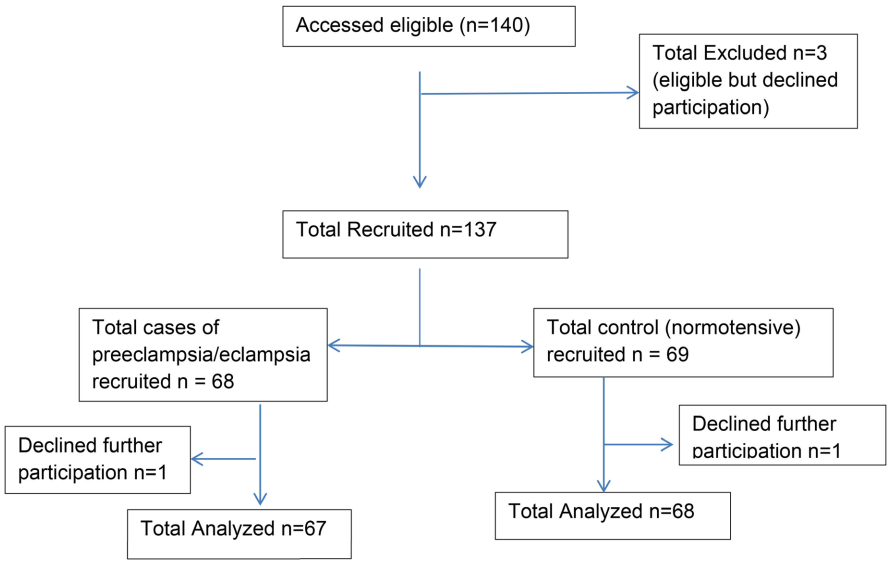

Figure 1. The flow chart of patients through the study.

Table 1. Socio-demographic data of participants.

Variables	Cases (n = 67)	Control (n = 68)	P value
Age			
<20	4	5	0.7 (ns)
20 - 24	13	14	
25 - 29	20	18	
30 - 34	17	16	
35 - 39	10	11	
>39	3	4	
Social Class			
0 - 1	13	15	0.5 (ns)
2 - 3	21	23	
4 - 5	33	30	
Parity			
P_0	37	36	1.0 (ns)
$P_{1\text{-}4}$	18	19	
$\geq P_5$	12	13	
Educational Status			
None	13	14	0.9 (ns)
Primary	14	16	
Secondary	22	21	
Tertiary	18	17	
Occupation			
House-wife	9	8	0.9 (ns)
Trading/Business	24	23	
Farming	14	15	
Civil Servants	15	16	
Others	5	6	
Marital Status			
Married	46	47	0.9 (ns)
Single	15	14	
Divorced	1	2	
Co-habiting	3	2	
Widow	2	3	
Religion			
Christian	56	57	1.0 (ns)
Muslim	7	6	
Traditional worshipers	3	4	
Others	1	1	

Cases = Preeclampsia/eclampsia, control = Normotensive, ns = not significant.

Table 2. Assessment of risk factors for preeclampsia/eclampsia and malaria among participants.

VARIABLES	CASES	CONTROL	OR (95% CI)	P value
Bed nets Usage				
Yes	18	05	4.6 (1.6 - 13.3)	0.005 (s)
No	49	63		
Social Class				
0 - 1	13	15		
2 - 3	21	23	0.3 (2.2 - 5.3)	0.47 (ns)
4 - 5	33	30		
Family Hx of Hypertension				
Yes	37	24	2.3 (1.1 - 4.5)	0.02 (s)
No	30	44		
Hx of Malaria				
Yes	38	23	2.3 (1.3 - 5.2)	0.01 (s)
No	29	45		
BMI				
<18	5	7		
18 - 25	26	46	1.6 (1.1 - 5.1)	0.6 (ns)
>25 - 30	28	11		
>30	8	4		

Hx = history, ns = not significant, s = significant.

Table 3. Univariate and Multivariate analysis of risk factors of malaria and preeclampsia in FETHA.

Variables	Univariate Analysis			Multivariate Analysis		
	OR	95% CI	P value	OR	95% CI	P value
Age	1.0	0.8 - 1.0	0.6	1.0	0.9 - 1.2	0.6 (ns)
Primigravidae	1.1	0.6 - 2.2	0.8	2.1	0.3 - 0.7	0.3 (ns)
Non-usage of Bed net	4.6	1.6 - 13.3	0.005	3.5	0.7 - 1.1	0.003 (s)
Regular IPT use during ANC	2.1	1.1 - 3.6	0.02	2.5	0.8 - 6.9	0.06 (ns)
Lack of Antenatal care	1.7	0.9 - 3.5	0.01	0.9	0.5 - 2.0	0.04 (s)
Family Hx of Hypertension	2.3	1.1 - 4.5	0.02	1.5	0.7 - 3.1	0.01 (s)
Hx of Malaria	2.6	1.3 - 5.1	0.01	2.0	1.0 - 4.3	0.07 (ns)
BMI	4.0	1.9 - 8.5	0.003	3.3	1.2 - 3.3	0.001 (s)
Blood group O vs Non-group O	0.9	0.5 - 1.8	0.8	1.0	0.5 - 2.1	0.3 (ns)
Placenta malaria	2.4	1.0 - 5.4	0.04	1.8	1.0 - 2.5	0.03 (s)

Hx = history, s = significant, ns = not significant.

Table 4. To access if placental malaria affects management and prognosis of preeclampsia/eclampsia.

Complications	PE with PM	PE without PM	Risk Ratio RR	95% CI	P value
Delivered by CS					
Yes	16	28	1.3	0.9 - 1.7	0.2 (ns)
No	5	18			
CVA					
Yes	4	0	19.2	1.1 - 341.7	0.04 (s)
No	17	46			
Abruptio Placenta					
Yes	11	10	2.4	1.2 - 4.8	0.01(s)
No	10	36			
PPH					
Yes	7	7	2.1	0.9 - 5.5	0.09 (ns)
No	14	39			
HELLP's Syndrome					
Yes	4	1	8.8	1.0 - 73.7	0.05 (ns)
No	17	45			
AKI					
Yes	2	1	4.4	0.4 - 45.6	0.2 (ns)
No	19	45			
DIC					
Yes	5	1	10.9	1.4 - 88.0	0.02 (s)
No	16	45			
Pulmonary Oedema					
Yes	5	2	2.7	1.1 - 25.9	0.03 (s)
No	16	44			
IUGR					
Yes	10	10	2.1	1.1 - 4.5	0.03 (s)
No	11	36			
IUFD					
Yes	7	4	3.8	1.3 - 11.7	0.02 (s)
No	15	42			
MM					
Yes	2	1	4.4	0.4 - 45.7	0.2 (ns)
No	19	45			

CVA = Cerebrovascular Accident, PPH = Postpartum Haemorrhage, AKI = Acute Kidney Injury, DIC = Disseminated Intravascular Coagulation, IUGR = Intrauterine Growth Restriction, IUFD = Intrauterine Fetal Death, MM = Maternal Mortality, s = significant, ns = not significant).

Table 5. Maternal preeclampsia/eclampsia and placental malaria and fetal outcome.

Variables	Cases Number		Control Number		RR	95% CI	P value
	With PM	Without PM	With PM	Without PM			
NICU admission	11	3	3	9	2.6	1.1 - 6.0	0.03 (s)
AS ≤ 7 at 1 minute	12	6	5	15	2.7	1.2 - 6.1	0.02 (s)
AS ≤ 7 at 5 minutes	9	4	3	10	3.0	1.0 - 8.6	0.04 (s)
NNJ	4	1	3	2	1.3	0.6 - 3.1	0.5 (ns)
Perinatal Mortality	6	2	5	1	0.9	0.5 - 1.5	0.7 (ns)

NICU = Neonatal Intensive Care Unit, AS = APGAR Score, NNJ = Neonatal Jaundice, PM = Placenta Malaria, s = significant, ns = not significant.

the placenta such as placenta ischemia, endothelial dysfunction and production of pro-inflammatory cytokines [2]. Malaria in pregnant women is a significant cause of obstetric morbidity especially when there is co-infection with human immunodeficiency virus (HIV) [22]. This study has established that the presence of placenta malaria among parturient with preeclampsia/eclampsia is associated with adverse outcome for both the mother and her fetus.

The prevalence of preeclampsia/eclampsia was 2.9% and this was greater than 0.4% reported by WHO for developed countries [23] and 1.2% recorded in University of Calabar Teaching Hospital [24], but this prevalence was comparable to 2.8% of live birth reported by WHO for developing countries [23]; it was however less than 6% reported in Sokoto [9]. This may be due to the regional variations in prevalence of preeclampsia/eclampsia in Nigeria. The proportion of placental malaria was 31.3% among parturients with preeclampsia/eclampsia (cases) and 16.2% among normotensive parturients (controls). These proportions were much more than the 6.3% among cases and 6.2% among controls reported in a similar study in Senegal [2]; and 19.6% among cases and 11.2% among controls reported by Adam *et al.* in Sudan [1]. This difference may be because the histology of placenta tissues were used in this study and has been proven to be more sensitive in the diagnosis of placenta malaria compared to peripheral and cord blood samples used in the previous studies. Also, the differences in socio-demographic data between two groups were not statistically significant, probably because of proper pair-matching of both cases and controls, hence the findings in the study may be related to the socio-demographic characteristics of the parturient.

From this study, logistic regression shows that the presence of placenta malaria significantly increased the odds of developing preeclampsia/eclampsia among parturient (OR = 2.4, 95% CI = 1.0 - 5.4, P value = 0.04). This finding is similar

to what was earlier reported by Adam *et al.* in Sudan [1] and Ndao *et al.* in Senegal [2]. From his work in Okigwe local government area of Imo State, Nigeria, Onuigbo *et al.* discovered that there was a strong association between placenta malaria and preeclampsia/eclampsia. He suggested that placenta malaria could be an independent risk factor for preeclampsia [18]. The probable reason for this association could be due to the ability of placenta malaria to cause placenta ischemia, endothelial dysfunction and production of pro-inflammatory cytokines [2]. These pro-inflammatory cytokines have been shown to increase the elaboration of anti-angiogenic factors such as soluble vascular endothelial growth factor receptors 1 (also known as fms-like tyrosine kinase 1) and soluble endoglins (sEng). These factors impede the remodeling abilities of vascular endothelial growth factors (VEGF) with subsequent development of preeclampsia/eclampsia [25] [26] [27]. Also, preeclampsia/eclampsia has been linked with hypo-albuminemia which causes in immune dysfunctions in the mother, with subsequent increase in susceptibility to malaria infections.

We also observed that placenta malaria is associated with adverse pregnancy outcomes among parturients with preeclampsia/eclampsia. It significantly increased the risk of developing cerebrovascular accidents, placenta abruption, disseminated intravascular coagulation, pulmonary edema, intrauterine growth restriction and intrauterine fetal death. This is probably because both placental malaria and preeclampsia/eclampsia can elaborate inflammatory mediators, which can cause anemia, endovascular damage,and decreased oncotic pressure which then lead to decreased end organs perfusions and subsequent end organs failure. Presence of placenta malaria in parturients with preeclampsia have no significant effect in the risk of delivery by caesarean section, risk of developing HELLP's syndrome and does not increase the risk of maternal mortalities. This may be due to low frequency of these pathologic processes in the index study.

Presence of placenta malaria among mothers with preeclampsia/eclampsia significantly increased the risk of admission of their fetuses into neonatal intensive care unit; and low APGAR scores at 1st and 5th minutes. The reason for poor neonatal outcomes among neonates delivered to mothers with preeclampsia/eclampsia could be because the presence of placenta malaria is associated with increased fibrosis at the placenta bed, which later results to worsening of placenta insufficiencies already caused by preeclampsia/eclampsia, with subsequent neonatal hypoxia.

6. Conclusion

There is a higher prevalence of placenta malaria among mothers with preeclampsia/eclampsia when compared with normotensive controls. Based on the findings of this study, the Null hypothesis is thereby rejected and Alternative hypothesis that states that "Placenta malaria parasites are associated with adverse pregnancy outcomes among parturient mothers with preeclampsia/eclampsia in AEFUTHA" is accepted.

7. Strengths

The pair-matching of the participants ensured elimination of bias, and this helped in improving the validity of the study. Histology of placenta tissues was used in the diagnosis of placenta malaria in this study and it is the most sensitive method for diagnosing placenta malaria. The slides were prepared and analyzed by a certified pathologist. Also, to ensure proper quality control, random samples from both cases and controls were sent to an independent pathologist.

8. Limitations of the Study

1) The staining and interpretation processes of the slides are labour intensive, time-consuming, and require considerable expertise. If these are not properly done, it may bring about errors in the result. To circumvent this, a pathologist was engaged and results of random samples were cross-checked by another pathologist.

2) Use of antimalaria and intermittent preventive treatment for malaria (IPT) within 4 weeks of sample collection is prone to recall bias and may affect the validity of the final result.

3) This was a hospital based study which may not be a true reflection of the situations in the community.

4) The sample size of this study may be so small to be a true representative of association between placenta malaria parasites and preeclampsia/eclampsia among parturient mothers in the general community.

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Recommendations

Presence of placenta malaria is associated with poor pregnancy outcome among mothers whose pregnancies are complicated with preeclampsia/eclampsia. Routine screening for malaria parasites during antenatal booking is recommended as this will help in early diagnosis and treatment of malarias. Also pregnant mothers are encourage to register their pregnancies at a maternity home or hospitals so that they can benefit from routine intermittent preventive prophylaxis for

malaria, as majority of participants in this study lack antenatal care.

Contribution to Knowledge

This study has established that Placenta malaria parasites are associated with adverse pregnancy outcomes among parturient mothers with preeclampsia/eclampsia in FETHA, and this is a new contribution to the body of knowledge. Routine screening for, and treatment of malarias will probably help to reduce severity of preeclampsia/eclampsia and its associated complications among parturient mothers.

Future Studies

There is need for multicenter studies to fully establish the association of placental malaria parasites with adverse pregnancy outcome among parturient mothers with preeclampsia/eclampsia. This will help to develop systemic review of literatures, and probably modify our guidelines for management of cases of preeclampsia/eclampsia in malaria endemic areas.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix. Questionnaire

Instruction: please tick as appropriate as any information given would be treated with utmost confidentiality.

Section A: Socio-Demographic Data

1) Hospital Number……

2) Age (years); <20 ( ), 20 - 24 ( ), 25 - 29 ( ), 30 - 34 ( ), 35 - 39 ( ), ≥40 ( )

3) Marital Status; Single ( ), Married ( ), Co-habiting ( ), widow ( ) Separated ( ), Divorced ( )

4) Parity; 0 ( ), 1 - 4 ( ), ≥5 ( )

5) Religion; Muslim ( ), Christian ( ), Traditional Worshipers ( )

6) Occupation; Civil Servant ( ), House-wife ( ), Trading/Business ( ), Farming ( ), Others ( )

7) Educational Status; None ( ), Primary ( ), Secondary ( ), Tertiary ( )

8) Husband's Occupation; Professionals ( ), Middle Level ( ), unskilled ( )

9) Booking Status; Booked ( ), Unbooked ( )

10) Social class; 0 - 1 ( ), 2 - 3 ( ), 4 - 5 ( )

Section B: Analysis of Risk Factors

11) Use of Bed-nets; Yes ( ), No ( )

12) Family History of Hypertension; Yes ( ), No ( )

13) History of Malaria; Yes ( ), No ( )

14) BMI: <18 ( ), 18 - 25 ( ), >25 - 30 ( ), >30 ( )

15) Blood Group; O ( ), Non "O" Blood group ( )

Section C: To Access If Placenta Malaria Patients' Management and Prognosis of Preeclampsia/eclampsia

16) Mode of Delivery; SVD ( ), C/S ( )

17) Abruptio Placenta; Yes ( ), No ( )

18) Postpartum Hemorrhage; Yes ( ), No ( )

19) HELLP's Syndrome; Yes ( ), No ( )

20) Acute Kidney Injury; Yes ( ), No ( )

21) Any Disseminated Intravascular Coagulation; Yes ( ), No ( )

22) Presence of Pulmonary edema; Yes ( ), No ( ).

23) Presence of Intrauterine Growth Restriction; Yes ( ), No ( ),

24) Any Intrauterine Fetal Death; Yes ( ) No ( )

25) Maternal Outcome; Alive without morbidity ( ), Alive with morbidity ( ), Dead ( )

26) Placenta Malaria Histology Result; Positive ( ), Negative ( )

Section D: Maternal Preeclampsia/Eclampsia and Placenta Malaria and Fetal Outcome

27) NICU Admission; Yes ( ), No ( )

28) If yes, Duration of admission in days; 1 - 5 ( ), 6 - 10 ( ), ≥10 ( )

29) If yes, indication for Admission; Birth Asphyxia ( ), Neonatal Jaundice

( ), Sepsis ( ), Others ( )

30) APGAR Score at 1 minute; <3 ( ), 4 - 7 ( ), 8 - 10 ( )

31) APGAR Score at 5 minute; <3 ( ), 4 - 7 ( ), 8 - 10 ( )

32) Fetal Outcome; Alive without morbidity ( ), Alive with morbidity ( ), Dead ( )