CHANGES IN PLACENTA AND CHORIOAMNIOTIC MEMBRANE HISTOARCHITECTURE IN PATIENTS WITH MALARIA IN PREGNANCY

A dissertation submitted in partial fulfillment of the requirements of Master of Science Degree in Anatomy, University of Nairobi

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DECLARATION

I hereby confirm that this dissertation is my original work and has not been presented elsewhere for review and approval:

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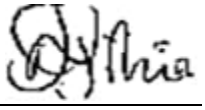
3. Sign:  Date: ___12th November, 2021___

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DEDICATION

With heartfelt regards, I dedicate this work:

To the Almighty God

For His mercies and grace that sustains me

To my Husband and my family

For the immense support, for believing in me, and for your unending prayers

ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

CAM	Chorioamniotic membrane
CSA	Chondroitin Sulphate A
CTB	Cytotrophoblast
CV	Chorionic villus
DNA	Deoxyribonucleic acid
EVT	Extra-villous trophoblast
FGR	Fetal growth restriction
HCG	Human Chorionic Gonadotrophin
LBW	Low birth weight
PCR	Polymerase chain reaction
RBCs	Red Blood Cells
STB	Syncitiotrophoblast
WHO	World Health Organization

LIST OF FIGURES

LIST OF TABLES

SUMMARY

Background: Malaria in pregnancy has adverse consequences for both fetal and maternal health. These outcomes result from improper trophoblast invasion concomitant with the reduced transformation of maternal spiral arteries due to inflammation and disturbances in maternal hormonal function. The events may further contribute to fetal growth restriction (FGR) by reducing the transport of maternal blood to the placenta. It is not fully understood how malaria in pregnancy alters the terminal villus and the chorioamniotic membrane and how these relate to pregnancy outcome.

Objective: To compare the changes in the placenta and the chorioamniotic membrane in patients with and without malaria in pregnancy

Materials and methods: One hundred and sixteen placentae were used for the study. The placenta from mothers who have had malaria in pregnancy and those from mothers without a history of malaria in pregnancy were collected using standard techniques. The placental tissue and the adjacent parts of the fetal membranes were obtained in an aseptic technique immediately after delivery and the specimen were labeled with a sequential study number. The tissues were macroscopically examined to determine any gross changes. Biopsies of both the placentas and the fetal membranes obtained were obtained immediately after delivery and fixed in 10% formalin for 24hrs. They were then prepared for paraffin wax embedding and stained with Hematoxylin and Eosin. Photomicrographs were taken using a Zeiss™ digital photomicroscope (Carl Zeiss AG Oborkochen, Germany) at ×400 magnification for morphometric analysis.

Data handling: Statistical analyses were performed using SPSS 23.0 (Version 23.0, Chicago, Illinois). Mean values, Standard Deviations, and frequency tables were generated from the data.

The independent-sample *t*-test was used to compare the mean values from the gross examinations by the placenta with malaria. Features of the placenta from the two groups were compared by a χ^2 test. A *P*-value of ≤ 0.05 was considered statistically significant

Results: The mean birth weights of the neonates delivered were 2663g ± 419 among the cases and 3198g ± 390 among the controls. The odds of having low birth weight neonates were significantly greater for those with malaria compared to those without malaria P=0.001, OR=7.602. The mean placental weights observed were 478.27g ± 40.95 among the cases and 511.55g ± 35.58 among the controls. The odds of having low placental weight was significantly higher for those with malaria compared to those without malaria P=0.001, OR=4.424. Placenta photomicrographs from women with malaria in pregnancy showed syncytial knotting, syncytial delamination, fibrin deposition and red blood cell agglutination and leukocyte infiltration. Morphometric analyses showed significantly higher counts of syncytial knot (p-value <0.001, CI: 1.549 - 4.596) and delamination (p-value 0.01, CI: 0.715 - 4.667), fibrin deposition (p-value 0.009, CI: 0.029 - 0.188), smaller intervillous space area (p-value 0.036, CI: -0.013- -0.362) and (p-value 0.004, CI: 0.846 - 3.98) in placenta from mothers with malaria in pregnancy compared to controls. Photomicrographs of the chorioamniotic membrane showed histological alterations. These included amniotic epithelial change to columnar and stratified types, epithelial delamination, fibrin deposition and leukocyte infiltration in women with malaria in pregnancy. Statistical analysis showed significant differences in epithelial type (p-value 0.001, x^2=17.9), epithelial denudation (p-value <0.001, x^2= 19.4) and fibrin deposition (p-value of 0.02 and x^2 =7.5).

Conclusion: This study gives more insight on the effect of malaria on the placental histoarchitecture. The findings provide a morphological basis for understanding adverse pregnancy outcomes in women who have had malaria in pregnancy. Measures should be put in

place aggressively to treat and hence reduce the severity of adverse feto-maternal outcomes of malaria infection in pregnant mothers.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

The malaria parasite *Plasmodium spp* has interacted with its invertebrate and vertebrate hosts since time immemorial (Ma et al., 2018). Malaria persists as the infectious parasitic disease with the highest mortality worldwide, with its burden being felt mostly in sub-Saharan Africa (WHO, 2016). Every year, an estimated 125 million pregnant women, comprised mostly of sub-Saharan Africans and Asians, are at risk of infection by malaria in pregnancy (Dellicour et al 2010).

Malaria has adverse consequences for maternal and fetal health during pregnancy. Increasing evidence suggests that malaria infection, especially in early pregnancy, enhances the risk of adverse pregnancy outcomes. Infection with Plasmodium *spp*. alters the trophoblastic invasion of uterine walls and reduces the transformation of maternal spiral arteries due to maternal hormonal and inflammatory disturbances that are induced (Unger et al., 2016). A combination of these factors contribute to FGR by significantly reducing the delivery of maternal blood to the placenta.

Malaria in pregnancy, defined as malaria infection in the placenta or maternal blood at any time during pregnancy is usually associated with a number of maternal-fetal complications, such as intra-uterine growth restriction (IUGR), anaemia and pregnancy loss (Chaikitgosiyakul et al., 2014). The normal structure of the placental villous tree changes majorly (stem, intermediate or terminal villi) during placental development due to hormonal influence (Huppertz, 2008).

There are very few studies to the best of our knowledge that show the impact malaria parasites have on the chorioamniotic membrane, the terminal villi and neonatal outcome correlates, this being important in understanding the mechanistic pathway of malaria in pregnancy. In this study we aimed to compare the differences between the structure of terminal villi and the chorioamniotic membrane in patients with and without malaria in pregnancy. The findings of this study are

important in shedding more light on pathological mechanisms that may be targeted towards reducing the severity of feto-maternal adverse outcomes resultant from malaria infection in pregnancy. This study aimed at describing how malaria in pregnancy alters the placental histoarchitecture which could influence the outcome of pregnancy.

1.2. LITERATURE REVIEW

Anatomy of the placenta

The human placenta at term is a circular discoid organ whose dimensions are about 22 cm diameter, 2.5 cm central thickness and weighs an average of 470 g (Huppertz, 2008). It originally develops from the trophoblastic cells but is later comprised of both maternal and fetal tissues. The chorion structure is mainly formed by fetal blood vessels that are embedded in stromal tissue and trophoblastic cells that are arranged as ramified structures termed chorionic villi (CV) (Ventura-Ferreira et al., 2018). The fetal part of the mature placenta is referred to as the chorionic plate while the maternal part of the placenta is called the basal plate. This chorionic plate carries blood vessels which are radial branches from the umbilical vessels. The major functional units of the placenta are highly branched and densely packed villous structures that contain fetal blood vessels. The majority of maternal-fetal exchange of nutrients and other substances occurs at the terminal regions of the villi (Benirschke et al., 2012; Gude et al., 2004).

Histologically, the trophoblast of the developing embryo consists of two distinct layers: cytotrophoblast (CTB) layer which is internally situated and is supported by a basal lamina and the syncytiotrophoblast (STB) layer forms the contact barrier with maternal blood in the maternal intervillous space. The CTB is the source of progenitor cells that later differentiate into STB, which is post-mitotic (Mori et al., 2007). The differentiation of STB cells from CTB cells is essential for placental growth and maintenance throughout pregnancy (Racca et al., 2015). CTBs further proliferate and form anchoring villi that interact with the uterine wall (Ji et al., 2013).

Huppertz (2008) described five types of chorionic villi; stem villi, mesenchymal villi, immature intermediate villi, mature intermediate villi, and terminal villi. Stem villi are the largest villi that

are distinguishable by a perivascular contractile system around their central vessels. Mesenchymal villi are the most primitive villi that sprout from the stem villi. They are characterized by an abundance of cells of mesenchymal origin and which show the syncytial type of sprouting. Immature intermediate villi develop from mesenchymal villi and are typified by stromal channels containing fetal macrophages termed Hofbauer cells. Mature intermediate villi, on the other hand, contain small vessels and capillaries which are organized in a loose stroma. Terminal villi portray sinusoids and capillaries with a thin vasculo-syncytial membrane. The maternal-placental and fetal-placental blood circulation has also been well elaborated (Wang and Zhao, 2010).

The syncytial layer has several functions. It controls the exchange of nutrients, gases and several other substances between maternal and fetal blood. It also acts as a barrier against the attack of the fetus by the maternal immune response. Besides, it is responsible for the production of hormones, growth factors, and proteins including human chorionic gonadotrophin (hCG) and pregnancy-specific glycoproteins (Ji et al., 2013).

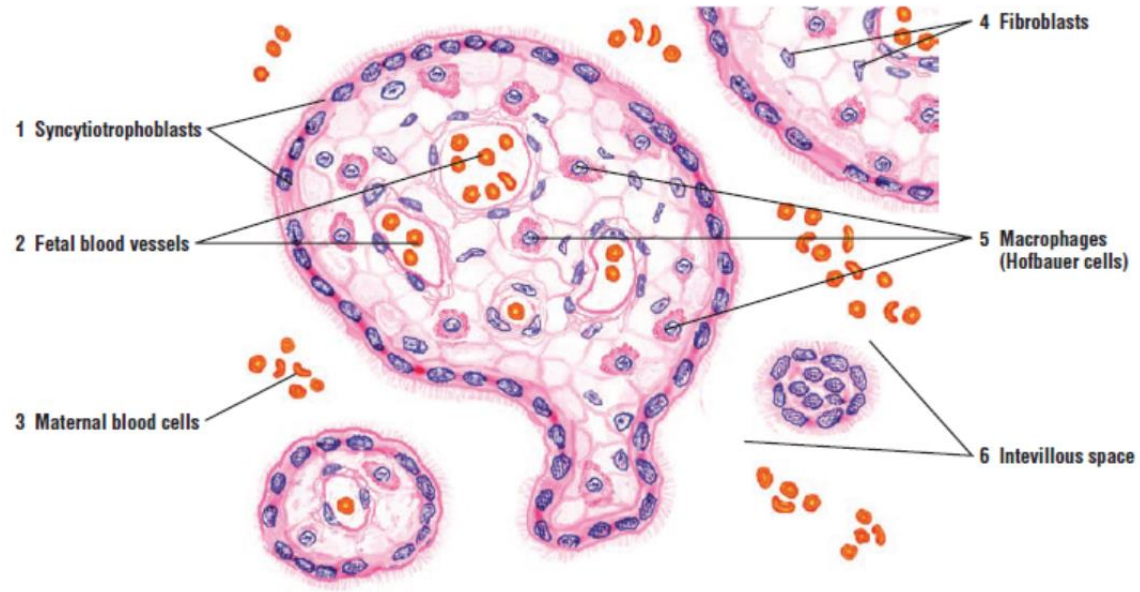


Figure 1: General structure of a chorionic villus (speechfoodie.com, n.d.)

Malaria and gravidity, birth weight and placental weight

Malaria is a life-threatening disease caused by intraerythrocytic protozoal parasite plasmodium that is transmitted to humans through vector female anopheles mosquito. Malaria is deleterious to both the mother and fetus. Pregnant women are at greater risk of malaria infection and of symptomatic malaria disease than non-pregnant adults (Rogerson et al., 2007). Studies have shown that the primigravid are more susceptible to malaria infection. This is because pregnant women develop immunity against malaria infection in a parity-dependent manner (Ataíde et al., 2014)

Studies have shown that newborns born with Low Birth Weights (LBW) by mothers who had malaria during pregnancy, usually had Intrauterine Growth Restriction (IUGR) and preterm births (Guyatt and Snow, 2004). High placental parasitemia has been associated with preterm births. Placental malaria could be associated with intervillous infiltration of monocytes and macrophages which could contain malaria pigment, hemozoin. Hemozoin is a parasite inorganic crystal metabolite released during schizogeny that is synthesized by plasmodium during heme detoxification process. High-density monocyte infiltrates are especially common in first pregnancy, and are associated with Low Birth Weight (LBW) (Jilly, 1969; Leopardi et al., 1996; Menendez et al., 2000a; Ordi et al., 1998; Rogerson et al., 2003). LBW was determined as birth weight below 2500g according to WHO guidelines. Low placental weight was determined as placental weight below the 95th percentile of the placental weight for both male and female neonates which was approximately 500g (Thompson et al., 2007)

Malaria and chorionic villous structure

Malaria caused by *Plasmodium falciparum* in pregnancy is an important preventable etiology of low birth weight (LBW) and neonatal mortality. It is largely as a result of fetal growth restriction

(FGR) (Umbers et al., 2013). The pathogenic mechanisms that underlie FGR in malaria are poorly defined, but it may involve placental insufficiency caused by poor development of the placenta (Desai et al., 2007). Boeuf et al (2013) cites impaired trans-placental amino acid transport as a key pathological mechanism that underlies FGR in malaria. The highest prevalence of *P. falciparum* infections in pregnancy occurs during the period between thirteen and eighteen weeks of gestation. This period corresponds to the development of placental circulation when extra-villous trophoblasts (EVT) invade the decidua and transform into spiral arteries to increase the blood supply of the placenta (Umbers et al., 2013).

Malaria infection, especially in early pregnancy, raises the risk of adverse outcomes of pregnancy by interfering with the capacity of trophoblasts to migrate and invade maternal decidua, an important determinant for the establishment of blood flow in the maternal placenta (Unger et al., 2016). According to Pereira et al., *P. falciparum*-infected Red Blood Cells (RBCs) in pregnancy preferentially bind Chondroitin Sulphate A (CSA) which results in sequestration of RBCs and antigen-antibody immune complexes in the placental intervillous spaces. This results in the deposition of RBCs, malarial pigments which eventually causes the placenta to turn black. The infected placenta also demonstrates fibrinoid deposits in the peri-villous space and Tenney-Parker changes (syncytial knots) (Ismail et al., 2000; Neres et al., 2008). Tenney-Parker changes are a major marker of maternal-vascular perfusion insufficiency and are characterized by an increase in syncitial knots (clustering of syncitial nuclei) and villous clustering. These changes may impair the transfer of nutrients from mother to fetus.

Malaria and chorioamniotic membrane

The chorioamniotic membrane (CAM) is a thin membrane that surrounds the developing fetus and forms the amniotic cavity. The CAM is a derivative of the fetal tissue and it is made up of two

layers: chorion (outer layer) and the amnion (inner layer). The chorion is a dense membrane that exists between the developing fetus and maternal tissue. The amnion is a lucid structure which provides nutrients to the amniotic membrane cells that secretes the amniotic fluid (Méhats et al., 2011). The CAM serves as the physical barrier between the fetus and the mother (Azagury et al., 2014).

Inflammation of the CAM termed as chorioamnionitis has been linked partly to ascending bacterial and fungal infection from the maternal genital tract (Czikk et al., 2011; Kim et al., 2015). Infectious agent from the maternal bloodstream such as HIV and other viruses have been implicated in chorioamnionitis but without consensus on their mechanisms (Ategeka et al., 2019; Baschat et al., 2003).

Acute chorioamnionitis is a well-recognized trigger of preterm delivery (Holzman et al., 2007; Mueller-Heubach et al., 1990). An emerging body of evidence has suggested that malaria infection, especially in early pregnancy, enhances the risk of preterm delivery but there remains no clear consensus on the mechanism (De Beaudrap et al., 2013; Elphinstone et al., 2019; Menendez et al., 2000b); (Abrams et al., 2004). Disruption of angiogenic, metabolic and inflammatory pathways are considered key causes of preterm birth (Elphinstone et al., 2019). Examination of CAM from women with malaria in pregnancy would provide some useful insight on the possible pathological mechanism of this infection in pregnancy and the related adverse pregnancy outcome.

1.3. JUSTIFICATION AND SIGNIFICANCE

JUSTIFICATION

Malaria, a leading cause of morbidity and mortality, is responsible globally for over one million deaths (WHO, 2016). In Kenya, 70% of the population is at risk of the disease with the greatest prevalence being in areas around Lake Victoria and the Coast (Ministry of Health, 2014, Kenya Malaria Indicator Survey, 2015). It is known that pregnant women and children under five years of age are most vulnerable to malaria (WHO, 2017). Malaria in pregnancy has been associated with adverse pregnancy outcomes including pregnancy loss and intrauterine fetal growth restriction (Umbers et al., 2013). These adverse pregnancy outcomes are partly attributable to alterations, by *Plasmodium spp*, in the placental histoarchitecture (Aagaard et al., 2014; Pelzer et al., 2017). Malaria parasites infect the placenta to induce inflammation, intervillous fibrin deposition and infarction (Eki-Udoko et al., 2021). There are very few studies that show the impact malaria parasites have on the chorioamniotic membrane and the terminal villi. Poor neonatal outcomes observed in malaria affected pregnancies correlated this being important to understand the mechanisms and placental changes that underlie the outcomes.

SIGNIFICANCE

The results of this study may help to explain the effect of malaria on the placental histoarchitecture hence further clarify the role malaria plays in adverse pregnancy outcomes. This may ultimately help develop therapeutic strategies towards reducing the severity of feto-maternal adverse outcomes resultant from malaria infection in pregnancy.

1.4 STUDY QUESTION, HYPOTHESIS AND OBJECTIVES

STUDY QUESTION

How does malaria in pregnancy alter the histoarchitecture of the placenta and the chorioamniotic membrane?

HYPOTHESIS

H_0 - Malaria in pregnancy does not alter placental and chorioamniotic membrane histoarchitecture

OBJECTIVES

Broad Objectives

To determine the alterations induced on placental structure by comparing patients with and without malaria in pregnancy

Specific Objectives:

To determine and compare in both patients who had malaria in pregnancy and those who did not

1. The clinical characteristics
2. Gross morphological features of the placenta
3. Light microscopic features of the terminal villi
4. Light microscopic features of the chorioamniotic membrane

CHAPTER 2: MATERIALS AND METHODS

2.1. Study design

The study was an unmatched case-control laboratory study.

2.2. Study population and setting

Placentas were collected during delivery from two groups of women, representing the control and experimental groups:

i. Women who had malaria during pregnancy
ii. Women who did not have malaria during pregnancy

The placenta was collected from the Maternity and Newborn Unit of the Jaramogi Oginga Odinga Referral and Teaching Hospital (JOOTRH). JOOTRH has been in existence for more than a century. It serves tmore than 10 counties in the western region of Kenya. Out of a bed capacity of 467 and the maternal and newborn unit has a bed capacity of 60 beds, twenty are in the maternity unit and 40 beds in the Newborn unit. Maternity and Newborn Unit has six (6) obstetric and gynecological consultants, four (4) medical officers, and four (4) midwives.

JOOTRH was selected preferentially due to its location in Kisumu; a malaria-endemic zone, where transmission is high throughout the year (Jenkins et al., 2015).

2.3. Materials

2.3.1 Sample size calculation

The sample size was calculated using the formula below recommended for unmatched case-control designs (Sullivan and Soe, 2009).

$$n_1 = \frac{\left(Z_{\alpha/2} + Z_{1-\beta}\right)^2 \bar{p}\bar{q}(r+1)}{r(p_1 - p_2)^2}$$

Key:

n_1= number of cases

n_2= number of controls

$z_{a/2}$ = standard normal deviation for a two-tailed test based on alpha level (relates to the confidence interval level)

z_p =standard normal deviation for a one-tailed test based on beta-level (relates to the power level)

r = ratio of controls to cases

p1 = proportion of cases with exposure and q1 = 1-p1

p2 = proportion of controls with exposure and q2 = 1-p2

Substituting into the equation with values from Walter et al., 1982 (in placentae collected from patients with malaria in pregnancy: the proportion of placentae with histological changes (0.42) and proportion of placentae without histological changes (0.24).

n_1 58 and n_2 = 58 and the total sample size n = 116

2.4. Methods

2.4.1 Selection criteria

Inclusion criteria

Placentas were only collected from women of confirmed African descent who are 18–35 years of age with a single live birth. The maternal ages above 35 years are associated with a significantly higher risk of gestational diabetes, hypertension, placenta previa among other obstetric complications associated with placental pathology(Fretts et al., 1995). Teenage pregnancy is associated with impaired placental development (Hayward et al., 2011).

Exclusion criteria

Women with concurrent obstetric complications or medical disorders including infections, cardiovascular disease, pre-eclampsia, diabetes, or malnutrition were excluded from the study.

3.4.2 Ethical consideration

Ethical approval for this study was obtained from the Kenyatta National Hospital (KNH)/University of Nairobi Ethics and Research Committee reference number KNH-ERC/A/297 and from Jaramogi Oginga Odinga Referral and Teaching Hospital Ethics and Review Committee, reference number ERC.IB/VOL.1/602. The study was conducted according to the guidelines provided by the committees.

After collection of specimens, measurements were taken with the organs intact and only small blocks were extracted for histology and finally, the tissue blocks and some of the tissue samples were stored in the bio-bank for future use.

2.4.3. Recruitment and informed consent

Qualified midwives identified participants who met inclusion criteria from the women who were admitted to labor and delivery units between April and June 2019. Jaramogi Oginga Odinga Referral and Teaching Hospital (JOORTH). A full explanation of the study was given to the participants by the principal investigator or trained research assistants and informed consent was obtained to enroll in the study

2.4.4. Samples collection procedure

Clinical data and placental samples were collected from JOORTH. The clinical data included maternal age, parity, gestational age and the mode of delivery. Placentas and parts of the fetal membranes attached to the chorionic plate were collected in an aseptic technique immediately after birth and designated a study sample number. Six biopsies (of the placentas and fetal membranes were obtained; two central biopsies from either side of the insertion of the umbilical cord and four from peripheral aspects of the placenta at twelve, three, six and nine o'clock positions. The specimens were fixed by immersion in 10% neutral buffered formalin for 24 hours.

2.4.5 Tissue processing for light microscopy

Tissue processing procedures were conducted at the Histology Section, Department of Human Anatomy, University of Nairobi. The membranes were removed from the placentas and made into rolls; subsequently, the rolls and placental biopsies were dehydrated in increasing concentrations of alcohol from 70% to 100% at an interval of 1 hour per concentration. The specimens were then dehydrated in ascending grades of ethyl alcohol, starting from 70% alcohol to absolute alcohol at one-hour intervals. The tissues were then placed in an alcohol toluene mixture (ratio 1:1), then cleared in toluene for two hours, followed by wax impregnation for 12 hours at 58°C. They were then embedded in paraffin wax and left to cool. Tissues were mounted on wooden blocks to

facilitate cutting into 7-micrometer thick sections on a microtome. The sections were floated on a warm water bath then picked on a clean glass slide. The slides were dried in an oven at 38℃ for 12 hours.

Hematoxylin and eosin

The dry sections were dipped in xylene to remove the paraffin wax for 5 minutes, followed by rehydration in descending grades of ethanol (100%, 95%, and 70%). After rehydration, the sections were dipped in a jar containing Ehrlich's Hematoxylin for 15 minutes then washed in running water for 45 minutes to remove excess stain. The sections were then stained in 1% Eosin solution for three minutes, followed by dehydration in ascending grades of ethanol from 70% to absolute alcohol. The sections were cleared in two changes of xylene before mounting.

2.4.6. Data analysis

Gross anatomical features, histological findings, and morphometry of placentas were compared among mothers who had malaria in pregnancy and those who did not have malaria in pregnancy. Numerical data were analyzed by SPSS, version 21.0. Mean values, Standard Deviations, and frequency tables were compiled using descriptive data. The independent-sample *t*-test was used to compare the mean values from the gross examinations by the placenta with malaria. Features of the placenta from the two groups were compared by a χ^2 test. A *P*-value of <0.05 was considered statistically significant

Gross examination

The macroscopic examination was performed to look for signs of tissue infarction or thrombosis, umbilical cord insertion site, placental shape, and membranes and color of the chorionic membrane. The weights of the placenta were also recorded.

Microscopic Evaluation

Five-micrometer serial sections were cut using a Leitz Wetzlar rotary microtome, floated in warm water, mounted on glass slides, and dried in a hot air oven at 40°C overnight. The sections were stained with Masson's trichrome and hematoxylin and eosin (H&E). H&E was used to demonstrate general histoarchitecture. Three blocks from each placenta were randomly selected for further processing through a simple random sampling technique. Slides were examined under a light microscope connected to a computer and monitor. The general structural organization of the terminal villus, the chorioamniotic membrane, the amount of fibrin deposition and syncytial knotting were noted.

Morphometric Analyses of Terminal Chorionic Villi

The villous structure of the placentas were further analyzed. Five placentas from the mother who had malaria in pregnancy and 5 placentas from the mother who did not have malaria in pregnancy were randomly selected with two blocks from each placenta. They were then subjected to morphometric analyses at 400×. Light microscopy slides were photographed using a Zeiss digital photomicroscope at various magnifications for analysis then downloaded on a computer using Scion Image Multiscan software (version 2.0 for windows) for analysis which enabled calculating the number of capillaries of the terminal villi (μm^2) and the Intervillous space.

Table 1: Evaluation method for morphometric analysis of the placenta

Pathological features	Evaluation methods	Staining
Syncytial knotting	Number of affected villi per field of view at 100x magnification	Heamatoxylin and eosin (H/E)
Syncytial delamination	Number of affected villi per field of view at 100x magnification	H/E
Fibrin deposition	Number of intersection points on a random grid that touched the areas with fibrin deposition multiplied by area of points set at 0.05mm^2	H/E
RBC agglutination	Number of intersection points on a random grid that touched the areas with RBC agglutination multiplied by area of points set at 0.05mm^2	H/E
Intervillous leukocytes	Number of Intervillous leukocytes per field of view at 1000x magnification	H.E
Terminal villus capillaries	Number of blood vessels per intermediate/ terminal villus per field of view at 100x magnification	H/E
Intervillous space	Number of intersection points on a random grid that touched the intervillous space multiplied by area of points set at 0.05mm^2	H/E

CHAPTER 3: RESULTS

A total of one hundred and sixteen (116) placentae were used for the study, 58 cases and 58 controls were collected and analyzed in the study. The ages of the participants ranged from 18 to 35 years. The gravidity ranged from primigravida to gravida 7. Most participants being primigravid (35/116, 30%) followed by gravida 3 (24/116, 21%), gravida 4 (21/116, 18%), gravida 2 (18/116, 16%), gravida 5 (11/116, 9%), gravida 7 (5/116, 4%) and lastly gravida 6 (2/116, 2%) [Fig. 2]. Of the 116 participants, 91/116 (78.4%) delivered vaginally while 25/116 (21.6%) delivered via cesarean section. Gestational age at delivery ranged from 38 weeks to 42 weeks based on the date of the last normal menstrual period [Fig. 3].

The mean birth weights of the neonates delivered were 2663 ± 419 g among the cases and 3198 ± 390 g among the controls. The odds of having low birth weight neonates was significantly greater for those with malaria compared to those without malaria P=0.001, OR=7.602. The mean placental weights observed were 478.27 ± 40.95 g among the cases and 511.55 ± 35.58 g among the controls. The odds of having low placental weight was significantly higher for those with malaria compared to those without malaria P=0.001, OR=4.424.

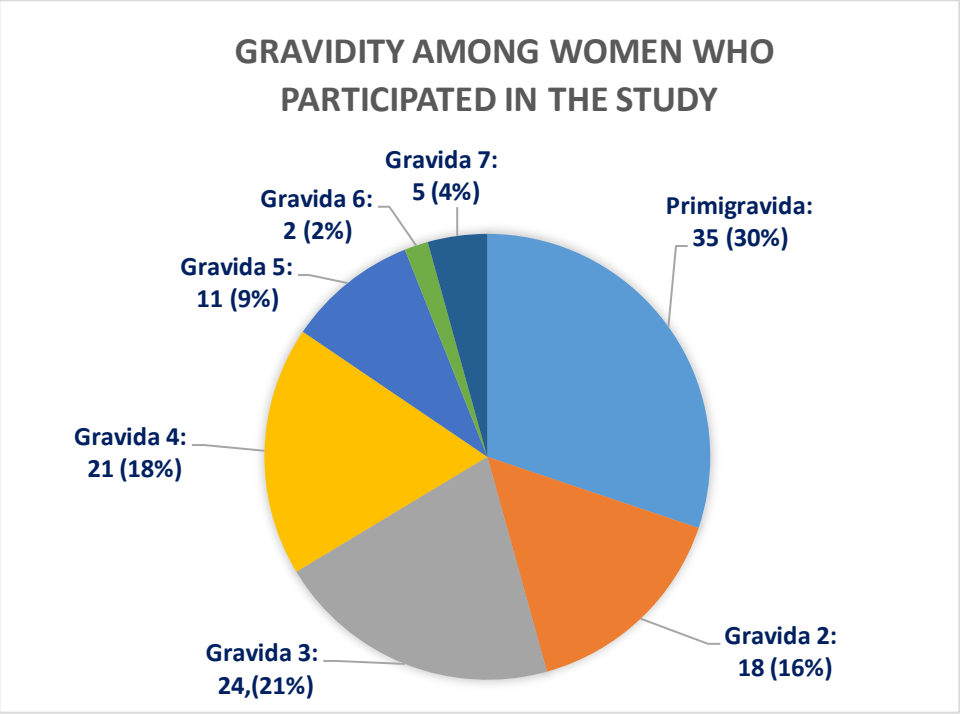


Figure 2: A pie chart showing distribution of patients based on gravidity

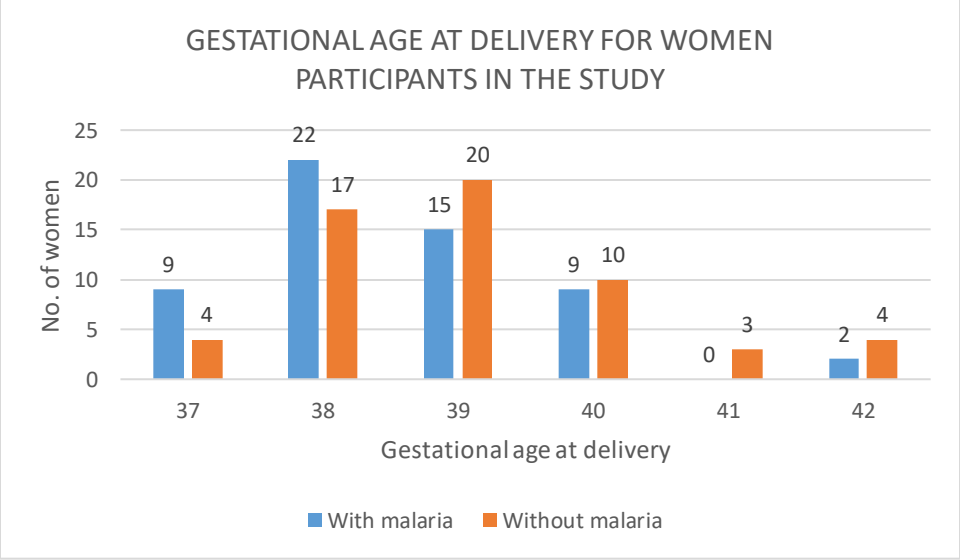


Figure 3:Gestational age at delivery for women participants in the study

Table 2: Comparison of Placental and Birth weights between cases and controls

	Serial Number	Mean	Std. Deviation	P-value
Birth Weight	**cases**	2663.7931	419.14628	**<0.001 (OR=7.602) ***
	controls	3198.2759	390.45245	
Placental Weight	**cases**	478.2759	40.95945	**<0.001 (OR = 4.424) ***
	controls	511.5517	35.58049	

Placental morphology

All the placenta that were examined had a discoid shape. Eccentric [Figure 4A], central [Figure 4B], marginal [Figure 4C] and velamentous [Figure 4D] types of cord insertions were observed, with the most prevalent types being central and eccentric insertions in both cases and controls [Figure 4]. Areas of white infarct and meconium staining were noted in some of the placenta from the women with malaria in pregnancy [Figure 4F]. There was no significant difference in cord insertion type between the two groups. [Fig. 5]

The color of the chorionic membrane was noted to be predominantly shiny maroon in both case and control groups (46/116; 79% and 42/116; 72% respectively) [Fig. 6]. There was no significant difference in color of the chorionic membrane between the two groups.

FIGURE LEGEND

Figure 4 A-F: Placental morphology and cord insertion types among participants in the study. Figures 4A-B are from women without malaria in pregnancy while 3C-F are from women with malaria in pregnancy.

Fig 4A: Macrograph of the placenta showing fetal membranes (white arrows). The umbilical cord has an eccentric type of insertion (EC).

Fig 4B: Photograph of the placenta showing central type of cord insertion (CC).

Fig 4C: Photograph of the placenta showing marginal type of cord insertion. Some white areas of infarct

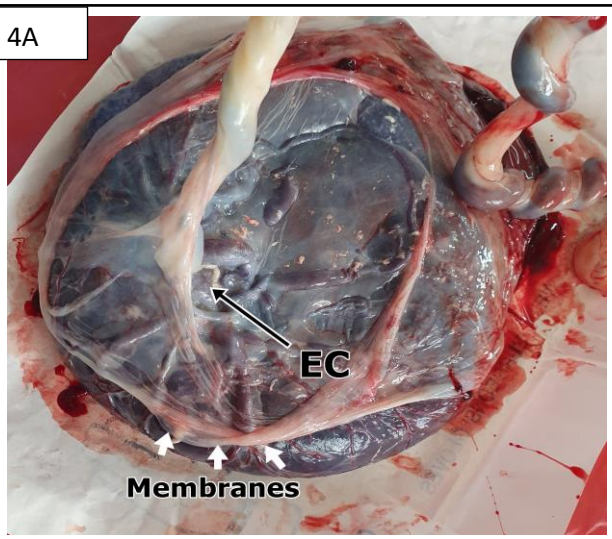
Fig 4D: Photograph of the placenta showing velamentous type of insertion (VC). Notice the chorionic vessels branching before reaching the placental surface.

Fig 4E: Photograph of the placenta showing Marginal type of cord insertion (MC).

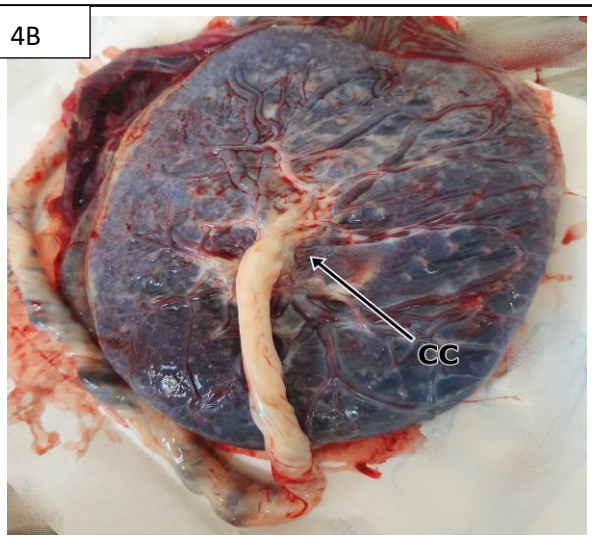
Fig 4F: Photograph of the placenta showing meconium staining of placental membranes with an eccentric type of cord insertion (EC). Notice the areas of white infarct (arrows).

FIGURES

4A



4B



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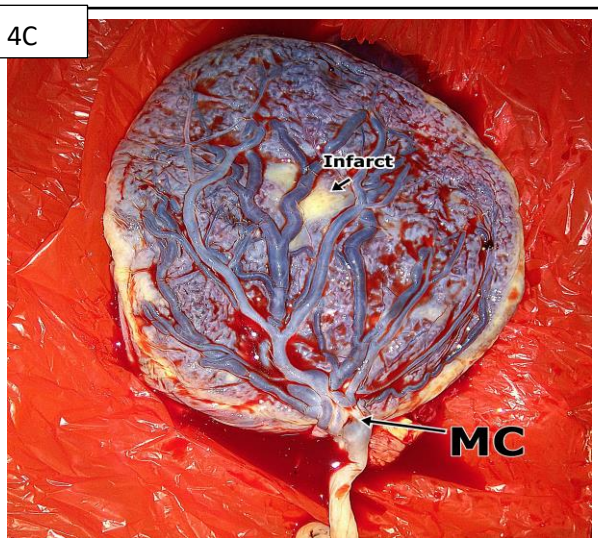

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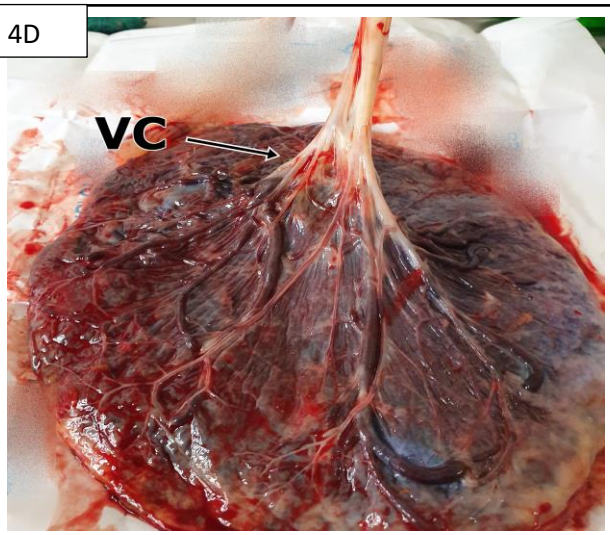

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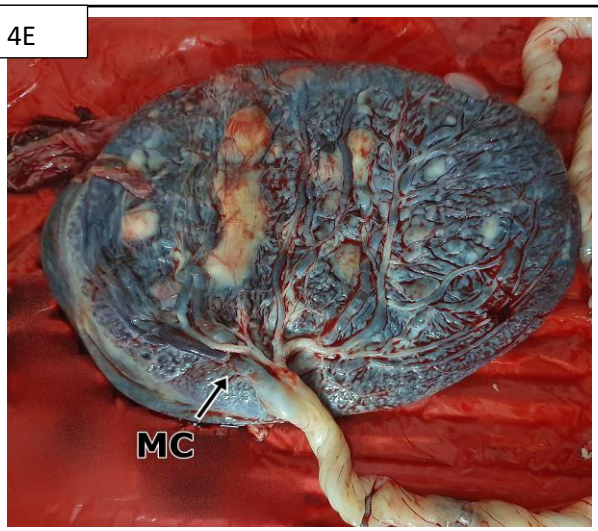

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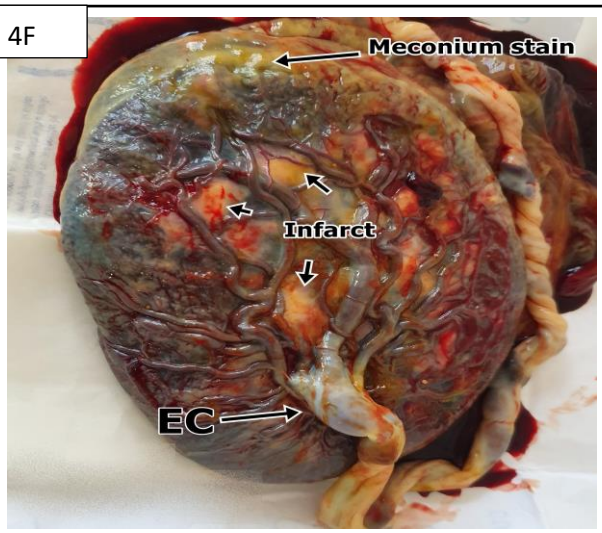


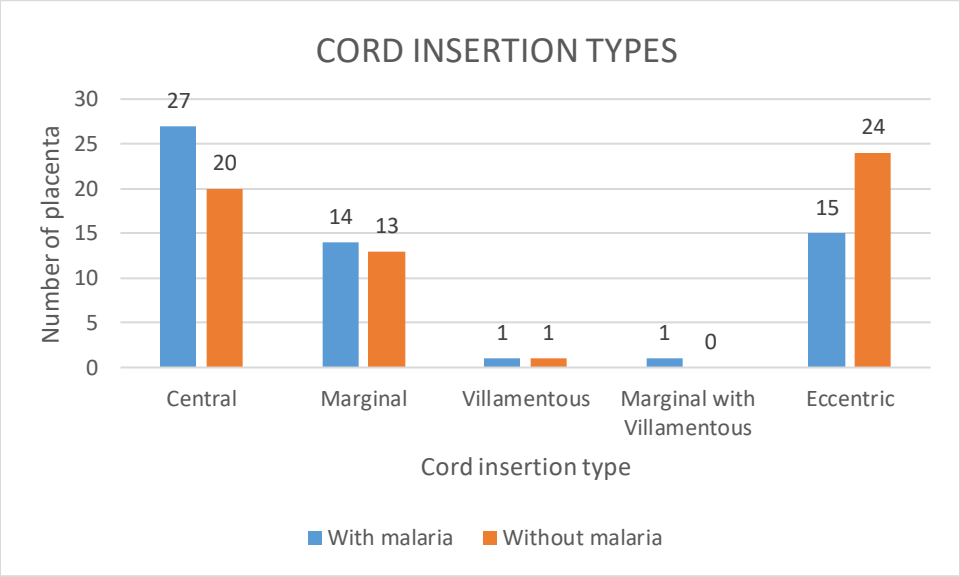


Figure 5:Cord insertion type among placenta from women participants in the study

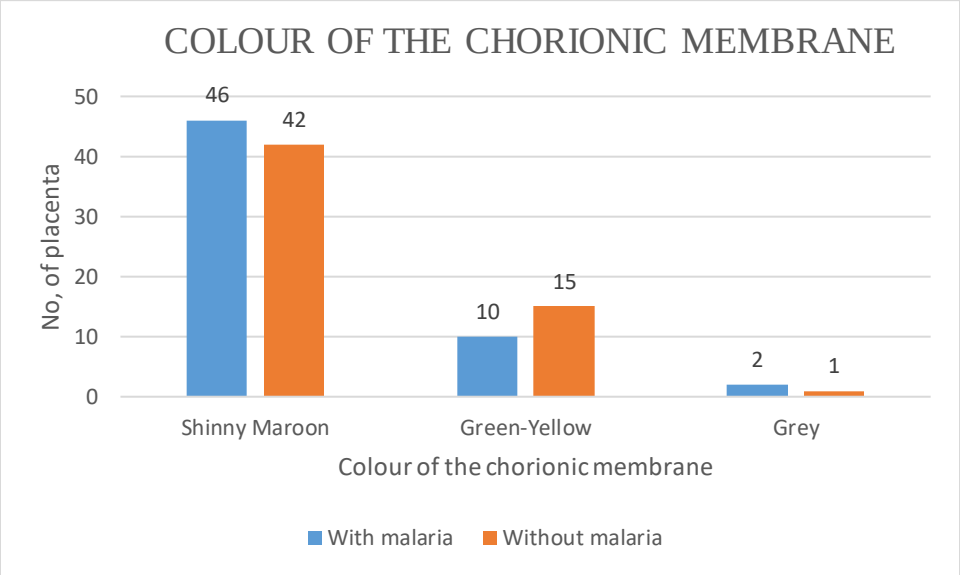


Figure 6: Colour of the chorionic membranes from women participants in the study

Placental histology

The microscopic features of the placenta were observed at both low and high-power magnification to determine the general histomorphology and detailed features [Figure 7]. At x40 magnification, the outlines of the basal part of the placenta [Figures 7A] and fetal chorionic plate [Figures 7C] of the placenta were noted. The distinction between stem villi and floating villi was also noted [Figures 7E]. Larger deposits of intervillous fibrin were noticeable at low magnification among the cases.

At x100 and x400 magnification, more detail of the trophoblastic shell and villi at the maternal part of the placenta [Figure 7B], and the chorionic plate [Figure 7D] could be seen. Intermediate and terminal villi could be noted by the presence of small arterioles and capillaries [Figure 7F]. The basal and chorionic plate morphology in placenta from women with malaria [Figure 8] did not show any different features from that in the placenta from women without malaria. The floating villi at low magnification showed areas of fibrin deposition [Figure 9A], which could be clearly seen at higher magnification [Figure 9B]. Larger areas of red blood cell agglutination and inter- and intra-villous fibrin deposition could be spotted in placenta from the women with malaria in pregnancy in comparison with the controls [Figure 9C]. The continuity of syncytiotrophoblast around the villi was observed. Syncytial knots [Figure 9D] and delamination [Figure 9E] were frequently observed in the cases compared to the controls.

At x1000 magnification, we examined the intervillous space and the characteristics of the blood cells present [Figure 10A]. Among the cases, malarial trophozoites could be observed within the red blood cells [Figure 10B]. Different degrees and types of leukocyte infiltration in the intervillous space could be seen especially in the placenta from the cases, ranging from few [Figure

10C] to moderate [Figure 10E]. There were more leukocyte infiltrates in the cases compared to controls. These included granulocytes and monocytes [Figure 10D].

Morphometric analysis of different parameters within the placental photomicrographs revealed statistically significant differences in syncytial knot and delaminated villi counts, fibrin deposits, intervillous space area and vascular profile between the case and control groups. There were significantly higher counts of syncytial knot (p-value <0.001, CI: 1.549 - 4.596) and delamination (p-value 0.01, CI: 0.715 - 4.667) in the cases compared to controls. Fibrin deposits covered a significantly larger area in the cases than in the controls (p-value 0.009, CI: 0.029 - 0.188) while the intervillous space area was noted to be smaller in the cases compared to controls (p-value 0.036, CI:-0.013- -0.362). There were significantly more vessels in the intermediate and terminal villi from the case placenta compared to the controls (p-value 0.004, CI: 0.846 - 3.98). The mean number of vessels per villi were 5.85 ± 2.10 and 3.43 ± 0.45 among the cases and controls respectively. There was no statistically significant difference in intervillous red blood cell agglutination and leukocyte counts between the two groups [Table 3].

FIGURE LEGEND

Figure 7 A-F: Microscopic features of the placenta from the women without malaria (control) group

Fig 7A: Photomicrograph showing the basal part of the placenta at x40 magnification with the decidua and trophoblastic shell. Areas of fibrin deposition are visible (F). H&E x40 magnification.

Fig 7B: Photomicrograph showing the trophoblastic shell and villi at the maternal part of the placenta at higher magnification. H&E x100 magnification.

Fig 7C: Photomicrograph showing the fetal chorionic plate at low magnification. Notice the large stem villus (Sv) arising from the chorionic plate. H&E x40 magnification.

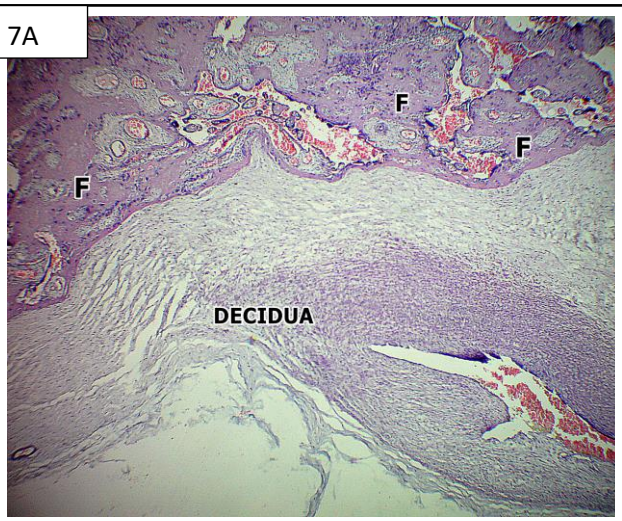
Fig 7D: Photomicrograph showing the chorionic plate at higher magnification. Sv – Stem villus, Fv – Floating villus. H&E x100 magnification.

Fig 7E: Photomicrograph showing the floating villi (Fv) of the placenta at low magnification. H&E x40 magnification.

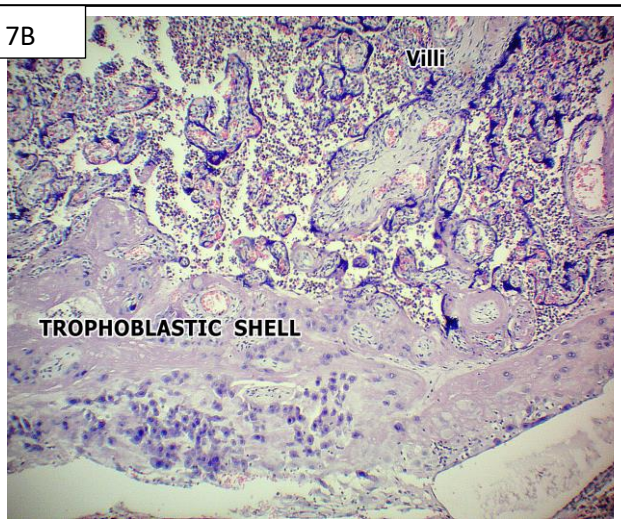
Fig 7F: Photomicrograph showing the floating villi of the placenta at a higher magnification. Iv – Intermediate villi, Tv – terminal villi. H&E x100 magnification.

FIGURES

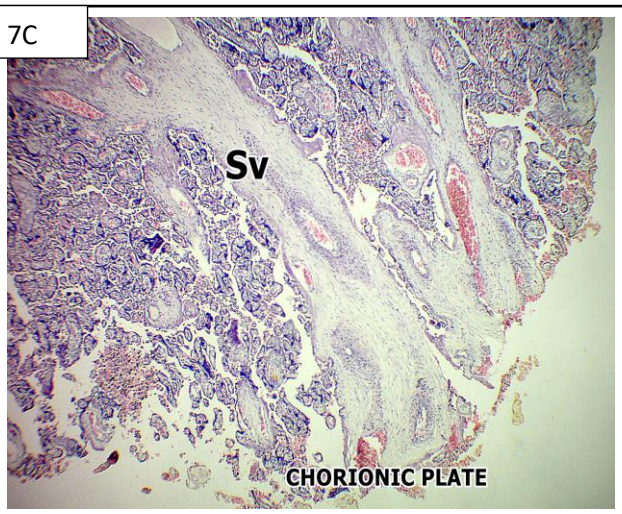
7A



7B



7C



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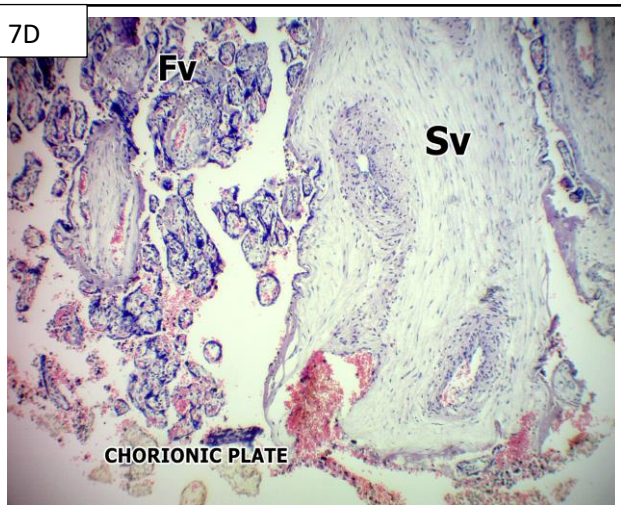

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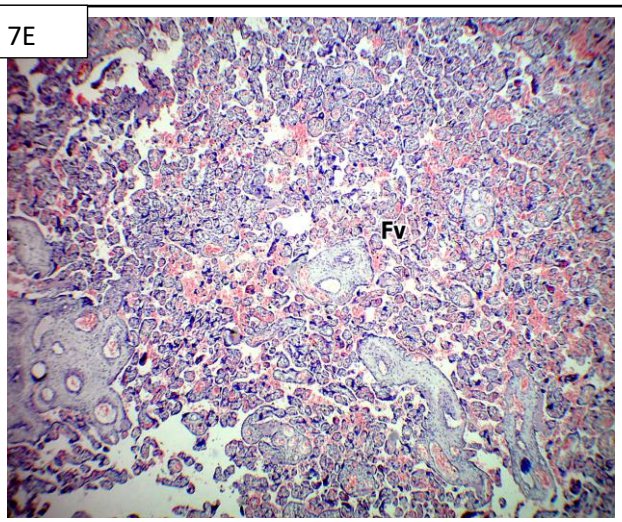

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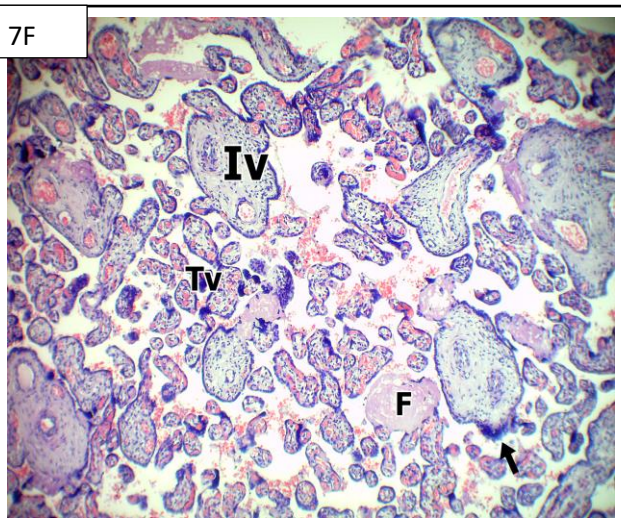


FIGURE LEGEND

Figure 8 A-E: Basal and chorionic plate morphology in placenta from women with malaria (cases).

Fig 8A: Photomicrograph showing the basal plate of the placenta at a low magnification. Fv – Floating villi. H&E x40 magnification.

Fig 8B: Photomicrograph showing the basal plate of the placenta at a higher magnification. Notice the large areas of fibrin deposition (F). Fv – Floating villi. H&E x100 magnification.

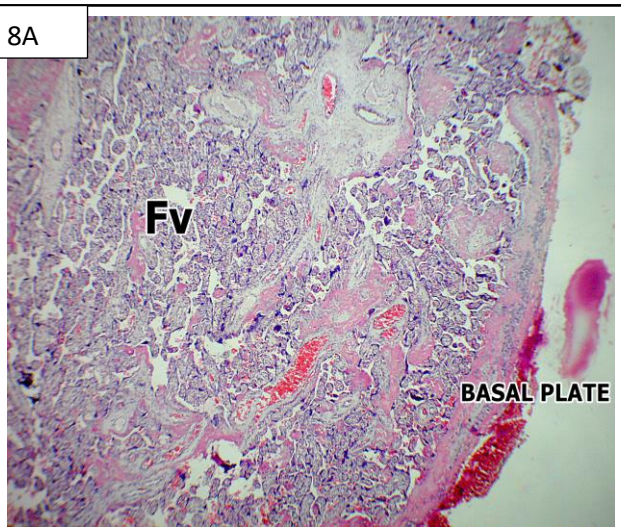
Fig 8C: Photomicrograph showing the trophoblastic cells (arrowheads) within basal connective tissue at very high magnification. H&E x1000 magnification.

Fig 8D: Photomicrograph showing the chorionic plate of the placenta at low magnification. A large stem villus can be noted arising from the chorionic late. H&E x 40 magnification.

Fig 8E: Photomicrograph of chorionic plate of the placenta at higher magnification revealing the Amnion and chorion layers and a stem villus (Sv). Areas of fibrin deposition are visible. H&E x100 magnification.

FIGURES

8A



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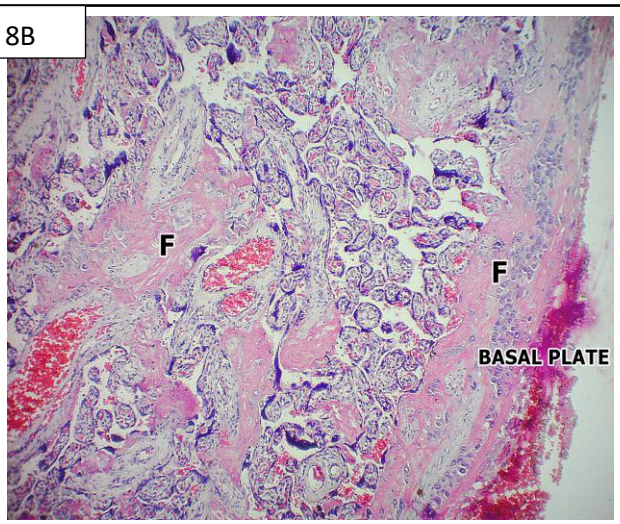

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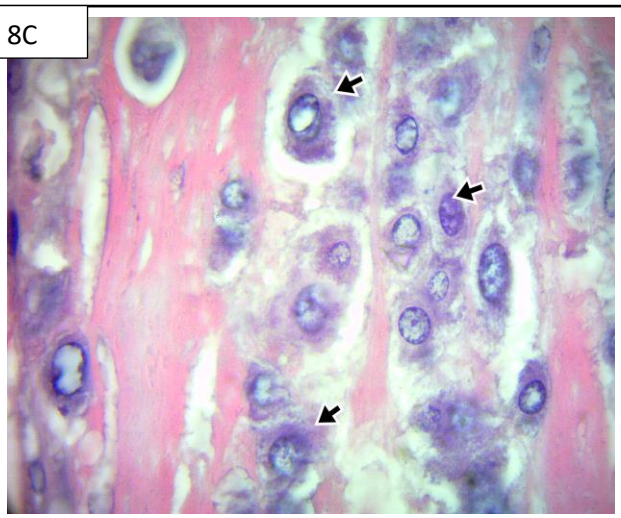


8D

8E

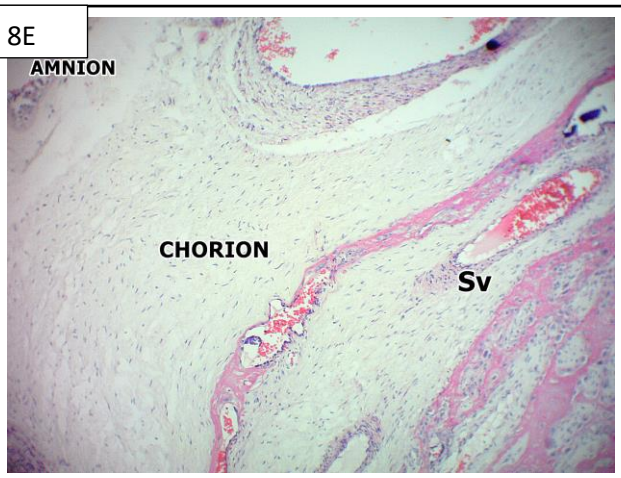


FIGURE LEGEND

Figure 9 A-E: Microscopic features of the floating villi in placenta from the women with malaria (cases)

Fig 9A: Photomicrograph showing the structure of the floating villi at low magnification. Notice the areas of fibrin deposition (F). Fv – floating villi, Sv – stem villus. H&E x40 magnification.

Fig 9B: Photomicrograph showing the floating villi at higher magnification with the intervillous fibrin deposition (F) clearly visible. Iv – Intermediate villi. H&E x100 magnification.

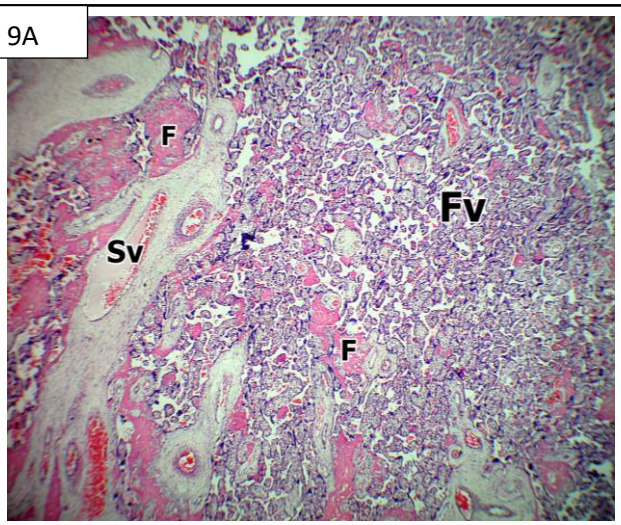
Fig 9C: Photomicrograph showing an intermediate villus (Iv) with areas of intravillous fibrin deposition (arrows). Red blood cell agglutination (Ag) is visible in the intervillous space. Tv – terminal villus, F – Fibrin. H&E x100 magnification.

Fig 9D: Photomicrograph showing the structure of a syncytial knot at very high magnification. Syncitiotrophoblast cells proliferate to form a tuft of cells. Tv – Terminal villus. H&E x1000 magnification.

Fig 9E: Photomicrograph showing an area of syncytial delamination at very high magnification. Arrows- White blood cells. H&E x1000 magnification.

FIGURES

9A



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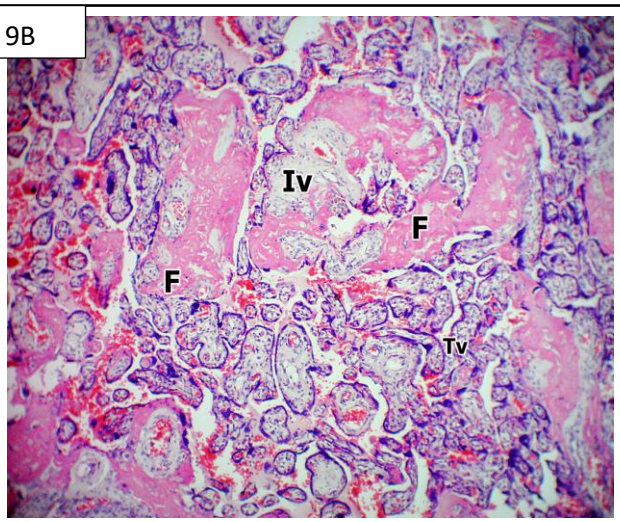

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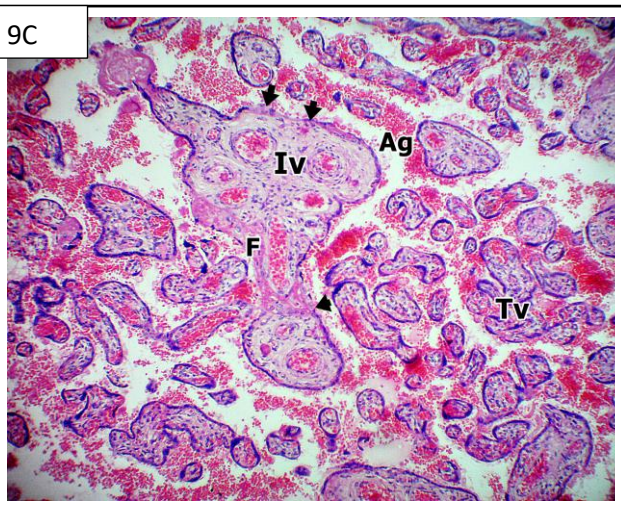

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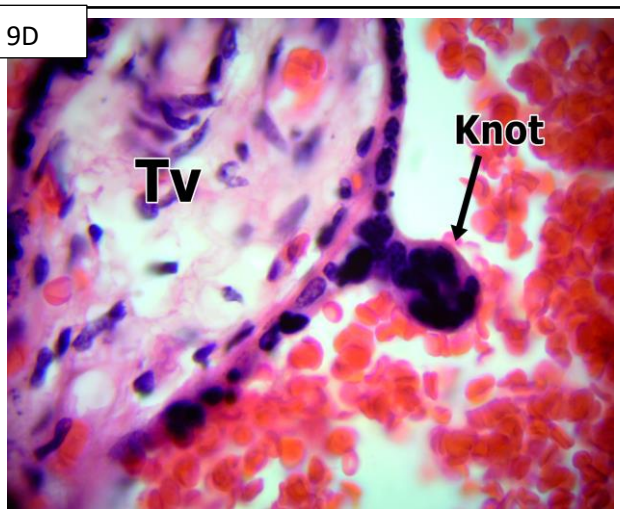

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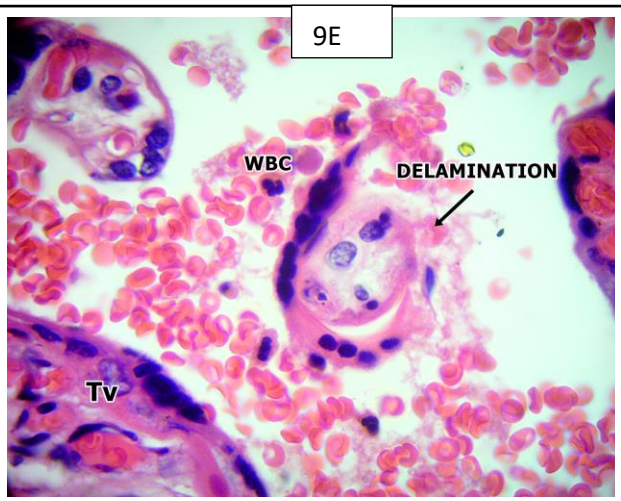


FIGURE LEGEND

Figure 10 A-E: Microscopic features of the intervillous space in placenta from the women with malaria (cases)

Fig 10A: Photomicrograph showing the intervillous space of placenta from women without malaria (control) group for comparison. White blood cells are generally absent. Tv – Terminal villus. H&E x1000 magnification.

Fig 10B: Photomicrograph showing the the intervillous space in placenta from the women with malaria (cases) group**.** A ring stage trophozoite of plasmodium falciparum is visible (arrrow). Ag – Agglutinated red blood cells, Tv – Terminal villus. H&E x1000 magnification.

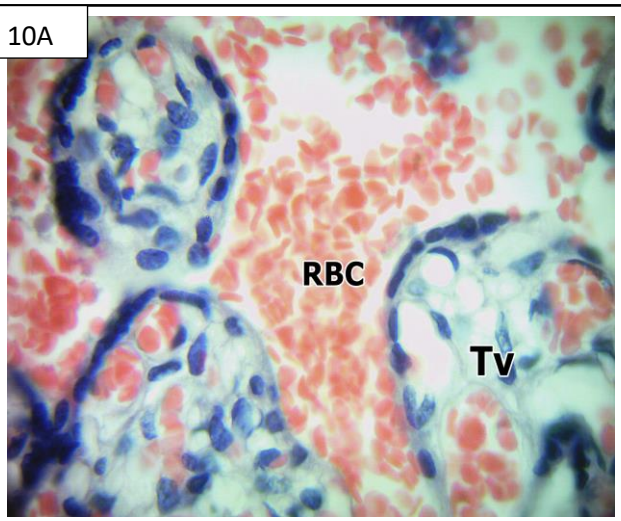
Fig 10C: Photomicrograph showing little infiltration of white blood cells (arrows) in the intervillous space of placenta from the cases. Ag – Agglutinated red blood cells. H&E x1000 magnification.

Fig 10D: Photomicrograph showing Photomicrograph showing a large infiltration of white blood cells (arrows) in the intervillous space of placenta from the cases. Ag – Agglutinated red blood cells, Mc - Monocyte. H&E x1000 magnification.

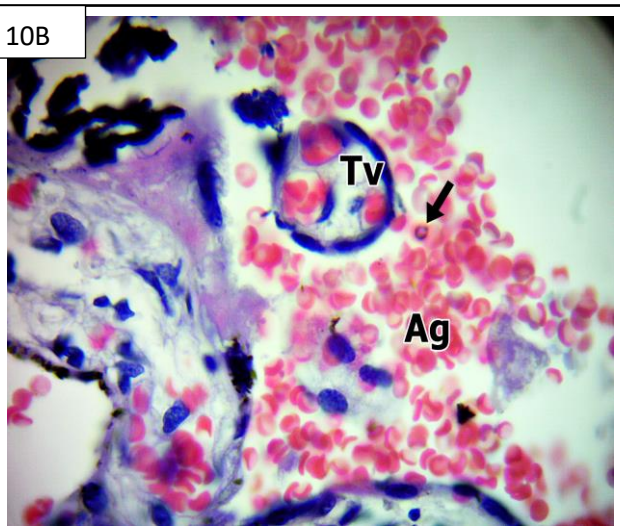
Fig 10E: Photomicrograph showing intervillous white blood cells infiltration (Arrows) in placenta from the case group. An area of extra-villous fibrin deposition can be seen (F). Ag – Agglutinated red blood cells. H&E x1000 magnification.

FIGURES

10A



10B



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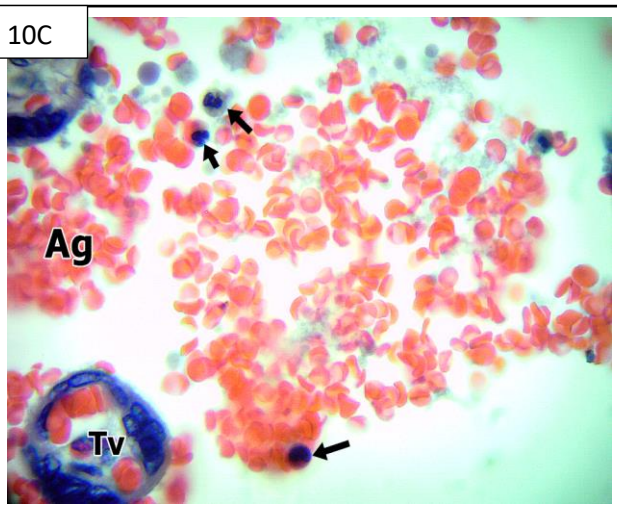

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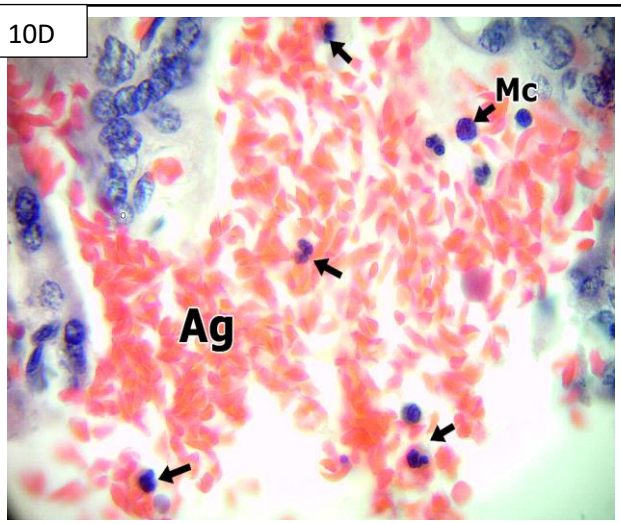

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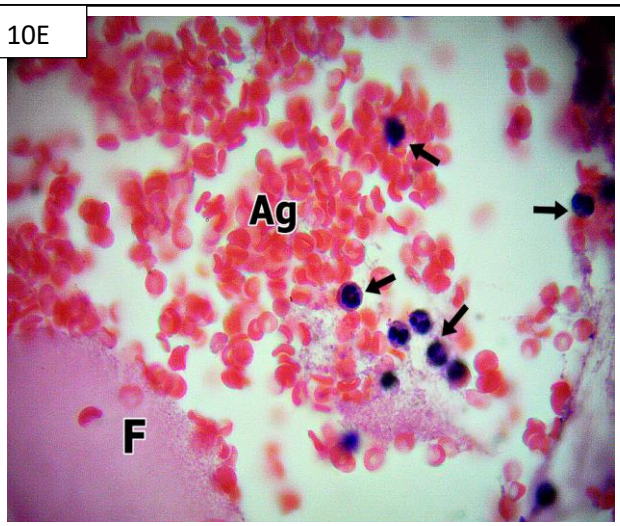


Table 3: Comparison of placental morphometric parameters between cases and control

	Case/control	Mean	Std. Deviation	P-value (CI)
Syncytial knots(number/field)	**Case**	6.4474	2.00510	**<0.001 (1.549 - 4.596) ***
	control	3.3750	.81196	
Delaminated villi(number/field)	**Case**	5.1158	2.46785	**0.01 (0.715 - 4.667) ***
	control	2.4250	1.68587	
Fibrin deposition (area in mm^2)	**Case**	.1842	.10194	**0.009 (0.029 - 0.188) ***
	control	.0750	.05657	
Intervillous red blood cell agglutination (area in mm^2)	**Case**	.1200	.117R90	0.206 (-0.032 - 0.145)
	control	.0638	.04373	
Intervillous space area (mm^2)	**Case**	.4774	.22313	**0.036 (-0.013- -0.362) ***
	control	.6650	.12762	
Vascular profile per villus per field (number/field)	**Case**	5.8505	2.10856	**0.004 (0.846 - 3.98) ***
	control	3.4375	.45962	
Intervillous leukocyte count per field (number/field)	**Case**	2.1526	1.96702	0.742 (-1.82 - 2.533)
	control	1.8000	3.54965	

Chorioamniotic membrane histology

The chorioamniotic membrane was observed at both low and high power magnification for both the cases and controls. At x40 magnification, the chorioamniotic membrane had the three layers; amnion, chorion and decidua [Figure 11A]. At higher magnification, the controls in most cases were observed to have a low cuboidal epithelium [Figure 11B&C] or simple squamous epithelium [Figure 11D].

The cases were also examined and the different types of epithelium were noted [Figure 12]. At X40 and X100 magnification for the cases, the amnion was observed to have cuboidal [Figure 12A], pseudostratified columnar epithelium [Figure 12B], columnar [Figure 12C], or squamous epithelium [Figure 12D]. The most common were pseudostratified and columnar epithelial types. Eosinophilic basement membrane with fibrin deposition was seen in some cases [Figure 13A & B]. Epithelial denudation was also observed in the majority of the cases [Figure 13C & D]. At X1000 magnification, the chorion was observed to have several reticular fibers and white blood cell infiltration [Figure 13E & F].

The different parameters within the CAM showed statistically significant differences in the type of epithelium (p-value 0.001, x^2=17.9) [Table 4] and epithelial denudation (p-value <0.001, x^2= 19.4) [Table 5]. The fibrin deposition also showed significant difference with a p-value of 0.02 and x^2 =7.5 [Table 6]. There was no statistically significant difference in leukocyte counts between the two groups [Table 7].

FIGURE LEGEND

Figure 11 A-D: Microscopic features of the chorioamniotic membrane in placenta from women without malaria (controls)

Fig 11A: Photomicrograph of the chorioamniotic membrane at low magnification to reveal the three layers; Amnion (AM), Chorion (CH) and decidua (D). H&E x40 magnification.

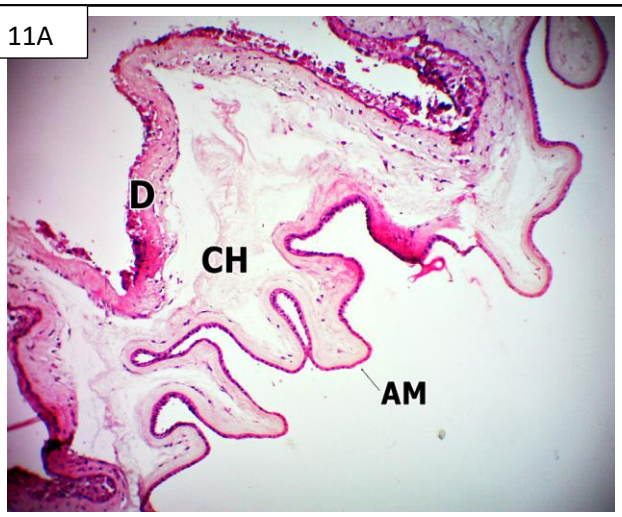
Fig 11B: Photomicrograph showing the low cuboidal epithelium at higher magnification as seen in some control placenta. AM – Amnion, CH – chorion. H&E x100 magnification.

Fig 11C: The cuboidal eithelium at high magnification. AM- Amnion. H&E x400 magnification.

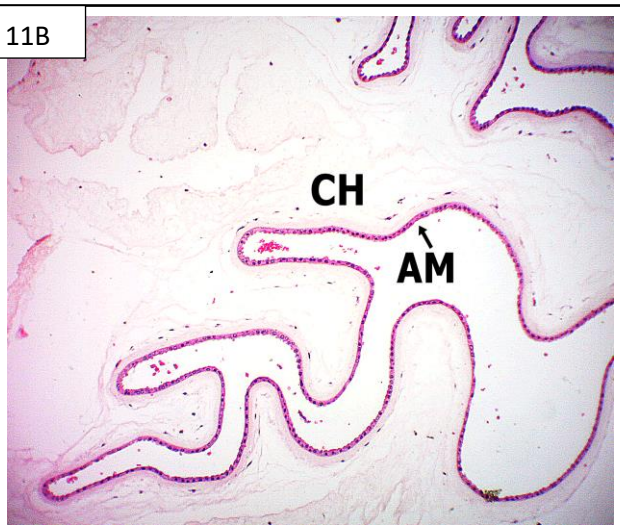
Fig 11D: Photomicrograph showing the squamous type of epithelium seen in some control placenta. AM- Amnion. H&E x400 magnification.

FIGURES

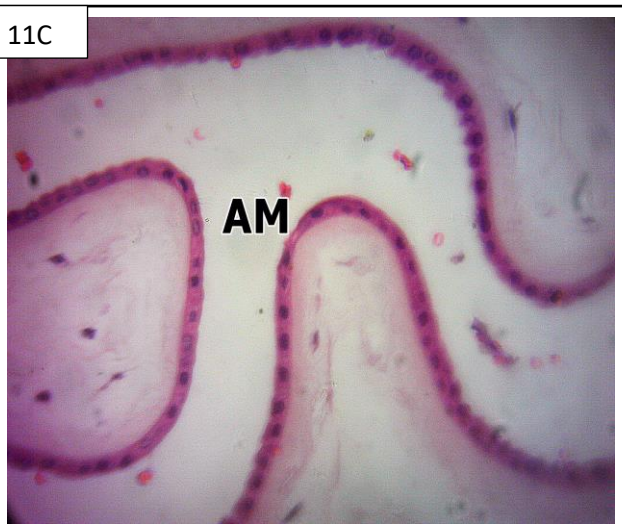
11A



11B



11C



11D

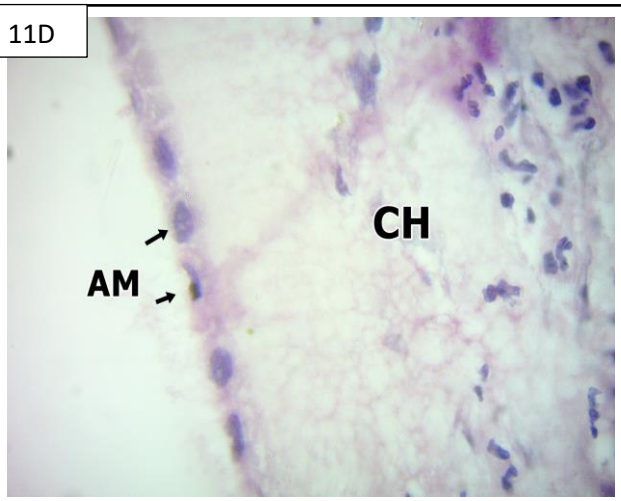


FIGURE LEGEND

Figure 12 A-D: Microscopic features of the amniotic epithelial types in placenta from women with malaria (cases)

Fig 12A: Photomicrograph showing the chorioamniotic membrane with both cuboidal and columnar types of amniotic epithelium. Asterisks -cuboidal epithelium, Arrows – columnar epithelium. H&E x100 magnification.

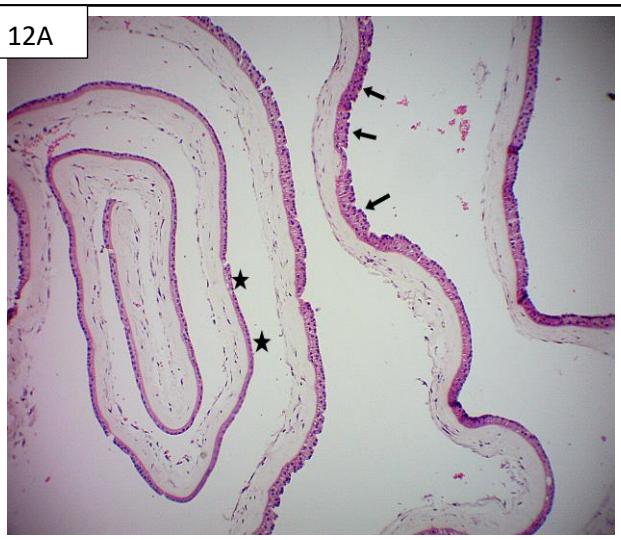
Fig 12B: Photomicrograph showing the stratified type of amniotic epithelium (arrows) as seen in some of the cases. H&E x400 magnification.

Fig 12C: Photomicrograph showing columnar type of amniotic epithelium (arrows) observed in some of the cases. H&E x400 magnification.

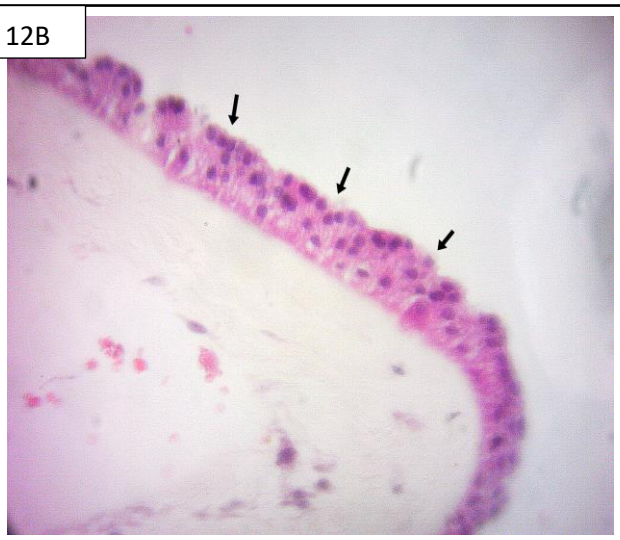
Fig 12D: Photomicrograph showing squamous type of amniotic epithelium (arrows) observed in some of the cases. H&E x400 magnification.

FIGURES

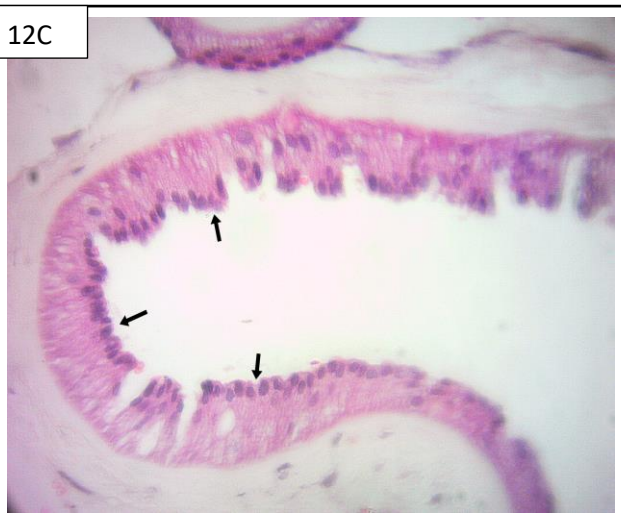
12A



12B



12C



12D

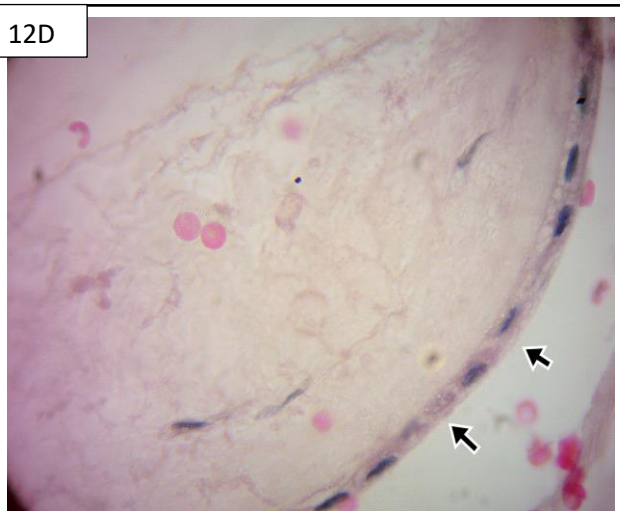


FIGURE LEGEND

Figure 13 A-F: Microscopic features of the chorioamniotic membrane in placenta from women with malaria (cases)

Fig 13A: Photomicrograph showing areas of fibrin deposition in the amniotic membrane beneath the epithelium (Arrows). CH – chorion. H&E x100 magnification.

Fig 13B: Photomicrograph showing fibrin deposition in the sub-epithelium at higher magnification. H&E x400 magnification.

Fig 13C: Photomicrograph showing areas of amniotic epithelial denudation (Arrows) at low magnificaction. AM – Amnion, CH – chorion. H&E x100 magnification.

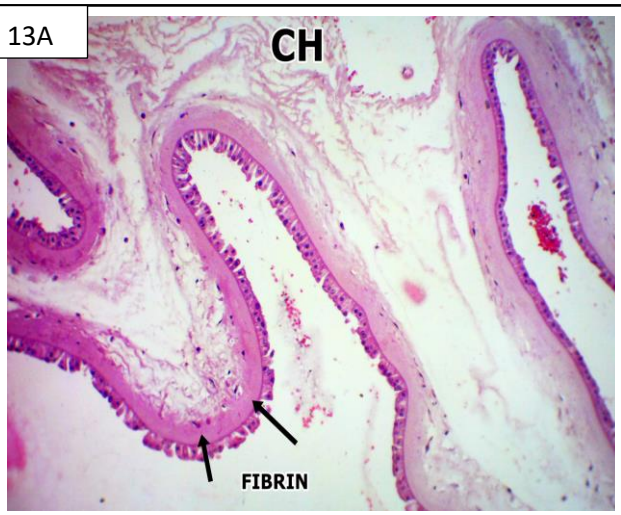
Fig 13D: Photomicrograph showing amniotic epithelial denudation (Arrow) at higher magnification. H&E x400 magnification.

Fig 13E: Photomicrograph showing leukocyte infiltration in the amnotic compact layer in the sub-epithelium. H&E x400 magnification.

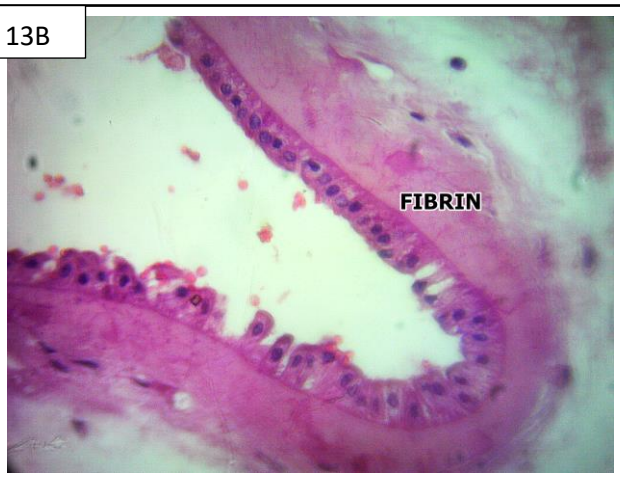
Fig 13F: Photomicrograph showing leukocyte infiltration in the chorion layer of the chorioamniotic membrane (arrow). WBCs – White blood cell, RBCs – Red blood cells. H&E x400 magnification

FIGURES

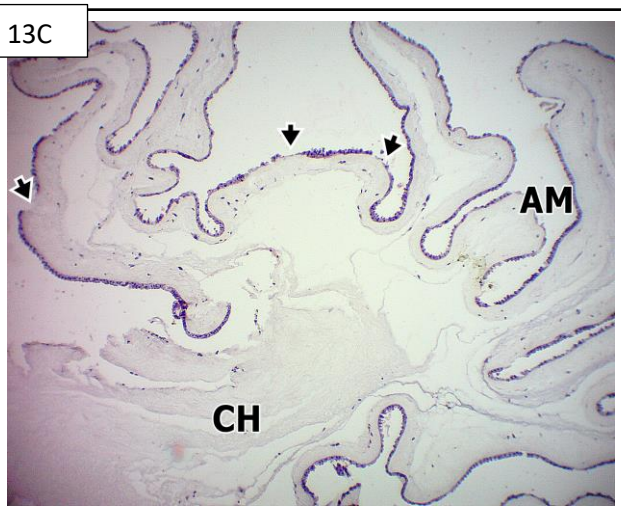
13A



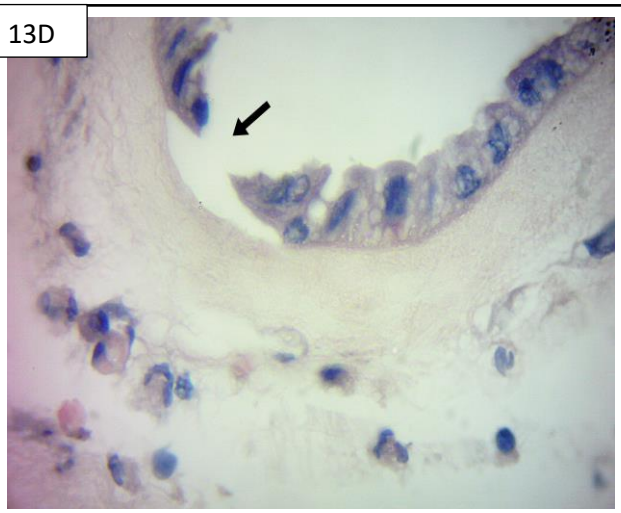
13B



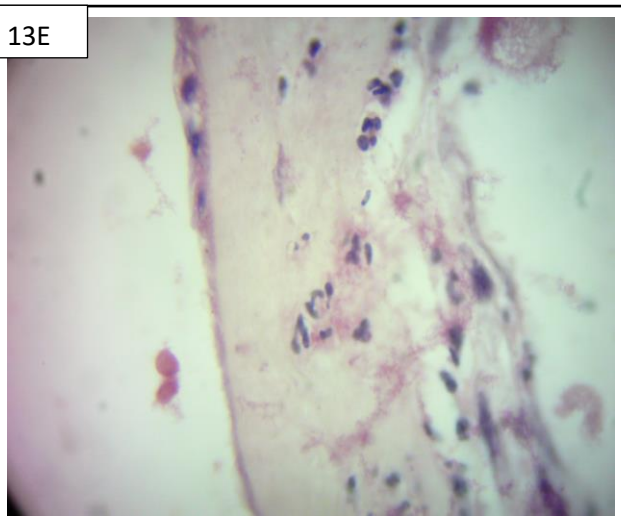
13C



13D



13E



13F

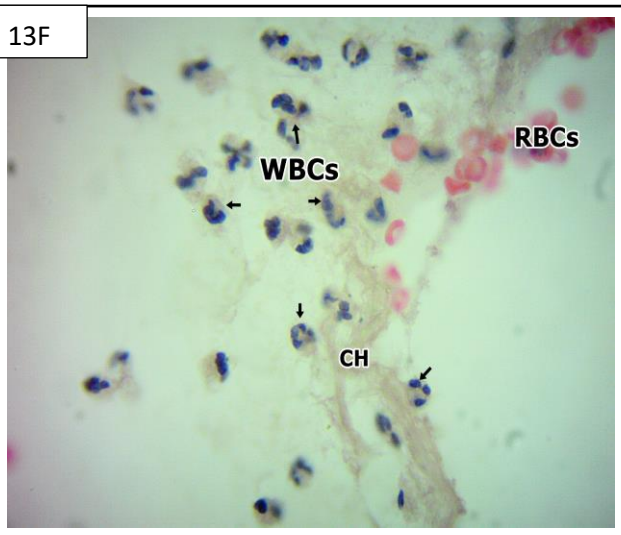


Table 4: Type of epithelium

Group(case or control)	Cases		Controls		χ2(p-value)
	Frequency	Percent	Frequency	Percent	
Cuboidal	18	31.0	38	65.5	17.932 (0.001) *
Columnar	23	39.7	0	0	
Both cuboidal and columnar	9	15.5	6	10.3	
Squamous	4	6.9	14	24.1	
Stratified	4	6.9	0	0	
Total	58	100.0	58	100.0	

Table 5: Epithelial denudation

Group (case or control)	Cases		Controls		χ2 (p-value)
	Frequency	Percent	Frequency	Percent	
No denudation	15	25.9	38	65.5	19.144 (<0.001)*
Minor denudation	32	55.2	17	29.3	
Major denudation	11	19.0	3	5.2	
Total	58	100.0	58	100.0	

Table 6: Fibrin deposition

Group	Cases (With malaria)		Controls (Without malaria)		$\chi 2$ (p-value)
	Frequency	Percent	Frequency	Percent	
No deposition	41	70.7	52	89.7	**7.501 (0.024)***
Minor deposition	14	24.1	6	10.3	
Major deposition	3	5.2	nil	Nil	
Total	58	100.0	58	100.0	

Table 7: Leukocyte infiltration

Group(case or control)	Cases		Controls		$\chi 2$ (p-value)
	Frequency	Percent	Frequency	Percent	
No infiltration	38	65.5	44	75.9	2.939 (0.230)
Minor infiltration	18	31.0	14	24.1	
Major infiltration	2	3.4	nil	Nil	
Total	58	100.0	58	100.0	

CHAPTER 4: DISCUSSION

4.1 Clinical characteristics of the women with malaria in pregnancy

The present study revealed that there were more primigravida among the women who had malaria in pregnancy compared to those who did not. This finding is consistent with what Ismail et al., (2000) stated that primigravidae showed chronic infections more frequently than multiparas. The severity of malaria has been postulated to be as a result of women becoming resistant to malaria in subsequent pregnancies. This is because they obtain antibodies against the CSA-binding placental parasite forms. As a result of acquired immunity, placental malaria is less severe and inflammatory in multigravida women as compared to primigravid women. These parity differences could account for the different outcomes these women experience (Duffy, 2007).

This study also demonstrated significantly lower birth weight neonates among women with malaria in pregnancy. These findings are consistent with what was presented by Sharma and Shukla (2017) and Patel et al. (2017). Low birth weight can be attributed to the neonates being small for gestation (that is birth weight below the 10th percentile which is expected at a given gestational age), a condition which usually results from intrauterine growth restriction (Kapisi et al., 2017; Aribodor et al., 2009)

This study further showed significant reduction placental weight in the women with malaria infection in pregnancy compared to those without. This was consistent with previous study according to Rijken et al., (2012) which demonstrated a reduction of placental volume using ultrasonography following malaria exposure. Reduction in placental weight and volumes in plasmodium infected placenta may be attributed to compromise on placental circulation through trophoblastic thickening, fibrinoid necrosis and other pathological lesions (Moshi et al., 1995).

Conversely, Iyare and Uneke (2018) found that there was increased placental weight and birth weight. This may be due to limitations in their study methodology, having not stated their sampling methods and sample sizes.

4.2 Malaria and the gross morphological features of the placenta

All the placenta in the present study had a discoid shape. This is consistent with standard observations made on placental shape in humans. The cord insertion types that were most common were central and eccentric with no statistically significant difference noted in those with and without malaria. Marginal and velamentous insertion types were observed in about a quarter with slightly higher frequency in cases than controls. Previous studies show that central and paracentral types of cord insertion are the most common types in normal pregnancy (Brouillet et al., 2014). This is in contrast to studies on HIV by Obimbo et al., (2019) which shows influence of the disease on cord insertion. Velamentous and marginal cord insertions are described as abnormal and may be associated with adverse fetal and neonatal outcomes including preterm delivery, low birth weight, intrauterine growth restriction, stillbirth, increased rate of emergency caesarean section, and low Apgar scores (Brouillet et al., 2014; Ebbing et al., 2013; Hasegawa et al., 2006). Velamentous-and marginal cord insertions may result from a process known as trophotropism in which the chorion frondosum of the early placenta migrates to a better vascularized zone of the uterine wall as pregnancy advances (Robinson et al., 1983).

There were no significant differences in the color of the chorionic membrane between the cases and controls from the present study with the normal shiny maroon color being the most frequent in both groups. Previous studies have however associated malaria, HIV and other puerperal infections with increased incidence of meconium stained greenish yellow placenta (Obimbo et al., 2019; Chawla and Manu, 2007; Tran et al., 2003). Meconium stained membranes is generally

associated with any condition that causes fetal distress (Djabanor et al 2017). Infections such as malaria in pregnancy may lead to placental insufficiency and hypoxic state leading to fetal distress. The grey color of chorionic membranes associated with chorioamnionitis was observed in very few cases, suggesting less possibility of concurrent chorioamnionitis. However, specific indicators for etiology of chorioamnionitis were not assessed in this study.

4.3 Malaria and the Light microscopic features of the placenta

Analysis of the photomicrographs of the placenta revealed significantly higher counts of syncytial knots and delaminated syncitiotrophoblast in those with malaria as compared to those without. This finding is consistent with reports from previous studies on placental malaria (Souza et al., 2013; Ismail et al., 2000; Bulmer et al., 1993; Yamada et al., 1989). Syncytial knotting is associated with hypoxia and oxidative stress, both of which have been determined to occur in placental malaria infection (Agudelo et al., 2014; Boeuf et al., 2008; Heazell et al., 2007).

Additionally, significantly large amounts of intervillous and intravillous fibrin deposition among placenta with malaria were observed in this study. Fibrin deposition is consistently observed in plasmodium infected placentas (Ahmed et al., 2014; Bulmer et al., 1993; Chaikitgosiyakul et al., 2014; Ismail et al., 2000; Souza et al., 2013). Fibrin deposition in placental malaria is associated with an upregulation of the extrinsic coagulation pathway, leading to increased thrombin generation, fibrin generation and platelet aggregation (Francischetti, 2008). Perivillous fibrin deposition may interfere with feto-maternal exchange at the placental barrier, causing adverse fetal outcomes including intrauterine growth restriction (Ismail et al., 2000); (Katzman and Genest, 2002; Taweevisit and Thorner, 2016)

There was significant reduction in the intervillous space area in the placenta with malaria compared to those without malaria in the present study. This finding corroborates that of Souza et al., (2013)

on *Plasmodium vivax* on the placenta. Reduction in the intervillous space area is due to extensive branching of immature intervillous villi as a compensatory mechanism in response to placental hypoxic states (Macara et al., 1995; Sankar et al., 2013). Similar observations have also been made in HIV and pre-eclampsia (Obimbo et al., 2019; Sankar et al., 2013). Hypoxia in placental malaria is caused by sequestration and agglutination of parasitized red blood cells. Intervillous red blood cell agglutination was significantly higher among placenta from participants with malaria in the present study, a finding which is consistent with published literature. Placental hypoxia due to malaria in pregnancy may also explain the increased density of blood vessels observed in intermediate and terminal villi. In placental hypoxia, angiogenesis is triggered by hypoxia inducible factor and vascular endothelial growth factors to compensate for reduced fetal oxygen supply (Eskild et al., 2016; Kadyrov et al., 1998; Ogino and Redline, 2000).

In our study, there was marked presence of white blood cells among the participants with malaria in pregnancy. This observation was in tandem with previous studies. According to Ismail et al. (2000), mononuclear inflammation is a frequent finding in placental malaria. Other studies also show involvement of polymorphonuclear cells in the inflammatory response in placental malaria. Infiltration by mononuclear cells is triggered by interferon gamma, interleukin-2 and tumor necrosis factor. (Davison et al., 2006; Maestre and Carmona-Fonseca, 2014). As a consequence, macrophages produce reactive oxygen species that cause cellular tissue damage in the placenta (Sharma et al., 2012).

4.4 Malaria and the Light microscopic features of the chorioamniotic membrane

In the present study, the normal epithelial type which is either simple squamous or low cuboidal was observed. However, other abnormal types of epithelia were noted including stratified cuboidal epithelium, pseudostratified columnar epithelium and both cuboidal and columnar types; which

were found to be significantly higher among the cases with malaria in pregnancy. These findings have not been reported by previous studies in malaria to the best of our knowledge. It was also noted that epithelial denudation was more prevalent in amniotic membranes from participant with malaria compared to the controls. Ho et al. (1982) reported that changes in amniotic epithelia occur naturally along the course of pregnancy, in that early pregnancy showed flattened cuboidal while late pregnancy showed cuboidal or columnar type of epithelia. Amniotic membrane senescence has also been observed in studies on rat placenta. This could lead to reduction in number of epithelial cells, reduction in basal lamina thickness and denudation.

Epithelial denudation was also marked among the cases with malaria. Studies with group B streptococci showed reduction in cytokeratin in affected amniotic epithelia leading to impaired integrity of epithelia which is postulated to cause epithelial denudation (Vanderhoeven et al., 2014). Similar observations have been noted in placenta with gestational diabetes, in which there was weakening of epithelial intercellular junction (Toğrul et al., 2017). Such degenerative changes in disease states may explain the loss of epithelial cells observed in the current study.

Placental malaria was significantly associated with fibrin deposition in the chorioamniotic membrane. Malaria induces fibrin deposition by promoting extrinsic coagulation pathway and thus an upregulation of the thrombin generation (Avery et al., 2012; Chaikitgosiyakul et al., 2014). Fibrin deposition resulting from dysregulated hemostasis leads to mid-gestation abortion or resultant low birth weight (Avery et al., 2012)

Leucocyte infiltration was higher in the cases with malaria in pregnancy. This is a common observation associated with chorioamnionitis (Redline et al., 2004, 2003). Chorioamnionitis can often occur without the evidence of microbial invasion of the membranes (Lannaman et al., 2017). To the best of our knowledge, there are no previous reports on evidence of histological

chorioamnionitis in malaria. Further studies may be done to investigate the mechanisms of malaria-induced leukocyte infiltration into chorioamniotic membranes and possible roles of bacterial co-infections.

CONCLUSION

This study has shown that women who have malaria in pregnancy have significantly higher odds of low placental and birth weights. These placentae also demonstrate increased syncytial knotting, fibrin deposition and other morphological changes that suggest placental insufficiency. Additionally, the chorioamniotic membrane undergoes histological alterations including changes in epithelial type and leukocyte infiltration. We therefore reject the null hypothesis in this study. These findings provide a morphological basis for understanding adverse pregnancy outcomes in women who have had malaria in pregnancy.

LIMITATIONS

Limitations:

There might have been some form of shrinkage of placenta during tissue processing; this may have affected the morphometry. Lack of a standardized system of nomenclature and criteria for the diagnosis and scaling of placental lesions may have affected the interpretation of placental lesions.

We acknowledge that the study was not designed to study chorioamnionitis but rather to determine histological changes in the CAM with malaria in pregnancy. Our study could not, therefore, determine the histological staging of chorioamnionitis in the CAM studies. Finally, our study did not assess the effect of timing of malaria infection on the histology.

Delimitation:

To overcome these problems preliminary comparison between these and freshly collected specimens were done to estimate the correction factor. Secondly, we used criteria developed by Fisher's lab to standardize the description of placental lesions as accurately as possible. We requested the midwives and staff in the concerned units to notify the researchers in case of appropriate candidates for the study. We matched the mothers who had malaria infection versus those who did not at the same trimesters of pregnancy.

RECOMMENDATIONS

This study has laid ground for further studies that may focus on the aspects of, correlation between severity of malaria infection and histoarchitecture of placenta, immunohistology and electron microscopic malaria infected preterm placenta.

Further studies may determine the association of malaria infection in pregnancy with the risk for ascending intrauterine infection. The timing of malaria infection in pregnancy may affect outcomes and should be examined in subsequent studies.

We also recommend that treatment with appropriate antimalarial regimen should strongly be adhered to forestall the likely effects of malaria infection in the placenta that can potentially lead to poor obstetrics outcome.

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APPENDICES

APPENDIX 1: CONSENT FORM

Study background

Malaria in pregnancy has important adverse consequences for maternal health. Emerging evidence from current research suggests infection with malaria in early pregnancy may increase the risk of adverse outcomes of pregnancy. These outcomes may be resultant from improper trophoblast invasion concomitant with the reduced transformation of maternal spiral arteries due to inflammation and disturbances in maternal hormonal function. The events may further contribute to Fetal Growth Restriction (FGR) by reducing the transport of maternal blood to the placenta.

The interaction between the maternal host and her microbiome has been shown to also influence the outcome of pregnancy. Maternal obesity, gestational diabetes, and antibiotics and probiotics have been shown to alter the microbiome, however, it is not fully understood how malaria in pregnancy alters the placental histoarchitecture and microbiome and consequences it has on pregnancy outcome.

Broad objective

To determine the differences in the placental histoarchitecture and microbiome in patients with malaria in pregnancy

Study procedures

If you choose to participate in the study, after delivery of the baby, the placenta will be collected and small sections will be taken from it for processing.

Confidentiality

We will do our best to keep your information anonymous and no information regarding her will appear on either the datasheets or the final thesis but we cannot guarantee absolute anonymity.

Benefits

The information obtained from the study may ultimately lead to reduced feto-maternal morbidity and mortality associated with malaria in pregnancy.

Risks of participation

There are no foreseeable risks to you for participating in the study. There is no cost or payment to you.

Voluntariness of participation

Your participation in this research is voluntary, and you will not be penalized or lose benefits if you refuse to participate or choose to stop

I, the undersigned, have been explained to and have understood the above and willingly accept to participate in the research study.

Signature/ thumbprint……Date……..

I the investigator, having explained in detail the purpose of this study, hereby submit that confidentiality of the data collected will be maintained and only details relevant to the study revealed.

Investigator: **Mercy Singoei** Telephone: **0729014757** Email: **mercy.singoei@gmail.com**

Signature…….Date……..

Lead supervisor: **Dr. Obimbo Moses** Telephone: **0721585906** Email: **obimbomad@gmail.com**

KNH-UoN ERC Secretary: **Prof. M.L. CHINDIA** Telephone: **(254-020) 2726300-9 Ext 44355**

E-mail**: uonknherc@uonbi.ac.ke** Website**: www.erc.uonbi.ac.ke**

APPENDIX 2: CHETI CHA RUHUSA

Lengo la uchunguzi

Lengo la utafiti huu nikuongeza ufahamu wa uhusiano kati ya vijidudu na zalio na ugonjwa wa malaria katika uja-uzito.

Manufaa

Ujuzi wa uhusiano huu utasaidia katika kupunguza vifo vya kina mama waja-wazito na vijusu wakati wa malaria unao husiana na uja-uzito.

Ombi

Hili ni ombi la ruhusa ya kutumia zalio ili kufanya utafiti huu. Mtafiti mkuu atakupa maelezo Zaidi kuhusu uchunguzi huu. Tafadhali fanya uamuzi kuhusu utumuzi wa zalio. Iwapo unaswali uliza mtafiti.

Kutumika kwa zalio katik auchunguzi huu sio lazima na hauna gharama yoyote.

Iwapo utakubali kutumika kwa zalio hili:

- Hakuna kiungo kizima kitakacho tolewa.
- Vipimo vya sehemu hii vitafanywa na vipande vidogo kuchukuliwa kwa uchunguzi kutumia darubini.
- Vipande vikibaki vita zikwa kwa heshima katika makaburi ya Lang’ata.

Usiri

Hatutafichua wala kuchapisha mambo yoyote kuhusu mama mja-mzito bali yale tu yanayohusiana na uchunguzi huu.

Nadhibitisha nimeyafahamu aliyonieleza mtafiti nanimekubali kwa hiari yangu mwenyewe zalio lisaidie katika uchunguzi huu.

Sahihi/kidole cha gumba……. Tarehe……

(Mama mja-mzito)

Mimi mtafiti nimemweleza mama mja-mzito kuhusu uchunguzi huu ipasavyo.

Mchunguzi: **Mercy Singoei** Nambari ya simu: **0729014757**

Barua pepe: **mercy.singoei@gmail.com**

Sahihi ya mtafiti…….Tarehe……..

Msimamizi mkuu: Dr. Obimbo Moses Nambari ya simu: **0721585906**

Email: **obimbomad@gmail.com**

KNH-UoN ERC Secretary: **Prof. M.L. CHINDIA** Nambari ya simu: **(254-020) 2726300-9 Ext 44355**

Barua pepe**: uonknherc@uonbi.ac.ke** Tuvuti**: www.erc.uonbi.ac.ke**

APPENDIX 3: TURNITIN ANTI-PLAGIARISM REPORT

CHANGES IN PLACENTA AND CHORIOAMNIOTIC MEMBRANE HISTOARCHITECTURE IN PATIENTS WITH MALARIA IN PREGNANCY

19
A dissertation submitted in partial fulfilment of the requirements of Master of Science Degree in

Anatomy, University of Nairobi

By:

Mercy Jepchirchir Singoei

H56/81441/2015

Department of Human Anatomy,

University of Nairobi

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APPENDIX 4: JOOTRH-IERC ETHICAL APPROVAL

Telegrams: "MEDICAL", Kisumu
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E-mail: ercjootrh@gmail.com
When replying please quote

JARAMOGI OGINGA ODINGA TEACHING & REFERRAL HOSPITAL
P.O. BOX 849
KISUMU

Ref: ERC.IB/VOL.1/602

Date: 27th August, 2019

Mercy Jepchirchir Singoei

Dear Mercy,

RE: REQUEST FOR ETHICAL APPROVAL TO UNDERTAKE A STUDY TITLED: "CHANGES IN PLACENTA HISTOARCHITECTURE AND NICROBIUOME COMPOSITION IN PATIENTS WITH MALARIA IN PREGNANCY."

The JOOTRH ERC reviewed your protocol and found it ethically satisfactory. You are therefore permitted to commence your study immediately. Note that this approval is granted for a period of one year (w.e.f. 27th August, 2019 to 27th August, 2020). If it is necessary to proceed with this research beyond approved period, you will be required to apply for further extension to the committee.

Also note that you will be required to notify the committee of any protocol amendment(s), serious or unexpected outcomes related to the conduct of the study or termination for any reason.

In case the study site is JOOTRH, kindly report to the Chief Executive Officer before commencement of data collection.

Finally, note that you will also be required to share the findings of the study in both hard and soft copies upon completion.

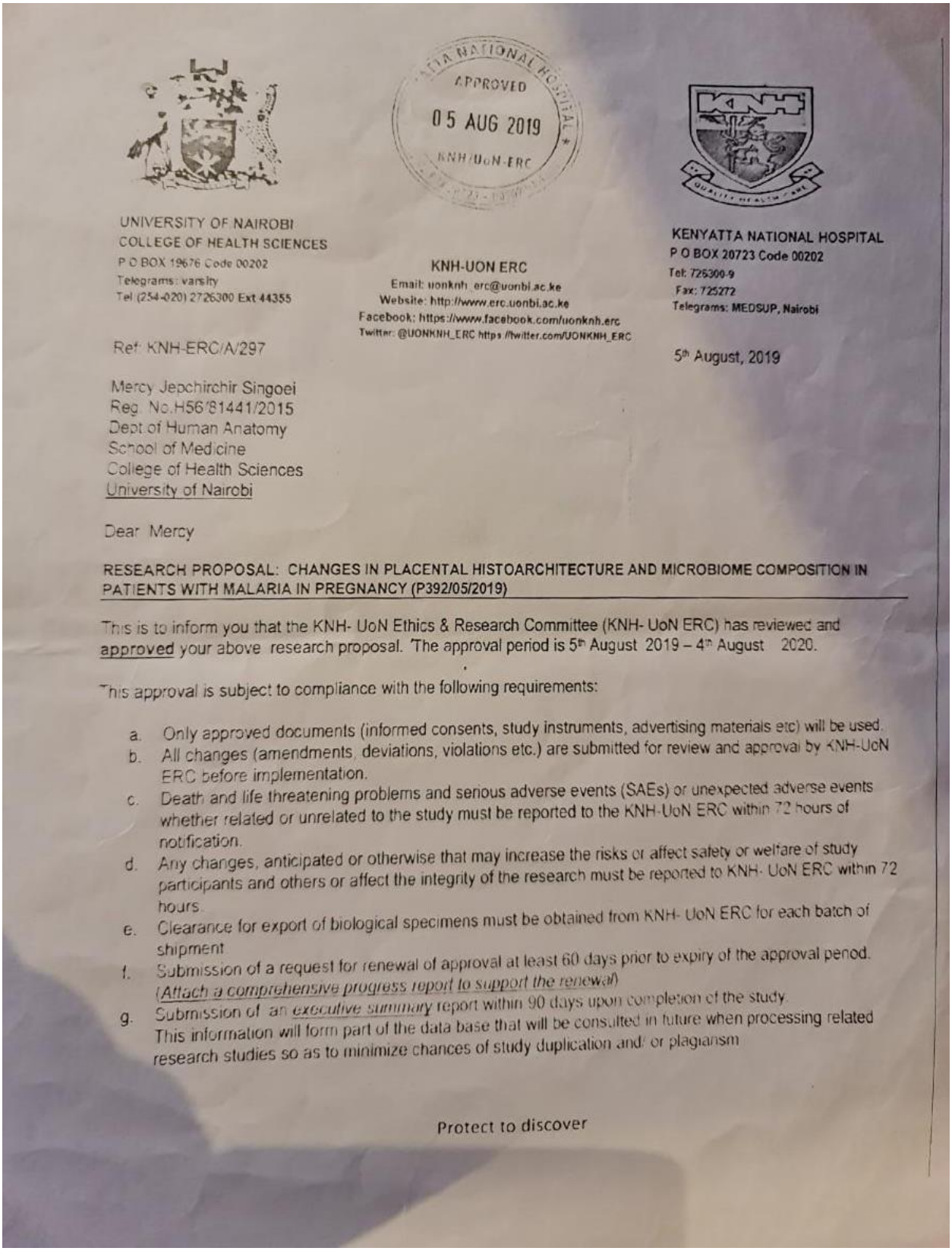
The JOOTRH – IERC takes this opportunity to thank you for choosing the Institution and wishes you the best in your endeavours.

Yours sincerely,

INSTITUTIONAL ETHICS & REVIEW COMMITTEE
27 AUG 2019

WILBRODA N. MAKUNDA
SECRETARY-IERC
JOOTRH - KISUMU

APPENDIX 5: KNH-UON ERC



APPROVED
05 AUG 2019
KNH/UoN-ERC

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5th August, 2019

Mercy Jepchirchir Singoei
Reg. No.H56/81441/2015
Dept.of Human Anatomy
School of Medicine
College of Health Sciences
University of Nairobi

Dear Mercy

RESEARCH PROPOSAL: CHANGES IN PLACENTAL HISTOARCHITECTURE AND MICROBIOME COMPOSITION IN PATIENTS WITH MALARIA IN PREGNANCY (P392/05/2019)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and approved your above research proposal. The approval period is 5th August 2019 – 4th August 2020.

This approval is subject to compliance with the following requirements:

a. Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b. All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
c. Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d. Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e. Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*)
g. Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

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For more details consult the KNH- UoN ERC website http://www.erc.uonbi.ac.ke

Yours sincerely,

PROF.M.L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Chairperson, KNH- UoN ERC
The Assistant Director, Health Information, KNH
The Dean, School of Medicine, UoN
The Chair, Dept.of Anatomy,UoN
Supervisors: Dr.Obimbo Moses Madadi(Dept. of Human Anatomy, UoN)
Dr.Paul Ochieng Odula, Dept.of Human Anatomy,UoN
Dr. Jesse Gitaka, Dept.of Human Pathology, UoN)

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APPENDIX 6: PUBLISHED MANUSCRIPT

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Changes in the structure of chorioamniotic membrane in patients with malaria in pregnancy

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ABSTRACT

Introduction: Malaria infection in pregnancy has adverse consequences for both fetal and maternal health. There is insufficient data on the effect malaria in pregnancy has on the structure of the chorioamniotic membrane. Our objective was to determine the structure of the chorioamniotic membrane in patients with malaria in pregnancy.
Methods: Specimens of the chorioamniotic membrane from 58 women with malaria in pregnancy and 58 women without malaria in pregnancy were used for this study. Biopsies of the fetal membranes were obtained immediately after delivery and processed for light microscopy. They were stained using H & E. Photomicrographs were taken for morphological analysis and statistical analyses were performed using Statistical Package for Social Sciences (SPSS, Version 23.0, Chicago, Illinois). The independent-sample *t*-test and odds ratios were used to compare the appropriate values between the two groups at a 95% confidence interval.
Results: Photomicrographs of the chorioamniotic membrane showed histological alterations, including a change of amniotic epithelium to columnar and stratified types, epithelial delamination, extensive fibrin deposition, and leukocyte infiltration in women with malaria in pregnancy. Statistical analysis found significant differences in epithelial type (p-value 0.001, $\times 2 = 17.9$), epithelial denudation (p-value <0.001, $\times 2 = 19.4$) and extensive fibrin deposition (p-value of 0.02 and $\times 2 = 7.5$) between the study groups.
Discussion: This study has demonstrated histological alterations in the chorioamniotic membrane in association with malaria in pregnancy. Further studies may be conducted to characterize chorioamnionitis in malaria in pregnancy and associations with adverse pregnancy outcomes.

1. Introduction

Malaria is one of the infectious parasitic diseases with the highest mortality worldwide, with its burden being felt chiefly in sub-Saharan Africa. Every year, an estimated 125 million pregnant women, mostly from sub-Saharan Africa and Asia, are at risk of infection by malaria in pregnancy [1]. Malaria in pregnancy, defined as malaria infection in the placenta or maternal blood at any time during pregnancy, is associated with several maternal-fetal complications, including intrauterine growth restriction (IUGR), anaemia, pregnancy loss, and other adverse outcomes [2]. Placental malaria is characterized by parasitized red blood cells, hemozoin pigments and monocytes in the placenta.

The chorioamniotic membrane (CAM), made up of the chorion, amnion and at some locations, maternal decidua, is a thin membrane that surrounds the developing foetus and encloses the amniotic cavity. The CAM serves as the physical barrier between the foetus and the mother [3]. Inflammation of the CAM termed chorioamnionitis has been linked partly to ascending bacterial and fungal infection from the maternal genital tract [4,5]. Infectious agents from the maternal bloodstream such as HIV and other viruses have been implicated in chorioamnionitis without consensus on their mechanisms [6,7].

Acute chorioamnionitis is a well-recognized trigger of preterm delivery [8,9]. An emerging body of evidence has suggested that malaria infection, especially in early pregnancy, enhances the risk of preterm delivery but there remains no clear consensus on the mechanism [10–12]. Examination of CAM from women with malaria in pregnancy

Abbreviations: CAM, chorioamniotic membrane.

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