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Congenital Malaria: Correlation of Umbilical Cord *Plasmodium falciparum* Parasitemia with Maternal Peripheral Blood *Plasmodium falciparum* Parasitemia in selected Hospitals in Jimeta Yola, Adamawa State, Nigeria

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ABSTRACT: The vertical (trans-placental) transmission of the parasite *Plasmodium falciparum* from pregnant mother to fetus during gestational period was investigated in a clinical research involving 43 full term pregnant women in selected Hospitals in Jimeta Yola, Adamawa State Nigeria. During the observational study, parasitemia was determined by light microscopic examination of umbilical and maternal peripheral blood film for the presence of the trophozoites of *Plasmodium falciparum*. Correlational analysis was then carried on the result obtained at $p<0.05$. Presence of *Plasmodium falciparum* was established in 19 and 20 participants each for the maternal and umbilical cord blood samples respectively. The 20 cases of umbilical cord *P. falciparum* parasitemia recorded represents vertical transmission of *P. falciparum* (congenital malaria) from mother to fetus. A strong positive correlation ($p<0.05$) was established between maternal peripheral blood and umbilical cord blood parasitemia with Pearson's correlation coefficient of 0.762. Thus, in a malaria endemic area like Yola, Adamawa State, Nigeria, with a stable transmission of parasite, there is a high probability of vertical transmission of *Plasmodium falciparum* parasite from mother to fetus during gestation that can be followed by the presentation of the symptoms of malaria by the newborn and other malaria related complications. Families are advised to consistently sleep under appropriately treated insecticide mosquito net to avoid mosquito bite and subsequent infestation.

Key words: Trans-placental, Congenital malaria, *Plasmodium falciparum*, Umbilical cord, maternal, parasitemia.

I. INTRODUCTION

Malaria over the years has become an issue of major public health concern especially in sub-Saharan African countries like Nigeria with greater adverse effect on pregnant women and children. It threatens more than 500 million people and affects more than 150 million people each year and consequently causes much human suffering and seriously impedes development effort in the way of draining away manpower resources while imposing a financial burden on endemic countries (WHO, 2016a). Studies have shown that of all four species of *Plasmodium*, the *Plasmodium falciparum* is the most implicated in most malaria outbreaks in Nigeria and other Sub-Sahara African countries due largely to the pathology of its infection and severity of physiological damage caused. Almost every malarial death is caused by *P. falciparum* (WHO, 2016b).

Malaria in pregnancy often referred to as placental malaria (PM) results from infected erythrocytes (iE) binding to chondroitin-sulphate A (CSA) in the placenta (Fried and Duffy, 1996) and consequently fetal exposure when the parasites are transmitted across the placenta (Redd *et al.,* 1996). In countries where malaria is endemic, this risk represents a major health problem that often results in severe maternal anaemia, low birth weight in newborns, increased infant mortality (Malhortra *et al.,* 2006), and other sub-clinical health issues.

The umbilical cord (*funiculus umbilicalis*) is a helical and tubular blood conduit (Spurway *et al.,* 2012) connecting the developing embryo or fetus to the placenta (Kliman, 2013). It serves as a lifeline between the fetus and the placenta. Compromise of the fetal blood flow through the umbilical cord vessels can have serious deleterious effects on the health of the fetus and newborn (Kliman, 2013). Umbilical cord blood simply referred to as "cord blood" is the blood retained in the placenta and is attached to the umbilical cord after childbirth. It is the blood that remains in the blood vessel of the placenta and the portion of the umbilical cord that remains attached to it (New York Cord Blood Centre, 2013). Since the umbilical cord is the conduit between the placenta and the developing fetus, exchange of materials consistently take place between the fetus and mother with the placenta effectively serving as a selective membrane to checkmate the materials exchanged.

With the recent manifestation of symptoms of malaria and other parasitic infections by neonates and the expression of placental specific phenotypes of *Plasmodium falciparum*, the ability of the placenta to completely

protect the fetus from harmful external environment becomes doubtful. Loss of integrity of the placenta (and consequently its impaired selectivity) can result in infiltration of particles which can be of adverse effect to the fetus. Umbilical cord blood *plasmodium* parasitemia is defined as the vertical transmission of malaria from mother to fetus through the placenta and umbilical cord (Ou'edraogo *et al.,* 2011) and is a major threat to the life and development of the fetus.

Although Fretes *et al.* (2012), reported that neonatal malaria parasite infection may occur by trans-placental passage of parasites during disruption of the placental barrier at the time of delivery, with subsequent clinical manifestation of the illness in the newborn baby; insight into the sequestration of *P. falciparum* – infected erythrocytes in the placenta during pregnancy (Malhotra *et al.,* 2006), and the presence of placenta specific phenotypes of *P. falciparum* (Malaria site, 2013), suggests the possibility of trans-placental transmission of the parasite.

However, it is unknown in Yola, Adamawa state whether malaria parasite – *Plasmodium falciparum* can cross the placental barrier into the cord blood and what association exists between maternal peripheral blood parasitemia and cord blood parasitemia. Thus an investigation into the possibility of malaria parasite infested erythrocytes crossing the placental barrier into the umbilical cord and the establishment of the association between maternal peripheral blood and cord-blood *P. falciparum* parasitemia forms the basis of this research work.

II. MATERIALS AND METHODS

Study Area and Population

This study was conducted in Yola metropolis, Adamawa state in Nigeria at five designated hospitals namely State Specialist Hospital, Peace Hospital, Jimeta Hospital and Daama Specialist Hospital. The study population constitute of the women due for delivery in the hospital's maternity ward. Study participant's selection, sample and sampling Technique

Participant's selection was carried out via simple random sampling of the full term pregnant women at the designated hospitals following the procedure of informed consent in accordance with international best practices. The study sample was venous peripheral blood of the participants and the corresponding cord blood of the neonates after delivery. Final analysis involved thirty (30) cord blood samples in the ratio of 17:13 infected and uninfected. Infection was determined by microscopic examination of Field stain A and B thick blood film slides of the maternal peripheral and cord blood samples for the presence of the trophozoites of *Plasmodium falciparum*, an indication of *P. falciparum* parasitemia. Grading is as follows: 1-10 parasites per 100 thick film high power field (HPF): +; 11-20 parasites per 100 thick film HPF: ++; >21 parasites per 100 thick film HPF: +++.

Specimen collection and sample Handling

Venous blood (5ml) was obtained from each of the participants by vein puncture of the ante-cubital vein using a 21 gauge hypodermic sterile needle and syringe. The blood samples were then transferred into different clean and appropriately labeled sterile anticoagulant specimen bottles and cocked for the analysis.

Thick blood film stained slides of the maternal peripheral and cord blood samples were prepared for the assay of the presence of the trophozoite of *Plasmodium falciparum* using Light microscopy. They were subsequently stained using Field stain A and B and observed at 100 HPF with immersion oil.

Experimental design

Two groups of participants were used in the experiment namely; infected and uninfected groups as already described above. Hematological and biochemical assay were conducted on the two groups simultaneously and the test result compared using statistical tools to draw inference and conclusions.

Statistical Analysis

The result obtained was analyzed using Pearson correlation at 0.05 level of significance.

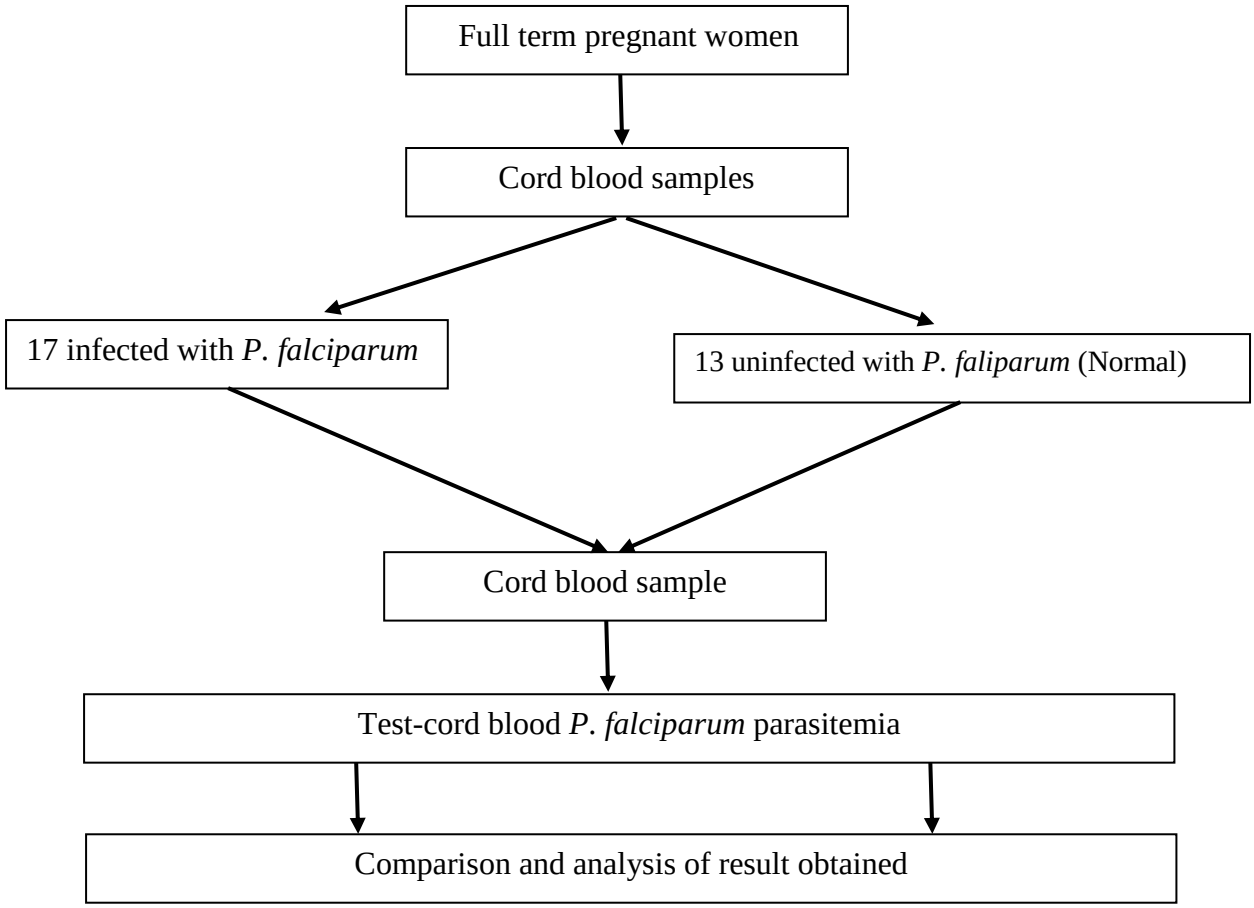

Figure 1: Experimental design

III. RESULT

Table 1 shows the result of maternal peripheral and cord blood *P. falciparum* parasitemia result with traditional microscopy. Out of the 42 maternal participants, 19 had Trophozoites of *P. falciparum* seen in their blood representing a total of 45% of the participants. A total number of 23 participants showed negative to *P. falciparum* assay under the microscope. This represents a total of 55% of the participants. The degree of infestation in the positive cases also varied. Low density infestation (+) with a total number of 17 accounted for 89% of the total positive cases, whereas medium density infestation (++) with a total number of 2 cases accounted for 11% of the total positive cases.

Similarly, in the sampled cord blood, a total of 20 cord blood samples recorded positive for *P. falciparum* representing a total of 48%. A total of 22 participants' cord blood samples tested negative to *P. falciparum*. Of the 20 positive cases, low density parasite load (+) was recorded in 19 cases representing 95% of the total positive cases while medium density parasite load (++) recorded in 1 case accounts for 5% of the total positive cases.

The 20 cases of umbilical cord *P. falciparum* parasitemia recorded represents vertical transmission of *P. falciparum* (congenital malaria) from mother to fetus.

Table 3: Maternal Peripheral and Cord Blood *P. falciparum* Parasitemia Result with Traditional Microscopy

S/N	Sample **code**	**Maternal**	**Cord blood**
1	SP01	+	+
2	SP02	+	+
3	SP03	+	+
4	SP04	-	-
5	SP06	+	+
6	SP07	-	-
7	SP08	-	-
8	SP09	-	-
9	SP10	-	-

10	SP11	+	+
11	SP12	+	+
12	SP18	++	+
13	SP20	-	-
14	SP21	-	-
15	SP23	-	-
16	SP24	-	+
17	SP25	+	+
18	SP26	+	+
19	SP27	+	+
20	SP45	-	+
21	SP46	-	-
22	SP47	+	+
23	SP48	-	-
24	SP49	-	-
25	SP50	-	-
26	SP51	+	-
27	SP53	+	-
28	SP54	-	-
29	SP55	+	+
30	SP56	-	+
31	SP58	-	-
32	SP59	-	-
33	SP67	-	-
34	SP60	+	+
35	SP62	-	-
36	SP64	-	-
37	SP68	-	-
38	DAM 04	+	++
39	DAM 05	+	+
40	PC 06	++	+
41	PC 08	-	-
42	PC 13	+	+

Keys: - negative (Trophozoites of *P. falciparum* not seen) + Positive (seen 1 to 10 parasites per high power focus), ++ Double positives (10 to 20 parasites per high power focus)

IV. DISCUSSION

The result of the *Plasmodium falciparum* assay with traditional microscopy for both maternal peripheral and umbilical cord blood and the co-relational analysis on the result obtained confirmed the possibility of congenital malaria via the vertical transmission of the parasite from mother to the developing fetus through the placenta and umbilical cord. This is in consonant with previous studies on the possibility of congenital malaria by Fretes *et al.,* (2012) and Malhotra *et al.,* (2006). Transfer of *Plasmodium falciparum* antenatally by trans-placental transmission of infected erythrocytes (IEs) is possible because of ability of the parasite-infected erythrocytes to accumulate in the microvasculature of various organs (Aikawa *et al.,* 1990). Also, Chen *et al.,* (2000), reported that erythrocytes infected with mature forms of *Plasmodium falciparum* do not circulate but are withdrawn from the peripheral circulation (sequestration); and then are bound to the endothelial lining (cytoadherence) and to uninfected erythrocytes (rosetting) in the microvasculature through multiple endothelial and erythrocyte receptors. These IEs then accumulate on the syncytiotrophoblastic membrane of the placenta through adhesive interactions in placental intervillous spaces. This is then followed by vascular obstruction, inflammatory events, and subsequently alterations of the syncytiotrophoblast layer (Crocker *et al.,* 2004) or impaired trophoblast invasion with accompanying infiltration of IEs into the placenta and umbilical cord, resulting in the clinical manifestation of the disease in the fetus and newborn. The findings of Malhotra *et al.,* (2006), are also in agreement with the result of the current study as they submitted that malaria-infected erythrocytes or their soluble products can transfer from the maternal intervillous placental blood (IVPB) to the fetus during gestation and that this may occur through the intact placenta, because maternal erythrocytes can cross to the fetus in normal pregnancies.

Although, Red *et al.,* (1996), reported that the likelihood of umbilical cord blood parasitemia was closely linked to the parasite density of placental malaria infection; this could not be readily established in the current study as it is known that *Plasmodium falciparum* status of the maternal peripheral blood at the time of assay

may not exactly represent the status of the mother at the exact time of the fetal infection. This is so because in a malaria endemic area with stable transmission of *Plasmodium falciparum* like Yola, Adamwa State; as the pregnant mother presents with the symptoms of the disease and begins to treat the infection, it is possible that IEs sequestration via cytoadhesion, rosetting, syncytiotrophoblastic layer alteration and subsequent infiltration of the parasite into fetus (Wiser, 2014) (usually in the second and third trimester of the gestational period) had already taken place. It may therefore occur that when the woman has succeeded in treating her infection, the parasites continue to thrive in the fetus and result in the subsequent manifestation of the symptoms of the infection in the fetus (congenital malaria) and the neonate of a supposedly healthy mother.

Moreover, asymptomatic malaria is usually prevalent in malaria endemic regions and has become a serious cause for concern as efforts are increasing towards eliminating the parasite (Laishram *et al*., 2012). Thus in an area with stable transmission of *Plasmodium falciparum* like Yola, Adamawa State, Nigeria, where adults have fairly high acquired immunity to the disease (Cross, 2014), there is the possibility asymptomatic malaria (a woman been a career of the parasite without presenting the symptoms of the infection). The knowledge of this and the submission by Laishram *et al*., (2012), that sub-patent malaria is still transmissible; there is then the possibility of trans-placental transmission of the parasite to the fetus, such that the fetus begins express the symptoms of the infection which was hitherto not supposedly present in the mother. The solution to this is prophylaxis for malaria before and during pregnancy.

The correlation between maternal peripheral blood and umbilical cord blood parasitemia ($p<0.05$) was found to be significant with a strong positive Pearson's correlation coefficient of 0.762. This implies that in a malaria endemic area like Yola, Adamawa State, Nigeria, with a stable transmission of parasite, there is a high probability of vertical transmission of parasite from mother to fetus during gestation that can be followed by the presentation of the symptoms of malaria by the newborn and other malaria related complications.

V. CONCLUSION

Congenital malaria (transfer of *Plasmodium falciparum* from mother to fetus) has been demonstrated. This is mediated by the action of PfEMP-1 and proceeds through via the sequestration of parasite infected erythrocytes by several mechanisms as cyto-adhesion, rosetting, syncytiotrophoblastic layer alteration and subsequent infiltration of the parasite into fetus (usually in the second and third trimester of the gestational period). Strong positive correlation ($p<0.05$) was also observed between maternal peripheral blood and umbilical cord blood *P. falciparum* parasitemia. Pregnant women who have malaria before or during pregnancy, therefore, have high chances of transmitting the parasite to their unborn child.

Prevention has always been a better choice than cure. Families are advised to consistently sleep under appropriately treated insecticide mosquito net to avoid mosquito bite and subsequent infestation

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