Influence of Gravidity (Parity) on Placental Parasitaemia in University of Port Harcourt Teaching Hospital, Rivers State Nigeria

R. U Akpiri* and P. I Agi

Abstract

**Background:** The influence of gravidity on placental parasitaemia was studied in the University of Port Harcourt teaching hospital. Because immunity is acquired over successive pregnancies, susceptibility to malaria is greatest during the first pregnancy and diminishes with increasing gravidity. Similarly, placenta inflammation and the sequelae of pregnancy malaria, such as severe maternal anemia and low birth weight, are most frequent during first pregnancies.

**Methodology:** Blood samples of the neonates, placenta, cord and the mothers were taken and malaria rapid immunodiagnostic tests were carried out using *Plasmodium falciparum* rapid test device. Tick films were examined microscopically for malaria parasite using oil immersion objective. Twenty six mothers and their placenta as well as the accompanying umbilical cord and 26 neonates were studied.

**Result:** The prevalence of placental malaria that lead to symptomatic first parasitaemia significantly decreased as the gravidity increased ($X^2=15.99, P<0.001$). Infants born from primigravidae were significantly more likely to be infected with *P. falciparum*($X^2=6.45 P<0.001$) as compared to infants born from multigravidae.

**Conclusion:** Immunity to malaria is lowest in primigravids, hence greater prevalence of placental inflammation and the sequel of pregnancy malaria, and associated complications.

**Keywords:** Malaria, pregnancy, immunity, Nigeria

1. Introduction

The hallmark of pregnancy malaria due to *Plasmodium falciparum* is the accumulation of infected erythrocytes (IEs) in the placenta. Placental IEs are a distinct parasite form that bonds to chondroitin sulfate A (CSA) on syncytiotrophoblast and in inter villous spaces. They do not adhere to CD36, a ubiquitous receptor on the microvascular endothelium that commonly supports adhesion of IEs in non-pregnant individuals. Adhesion to CSA allows parasites to sequester in the placenta, where dense accumulations can often occur with little or no parasitaemia detectable in the peripheral blood and because CSA-binding parasites do not commonly infect non-pregnant individual, women usually lack immunity to this parasite form prior to the first pregnancy. Immunity is acquired over successive pregnancies as a consequence of repeated exposures. These are associated with reduced risk of maternal parasitaemia and improved pregnancy outcomes. Because Immunity is acquired over successive pregnancies, susceptibility to malaria is greatest during the first pregnancy and diminishes with increasing gravidity (parity). Similarly, placenta inflammation and the sequelae of pregnancy malaria, such as severe maternal anemia and low birth weight, are most frequent during first pregnancies. Thus, pregnancy malaria is estimated to cause tens of thousands or hundreds of thousands of infant deaths each year. However, these estimates are extrapolated from the incidence of malaria-related outcomes such as low birth weight and maternal anemia that increase infant mortality risk. This present study, therefore seeks to investigate the prevalence of placental malaria in University of Port Harcourt Teaching Hospital and to determine the influence of parity on placental malaria prevalence. So far it has been shown that placental parasitaemia that can lead to pregnancy malaria is prevalent in this region and that parity is a key determinant.

2. Materials and Methods

2.1 Study Area: Port Harcourt city lies within 4°45’N 7°00’E/4.75°N4°00’E, in mangrove swamp forest Zone. Port Harcourt had a human population of 1,620,214 as at 2007(last population census) and it is a malaria holo-endemic area, as it is characterized by constant rainfall and mangrove/swampy vegetation.

2.2 Study Population

The study population involved 60% primigravidae and 20% multigravidae between the age bracket of 24-45 years. The study was carried out between 2009 and 2010. Written informed consent was obtained from the women prior to recruitment into the study.

2.3 Ethical Consideration

The studies conducted were approved by the university of Port Harcourt teaching Hospital ethical committee (UPTH/CS&T/118/VOL. XII/132). The participants were adequately informed on the modality and the purpose of the research.
2.4 Collection Tools

The tools used for this work were 2ml syringes, hand gloves, EDTA anticoagulant bottles (2.5ml capacity for holding 0.5ml anticoagulant and 2ml of blood), with mauve cap, light microscope, needles, lancets, (prickers), cotton wool swabs, etc.

2.5 Sample Collection

Maternal blood samples were collected by gynecologists on setting hydration lines. Samples from the placenta and umbilical cords were collected by the researcher through the assistance of the nurses (Figure 1). Prior to collection, personal data information on maternal age, address, history of intermittent presumptive therapy (IPT) against malaria during pregnancy were obtained from the patient’s folder.

![Figure 1: Blood Sample Collection from Placenta and Umbilical Cord](image)

(Fresh placenta were collected by the researcher, from post-natal labour ward of the University of Port Harcourt Teaching hospital immediately after delivery, from the volunteers. Blood samples were obtained from each for analysis).

2.6 Sample Analyses

Blood samples collected were screened, both quantitatively and qualitatively. For qualitative analysis, rapid immune chromatographic test kit for plasmodium parasites malaria antigen was used according well established method[13].

2.6.1 Diagnosis with Malaria Test Kit

A drop of blood was added using the micro-pipette into the pad in the depression (hole) at the anterior and of the card. The pad contains antibodies specific to Pf HRP2 (Plasmodium falciparum histidine-rich protein 2) antigen; buffer solution was also added on the pad; to lyse the blood. HRP 2 antigens join with the dye coloured antibody and travels up the strip. About half way up the strip a second antibody specific to Pf HRP2 antigen is impregnated in a line across the strip. As the blood crosses this antibody line, the dye coloured antigen-antibody was bound to it (captured). The buffer reagent on the cleaning pad clears the lysed blood leaving a white background against which the captured HRP2 antigen can be viewed as a pink mauve line, indicating a positive test for *P. falciparum* malaria. Positive control contained on the strip is seen as a solid pink-mauve line above the test line. For patient test that are negative, only the control line were seen visibly.

2.6.2 Qualitative Analysis

Two to three drops of blood were dropped at the center of the grease-free microscope slide and a thick smear of blood was made to occupy an area if about 15mm in diameter. The thick film was used to detect the presence of malaria parasite.

2.6.3 Staining

The thick film of blood was allowed to dry thoroughly (to avoid being washed off). The films were dipped in field stain A for 15 minutes, water for 7 minutes, field stain B for 3 minutes and water for 6 minutes. The slides were again allowed to dry properly using low power ovum and air drying.

2.6.4 Microscopy

Cedar wood immersion oil was dropped at the centre of the thick film of blood. The film was examined microscopically using the immersion oil objective lens (X100). The identification of *Plasmodium* sp was undertaken based on specific characteristics, including morphological features with respect to size and shape of infected red blood cells, chromatin dot pattern or ring form trophozoits, number of rings formed per cell and the shape and features of schizont and gametocytes in peripheral blood[16].

<table>
<thead>
<tr>
<th>Gravidity</th>
<th>Placental parasitaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primigravidae</td>
<td>13 (65%)</td>
</tr>
<tr>
<td>Secundigravidae</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>Multigravidae</td>
<td>3 (15%)</td>
</tr>
</tbody>
</table>

(Placental parasitaemia, were recorded in the different groups, using the conventional parasite load sign of “+”, number of rings formed per cell and the shape and features of schizont and gametocytes in peripheral blood were used to count the parasite load. Where IEs are more than 20(twenty), they were recorded as “++”, and more than 30(thirty) were recorded as “+++”. Thus, the figures indicated on the table were obtained.)

3. Placenta malaria outcome

The prevalence of placenta malaria that lead to symptomatic first parasitaemia significantly decreased as the gravidity increased ($X^2 =15.99, P<0.001$). Infants born by primigravidae were significantly more infected with *P. falciparum* ($X^2=6.45, P<0.001$) as compared to infants born by multigravidae.

4. Discussion

Of the 13 primigravid positive cases, 8 had scanty parasite load, 4 had a parasite load up to ++ and 1 was heavily affected with a parasite load of up to +++. Parasite load in secundigravidae was reduced in comparison to primigravidae. Thus infants born to primigravidae
recorded higher parasite load in their peripheral blood smear; indicating higher risk in this group. Placenta parasitaemia among the multigravidae was almost non-existent in this study. These observations are consistent with the findings in previous studies in malaria endemic regions were among several factors gravidity independently influenced the occurrence of placental malaria. It has been suggested that multigravid mothers develop malaria antibodies which block adhesion of parasites to chondroitin sulphate-A receptors in the placenta in subsequent pregnancies. Placental malaria has been observed to have a negative outcome to the newborn. Similar negative outcomes (such as foetal death, prematurity, jaundice, neonatal sepsis etc.) were observed in this study. These findings suggested that women who had pre-term delivery could have been due to parasitization of the placenta.

Factors such as area of residence and season of observation are observed to contribute to the parasite load, this agreed with the report of other researchers. Because immunity is acquired our successive pregnancies, susceptibility to malaria is greatest during the first pregnancy and diminishes with increasing gravidity. Similarly, placenta inflammation and the sequel of pregnancy malaria, such as severe maternal anaemia and low birth weight, were observed to be most frequent among the primigravid.

In conclusion, Gravidity is a major factor that influences placental parasitaemia prevalence; primigravid in this study and in previous studies were parasitized more frequently and experienced higher parasite densities, more severe inflammatory responses and greater clinical sequelae than women in other gravid group. For control and prevention of malaria sequelae, intermittent presumptive treatment (IPT) should be a top priority during antenatal.

References