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Research Article

Placental Malaria and Its Relationship with Neonatal and Placental Birthweight among Pregnant Women Attending Selected Hospitals in Katsina State

Yaradua, Z. A. and Yusuf, A. M.

Department of Biological Sciences, Faculty of Natural and Applied Sciences, Umaru Musa Yaradua University, Nigeria
***Corresponding Author's email*: ahmedzainab39.za@gmail.com**

ABSTRACT
Placental malaria has been recognized as a complication of malaria in pregnancy in areas of stable transmission. Women in zones of high malaria transmission are often asymptomatic, thus leading to chronic placental malaria (PM) with insidious consequences to both the mother and the unborn faetus. The present study was conducted to assess the relationship of placental malaria with placental and neonatal birthweight among pregnant women in Katsina State. A cross-sectional study involving 300 parturients was carried out using the biopsy pool method. Placental blocks were taken from the pericentric area of the maternal surface of the placenta, and the birth weights of the neonates and the placenta were recorded. The samples were fixed in 10% neutral-buffered formalin, and histopathological analysis was performed. Overall, 76% of the study participants were observed to harbour a high level of monocytes infiltrations with in the placental tissue, which shows a positive significant association with the presence of malaria parasitaemia in placental blood (r =0.735) and parasitaemia in peripheral blood (r = 0.385), followed by underweight babies (r = .372), and underweight placentas (r = .446). shows a weak association, while for normal weight babies (r = -.272) and normal placental weight (r = 0.277) a weak negative correlation was observed. However, all parameters measured show statistically significant associations (p = 0.000). The effects of a high presence of malaria parasite embedded within the placentas were found to be independently correlated with the underweight babies and underweight placentas.

Keywords: Neonatal weight; Placental malaria; Placental weight; Public health; Relationships

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INTRODUCTION

Malaria during pregnancy is a major global public health problem, with each of the most prevalent Plasmodium species causing substantial maternal and infant morbidity and mortality (Barber *et al.* 2015). The susceptibility of malaria infection in pregnant women was initially established about 65years ago (Steketee *et al.* 2001), where descriptive studies in Sub-Saharan Africa between 1950 and 1980 focused on the prevalence and virulence of *Plasmodium falciparum* infection among pregnant women (Archibald, 1956., Cannon, 1958., Gielles *et al.* 1969). Malaria during pregnancy in sub-Saharan Africa accounts for an estimation of about 10,000 maternal deaths and about 200,000 infant mortalities annually (Omer *et al.* 2017). Most of these deaths are caused by *Plasmodium falciparum*, which is one of the most abundant plasmodium species in tropical and subtropical regions (Omer *et al.* 2017).

Placental malaria has been recognized as a major complication of malaria in pregnancy. The placenta is known to preserves pregnancy and fetal development by ensuring maternal-fetal exchange (Brett *et al.* 2014) and maternal tolerance of feto-paternal antigens (Sharma and Sukla, 2017). However, during malaria episodes, the *P.falciparum*

Infected erythrocytes (IEs), including mature trophozoite and schizont tend to sequester within the intervillous spaces of the placenta around the 3rd month of gestation (Walker *et al.* 2012). The excessive sequestration of infected erythrocytes (Kidima, 2015), yields massive infiltration of monocytes and macrophages (Salih *et al.* 2011), and the deposition of fibrins and trophoblast, thus, leading to excessive pathological changes that could be detrimental to the mother and fetus (Djabanor *et al.*2017).

Different studies reported certain histological changes in placentas infected with the Plasmodium parasite. There were moderate to severe mononuclear intervillositis (Dimasuay *et al.* 2017), polymorphonuclear leukocytosis (Djabanor *et al.* 2017) and focal placental calcifications in the stroma which were associated with an increased risk of inter-uterine growth retardation (IUGR), low birth-weight (LBW) and preterm delivery (Djabanor *et al.* 2017). Another study also reported that inflammatory infiltration of the intervillous spaces is associated with LBW especially when mononuclear cells are highly increased (Bardaji *et al.* 2011).

In Nigeria, the burden posed by placental malaria to the health sector is growing at an alarming rate (Ezebialu *et al.* 2012) due to a lack of available tools that could be used in detecting and identifying the magnitude of placental malaria before delivery. The prevalence of placental malaria among pregnant women has been reported from different parts of Nigeria among which includes; 69.9% in Southeastern Nigeria (Ezebialu *et al.* 2012), 22.4% in Kaduna state (Aliyu *et al.* 2017), 41.6% in Northwestern Nigeria (Fana *et al.* 2017), 59.8% was also reported in Ogun state (Babalola *et al.* 2017). However, there is a paucity of such data in Katsina state, hence the present study aimed to provide more insight on the prevalence of placental malaria and provide insight on the effect placental malaria poses on neonatal weight.

MATERIAL AND METHODS

Study Area

The study was conducted in Katsina metropolis of Katsina State. The state covers a total area of 24, 192 km^2 spanning between longitude 10°33'59'' to 13°18'30''N and latitude 6°59'32'' to 9°00'0.1''E with an estimated population of 6.5 million people (National census projection, 2017). The climate of Katsina state is a tropical continental climate classified by Koppen as AW climate (winter dry Season) (Abaje *et.al* 2014). The dominant vegetation is Sudan savannah, which combines the characteristics and species of both Guinea and Sahel savannah (Tukur and Amadi, 2014). The mean annual temperature ranges from 29°C – 31°C, the highest air temperature normally occurs in April/May and the lowest occurs in December through February. Annual rainfall in Katsina state normally falls between July and October with a range of 500mm – 920mm (Ogungbenro and Morakinyo, 2014). Malaria transmission is endemic in Katsina state with all year-round transmission at levels below the National average, with a seasonal peak (60% of annual malaria cases) coinciding with the raining season (Stranchan *et al.* 2016).

Study Population

The study populations (subjects) involved were drawn from pregnant women (Primigravidae, Secundgravidae and Multigravidae) with a gestation period of between 39 to 40 weeks. Majority of people in Katsina predominantly speak Hausa and Fulfulde language with Islam as the dominant religion practiced. The populace largely engaged in subsistence farming (James *et al.,* 2018), trading and cattle rearing (Ladan, 2019).

Selection of Study Subjects

The study subjects were selected using a purposive sampling method, where pregnant women in their 3rd trimester between 39 to 40 gestational weeks and are willing to participate by delivering at either of the respective hospital units were involved. The selection of the study subjects relies on pregnant women fulfilling the inclusion and exclusion criteria.

Inclusion Criteria

For the purpose of the study only pregnant women with live birth and singletons delivered vaginally were involved in the study.

Exclusion Criteria

For the study pregnant women with a history of pre-eclampsia and diabetics were not included among the study subjects. In addition, women that are hypertensive or have had a caesarian section before were also not included.

Determination of Sample Size

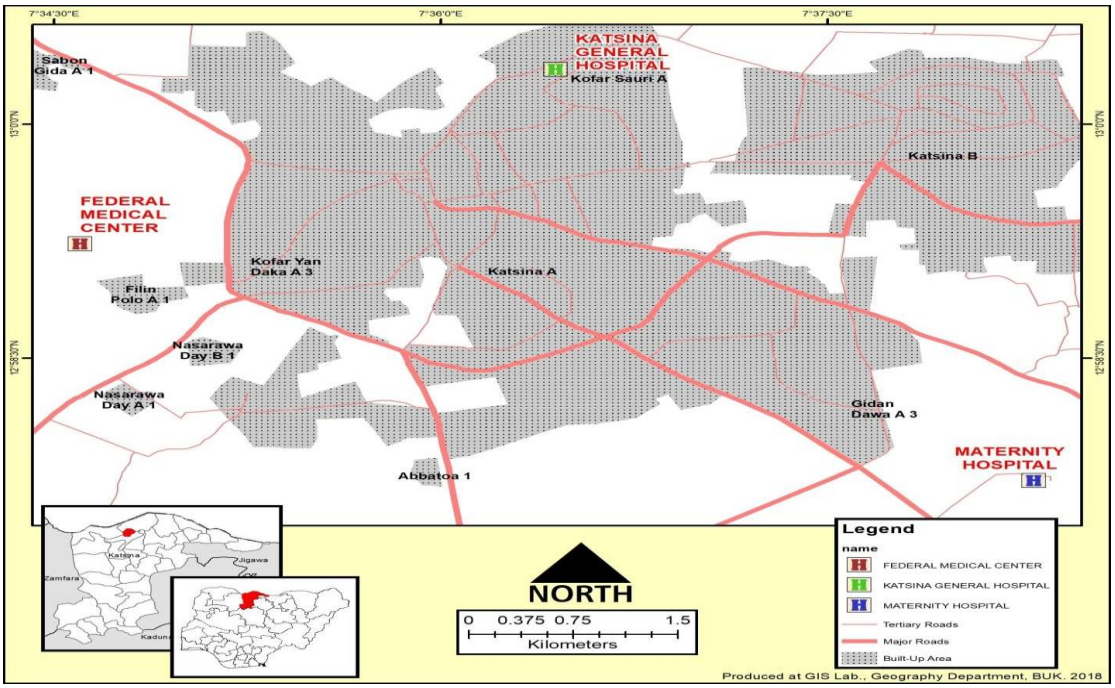
The sample size of the study was calculated based on Fisher 's population formula as reported by Charan and Biswas, (2013), using previous prevalence of 22.4% as reported by Aliyu *et al.,* (2017). The formula used for the calculation of the sample size is;

$$n = \frac{Z^2 P (1- P)}{d^2}$$

Where;

n = Sample size,
Z = Confidence level (95%) = Z-value is 1.96
P = expected prevalence or proportion (22.4% = 0.224)
(Aliyu *et al.,* 2017).
d = precision (5% = 0 .05)

Based on the above relationship, a figure of 267 was arrived at as the study sample size. However, to adjust and take care of anticipated dropout of consented study subjects and to be more precise, the figure 267 was rounded up to the nearest 100, thus 300 study subjects were recruited as the study sample size.



Produced at GIS Lab. Geography Department BUK. 2018.

Figure 1: Map of Katsina Metropolis showing the three hospitals.

Ethical Clearance and Informed Consent

Ethical clearance certificate with reference number (MOH/ADM/SUB/1152/1/194) and (FMCNHREC.REC.NO.03/082012) respectively were obtained from the State Ministry of Health in Katsina and from the management of Federal Medical Center Katsina, with which permission was obtained from the management of the three selected hospitals, with which written and oral informed consent was obtained from the study participants prior to collection of samples.

Study design

The study was a cross-sectional study, where 300 consenting pregnant women were randomly selected using purposive sampling method across the three major hospitals within Katsina metropolis. Between the months of June - August of 2024.

2.4 Sample Collection

To ensure an accurate weight the infants were placed completely naked on the scale using a clean cloth covering for hygiene and warmth following a technical guide designed by W.H.O. Using separate weighing scales (RGZ 20A, mechanical scale), the weight of the baby and the placenta was measured and recorded as Low-birth-weight in babies = < 2.5 kg, while heavy placenta was defined as weight >0.75kg, normal placental weight = 0.33kg – 0.75kg, while low placental weight was defined as weight <0.33kg as described by Iyare and Uneke, 2019.

Following the biopsy pool method described by Conroy *et al.* (2012), immediately after the safe delivery and weighing of the baby and the placenta, a full-thickness placental biopsy (2cm ×2cm) was excise from the maternal surface of the placenta approximately a third of the distance from the umbilical cord and at the edge of the placental disc (Conroy *et al.* 2012).

Placental tissue examination by Histology Assay and microscopy

All the placental tissue samples collected and preserved in 10% buffered formalin, pH 7.29, were resized and placed in well-labeled cassette. All procedures were carried out in accordance with the

protocol designed by John and El - Nageh (WHO, 2003).

The cassettes were placed in an automated tissue-processing machine (BK – TS3D Model, Biobase Biodustry. Shandong. Co, Ltd) where the tissues were dehydrated through different graded levels of ethanol, ranging from 70% through 95%. All the different tissue samples were embedded in molten wax forming wax blocks ready for sectioning.

Using an automated microtome (BK – MT398A, Biobase Biodustry, Shandong. Co., Ltd) all the 300 wax – blocked placental tissues were first trimmed to a thickness of ten micrometers (μm), which were further trimmed down to five μm. From each tissue sectioned, with the use of an automatic Biological – Tissue Slice Spreader (BK – TFI Model, Biobase Biodustry, Shandong. Co., Ltd) and a Tissue Hot Plate (BK – SDI) four sections were selected, four sections were selected, of which 2 sections were stained with Giemsa solution as described by Liu *et al.* (2016) based on a protocol by the WHO, 2003. The remaining two sections were stained with Hematoxylin and Eosin (H and E) (John *et al.,* 2019).

Using a light microscope (100×) under polarized light to increase the invisibility and minimize false positivity by formalin crystals. From the slides stained with H&E, monocyte 's accumulation was observed and expressed as geometric mean and standard deviation cell count per square millimeter (Salih *et al.* 2011). While the presence of parasites and malarial pigments in the intervillous spaces was observed from slides that were stained with Giemsa and classified according to Bulmer 's description (Bulmer *et al.,* 1993) and the methods employed by Ezebialu *et al.,* (2012). As either: (i) no infection (no parasites or pigments found); (ii) active infection (presence of parasites); (iii) active chronic infection (presence of malaria pigments with malaria parasites), and (iv) past chronic infection (presence of malaria pigment without malaria parasites).

Data Analysis.

The result was analyzed using Software for Social Science (IBM SPSS statistics 23), Simple descriptive statistic of frequency was used to analyze the occurrence of monocytes embedded within the placental tissues with respect to age group and parity level. Bivariate Pearson Correlation analysis was used to find out the relationship that exist between the presence of Monocytes caused by placental malaria and the varying weight of the placenta and the babies.

RESULTS AND DISCUSSIONS

Histological Analysis of Placental Biopsies

Monocytes / macrophages are one of first responses involved in immune cascade of patients with placental malaria, as a result of placental inflammation caused by the malarial sequestered red blood cells (Feeney, 2019). From the result obtained (figure 1.0), 25 (8.1%) of the placental samples examined shows present infection, 182 (60.7%) of the biopsies have an active infection, while 93 (31.1%) appeared to have a past infection. Also figure 2.0 reveals that about 76% of the study participants harbors high level of monocytes infiltrations with in the intervillous space of the placenta, which is in line with the findings of a study based in India (Ahmad *et al.* 2014) where 86% of the placentas examined harbors monocytes infiltration with varying capacity. However, the result obtained from this study is in contrast with the study conducted in Eastern Sudan where only 31.2% of the participants had presence of monocytes (Salih *et al.* 2011). The high presence of monocytes observed in this study could be attributed to high level of placental malaria observed among the study participants in figure 1.0. One of the pathogeneses of malaria parasites embedded within the placenta is the recruitment and activation of monocyte and macrophages, from different studies the activation of those monocytes is known to elicit different inflammatory response pathways that could contribute to either host protection by reducing the number of malaria parasite through opsonic or non-opsonic phagocytosis or pathogenesis which could lead to much complication to both the mother and the fetus. (Chua *et al.* 2021).

The high presence of monocytes observed in the present study could further be attributed to the fact that majority of the placentas found with monocyte infiltrations are placentas of young primigravidae and secundigravidae of younger age group as shown on figure 3.0 and figure 4.0 below where placental monocytes were observed in 97.2 % of primigravidae, 90.8% of secundigravidae and 98.9 % of study participants within 14 – 25 years. The high presence of monocytes observed among young primigravidae and secundigravidae from this study could be attributed to the ability of monocytes to have memory retention which is accentuated with subsequent pregnancies (Iyare and Uneke, 2018).

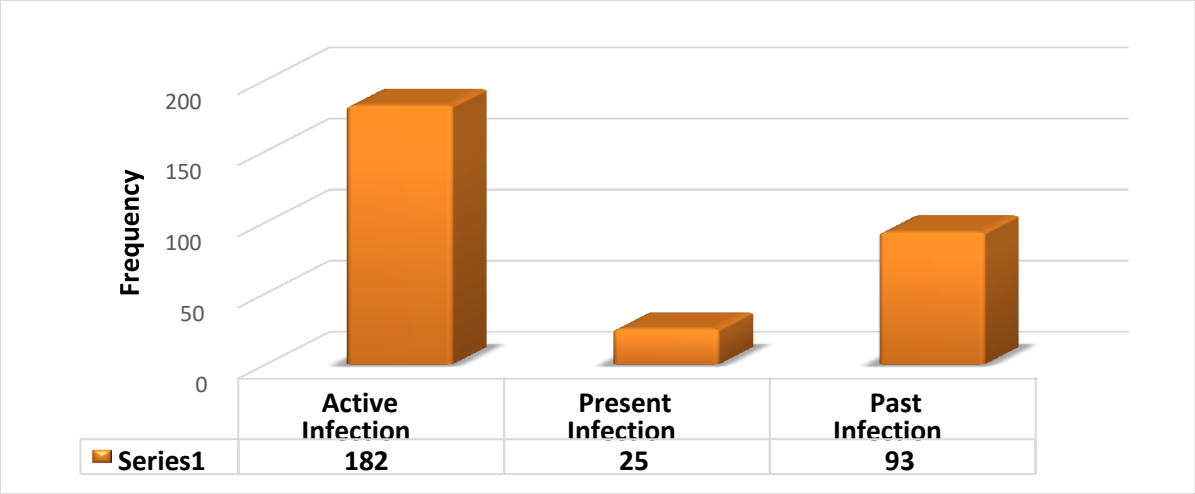


Figure 2: Occurrence of Malaria Parasites and Malaria Pigments within the Placental Biopsies

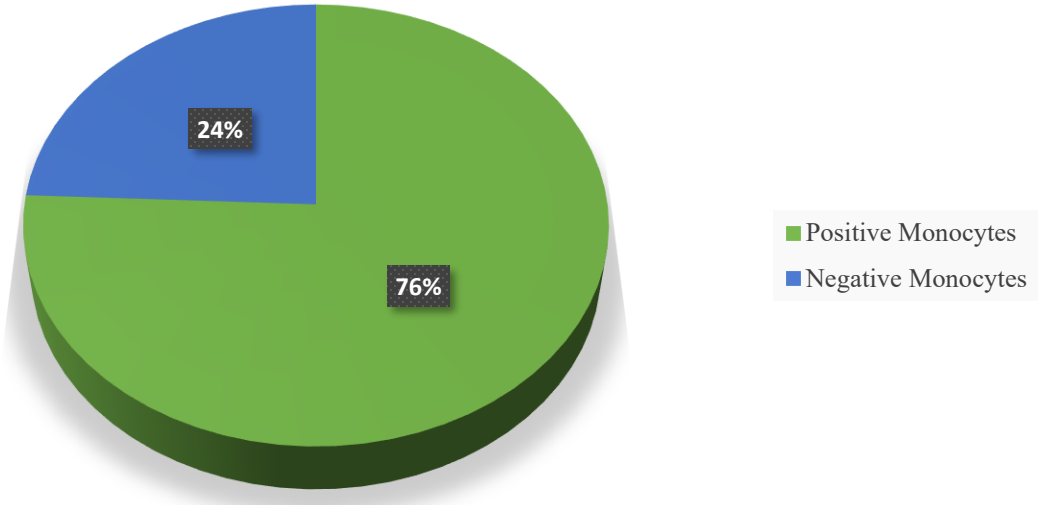


Figure 3: Prevalence of Monocytes embedded within placental tissue samples of all the study participants.

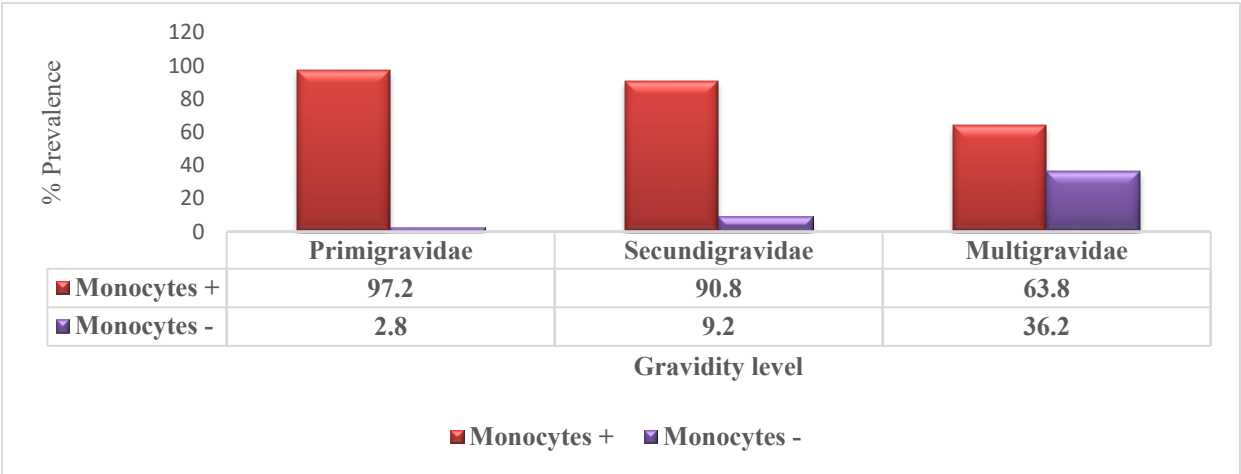


Figure 4: Prevalence of Monocytes embedded within placental tissue biopsies in relations to Gravidity level of study participants

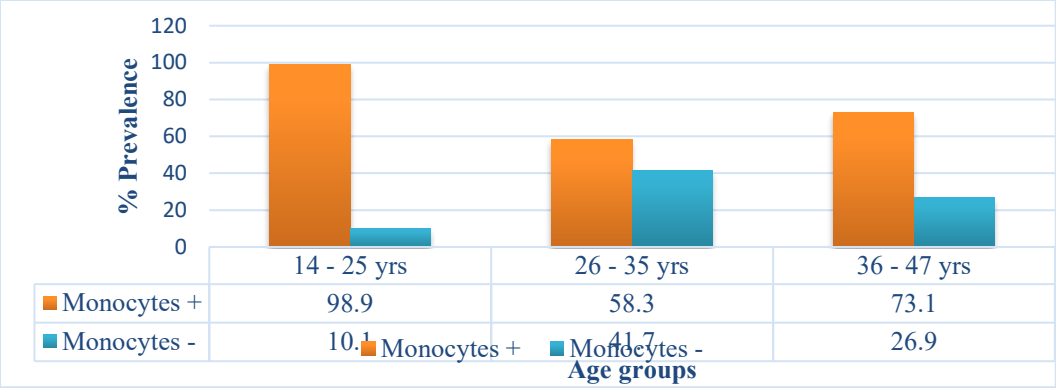


Figure 5: Prevalence of Monocytes present within placental tissues of study participants in relation there to Age group

In different autopsy studies of pregnant women with malaria, massive sequestration of infected erythrocytes in early pregnancy might interfere with trophoblast invasion into the uterus (Franke-Fayard *et al.* 2010) thus, reducing the amount of nutrients received by the fetus.

Adolescence tends to have the highest population in all societies. Due to their immaturity, they also tend to constitute the largest group with different health problems. From this research adolescents and younger women age 14 -25 years appears to have the highest number of underweight babies (59.4%) and subsequently the most number with underweight placenta (67.4%) as compared to other women in the study within higher age group as shown on table 2.0 below. The results obtain in this study is in line with several others which suggest younger age as a factor in pregnancy could be a predisposing factor underlying low birth weight and pre-term deliveries (Iyare and Uneke 2019).

Table 1: Occurrence of monocytes in different histopathological placental tissue samples examined in relation to gravidity, placental weight and baby weight

Gravid	Sample size	Baby weight no. (%)	Placental weight no. (%)
Primigravidae	72	Underweight 50 (69.4)	Underweight 57 (79.2)
		Normal weight 22 (30.6)	Normal weight 15 (20.8)
Secungravidae	51	Underweight 27 (52.9)	Underweight 31 (60.8)
		Normal weight 24 (47.1)	Normal weight 20 (39.2)
Multigravidae	177	Underweight 43 (24.3)	Underweight 86 (48.6)
		Normal weight 134 (75.7)	Normal weight 91 (51.4)

Table 2: Occurrence of monocytes in different histopathological placental tissue samples examined in relation to age differences, placental weight and baby weight

Age group	Sample size	Baby weight no. (%)	Placental weight no. (%)
14 – 25 yrs	138	Underweight 82 (59.4)	Underweight 93 (67.4)
		Normal weight 56 (40.6)	Normal weight 45 (32.6)
26 – 35 yrs	103	Underweight 49 (47.6)	Underweight 57 (55.3)
		Normal weight 54 (52.4)	Normal weight 46 (44.7)
35 – 47 yrs	59	Underweight 24 (40.7)	Underweight 31 (52.5)
		Normal weight 35 (59.3)	Normal weight 28 (47.5)

Bivariate Analysis of the relationship between Presence of Monocytes within Placental Biopsies and other Factors associated with Placental Malaria

From the results obtained on table 3.0 the presence of monocytes embedded within the placental tissue 's samples examined showed positive significant association with presence of malaria parasitemia in placental blood (r =0.735), followed by under weight babies (r = .372), and underweight placentas (r = .446) shows a weak association, while for normal weight babies (r = -.272) and normal placental weight (r = 0.277) a weak negative correlation was observed. However, all parameters measured shows statistically significant associations (p = 0.000).

Also, from the same result of Table 3.0 the relationship between placental malaria and

peripheral malaria (r = 0.242), underweight babies (r = 0.276) and underweight placentas (r = 0.209) show weak positive correlation, relationship with normal baby weight (r = -0.275) and normal placental weight (r = -0.243) shows weak negative correlation even though the association for all variables were statistically significant (p = 0.000). Conversely, the association of presence of monocytes in placental tissue, placental blood parasitemia and overweight placenta also shows a positive correlation (r = 0.274 and 0.283) which is also statistically insignificant at 99% level of significance (p = 0.003 and 0.009), respectively.

The bivariate analysis shows that a strong positive correlation exists with the increased prevalence of placental malaria among the study participants. This is consistent with the findings of a previous study conducted by Iyare and Uneke (2018).

Similarly, a positive correlation was observed between the presence of placental parasitemia and presence of monocytes within the placentas of the study participants and having low weight placentas and babies with low birth weight, which conforms with the findings of many studies around the world among which includes Patel *et al.* 2017, Dombrowski *et al.* 2018, and Solomon *et al.* 2020. During normal pregnancy the placenta is known to express seven isoforms of glucose transporters (GLUT 1, 3, 4, 8, 9a, 9b, and 10) which aid in the transportation of glucose from the maternal circulation through to the fetal circulation (Illsley and Baumann, 2020). Different investigations carried out on *Plasmodium*-infected placentas revealed that the over expression of monocytes and macrophages with in the malaria infected placentas could bring about PM associated intervillositis which is known to reduce the expression of glucose transporter isoform 1 (GLUT-1) on the syncytiotrophoblast basal membrane (Seitz *et al.* 2019), which explains the high prevalence of low birth babies.

Also, the presence of placental malaria and mononuclear infiltration is known to be associated with placentomegaly and high placental weight ratio as was observed in the present study, suggesting a less efficient placenta that has failed to translate its growth into proportionate fetal growth. From the findings of this present study, it was also observed that the presence of malaria parasitemia and the infiltration of monocytes within the intervillous space of the placenta to be positively correlated with over placental weight as what was observed from the study conducted by Iyare and Uneke (2018).

Table 3: Bivariate analysis of the relationship between Presence of Monocytes within placental biopsies and other factors associated with placental malaria

Variables	Placental parasitemia	Under weight babies	Normal-weight babies	Underweight placentas	Normal-weight placentas	Overweigh t placentas
Presence of Monocytes (r)	0.735**	0.372**	-0.272**	0.446**	-0.277**	
P - value	0.000	0.000	0.000	0.000 0.309**	0.000	0.274 0.003
Placental malaria (r)	1	0.276**	-0.275**		-0.243**	0.283
P – value	-	0.000	0.000		0.000	0.009

CONCLUSION

Findings from the present study reveals that about 76% of the study participants harbors high level of monocytes infiltrations with in the intervillous space of the placenta which positively correlate with the high prevalence of babies with low birthweight and underweight placentas.

Due to the high prevalence of malaria parasites and monocytes infiltration observed among the parturient in the study area, there is need to introduce and sustain mass literacy classes among not only pregnant women. This will help in mass enlighten of the different dangers posed by placental malaria and the different malignancies caused by the disease severity.

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