Full Length Research Paper

Malaria infection among pregnant women in South–Eastern Nigeria using peripheral blood smear, placental blood smear, and placental histology.

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Millions of women who become pregnant annually are at increased risk of being infected with malaria parasites which has obvious implication on foetal and maternal health. Various control measures have been put in place to reduce the parasite burden in these women. In the understanding that diagnosis is an important component of malaria control programme, we carried out this study among pregnant women in three locations (Okigwe, Afikpo, and Umuahia) to evaluate the diagnostic values of peripheral blood smear, placental blood smear or placental histology. 844 women were involved in the study. From each participant three samples were made (peripheral blood smear, placental blood smear, and placental histology). Placental histology identified 60.7% infections with plasmodium parasites while placental blood smear identified only 24.9%. The least infection rate was observed in peripheral blood smear 15.9%. In the three locations the same trend was observed. Placental histology showed highest infection rate followed by placental blood smear. Peripheral bloods smear showed the least infections. Statistical analysis was done using anova and the result was highly significant (F = 134.166, P<0.05). Multiple comparisms of the diagnostic methods were done using least significant difference (LSD) and the mean differences were significant at 5% probability and 95% confidence interval confirming the anova result. Sensitivity test revealed 40.4% for peripheral blood smear, 63.3% for placental blood smear, and 98.1% for placental histology. Infection was highest among primigravid (69.5%) and lowest among the multigravid (53.1%) but not statistically significant (X² = 0.6556, P<0.05). The result showed that placental histology is a more effective diagnostic tool for assessment of malaria–related morbidity during pregnancy. It also supported parasite sequestration in the placenta, a reason for diverse maternal and foetal outcomes.

Keywords: Blood smear, Placental blood smear, and Placental histology

INTRODUCTION

Malaria in pregnancy has become an issue of global worry. This is because the public Health problems associated with it is enormous especially in malaria endemic areas (Gamble et al 2006; WHO/AFRO 2004).

In endemic areas of Africa it has been observed that the frequency and severity of infection by P. falciparium are greater in pregnant women than in their non – pregnant counterparts (Gilles et al 1984). Women who become pregnant in this area, if unprotected are likely to be infected and to remain so till the time of delivery if untreated. Infection rates and its consequences are greater among the primigravidae than the multigravidae (Brabin 1991, Okoko et al 2000). The placenta which
develops as a new organ of pregnancy has been associated with severe parasitization by *P. falciparum* which prefers, replicates, and sequesters in the organ after binding and adhesion (Duffy and Fried 2003; Winter et al 2003).

The adhesion molecules which play a role here include chondroitin sulphat – A (CSA) and Hyaluronic Acid (HA) (Duffy and Fried 1996; Kakkilaya, 2006). Sequestration of infected erythrocytes in the placental tissues interferes with oxygen and nutrient supply to the foetus and consequently leading to diverse placental pathologies that determine pregnancy outcomes. Poor pregnancy outcomes associated with malaria in pregnancy include, maternal anaemia, low birth weight, preterm delivery, still bath and jaundice (Okoko et al 2003, Singer et al 2004).

Evolution of diagnostic methods through time has contributed greatly to malaria case management and control. Clinical diagnosis using peripheral blood smear has been the oldest and fastest. Other methods evolved included among others the placental blood smears. Leke et al (1999) observed that detection of malaria parasitemia by peripheral blood smear does not provide accurate estimation of placental parasitemia. Blood smears from the maternal sides of the placenta has been studied but not without some difficulties.

Recent works in placental histology done in many parts of Africa and other countries have revealed that absence of peripheral parasitamia does not mean absence of infection as well as giving credence to the cause of placental pathologies which are responsible for unpalatable maternal, foetal and post natal outcomes of the infection. In Nigeria, no record has been shown that such work has been done in any part of the country. This work was therefore done in the south east to:

Determine levels of malaria infection among pregnant women using peripheral blood smear, placental blood smear and placental histology. Show the different rates of infection in the three diagnostic methods with the view to identify or support parasite sequestration in the placenta. Determine any influence of parity on prevalence.

**MATERIALS AND METHODS**

**STUDY AREA**

The study area is South – East geopolitical zone of Nigeria. It is situated on the coastal hinterland of Nigeria east of the Niger and occupied by the Igbo speaking tribe. The zone is made up of five states which includes Abia, Anambra, Ebonyi, Enugu and Imo States.

Three states and three locations were randomly selected for the study. Consequently umuahia (Abia), Okigwe (Imo) and Afikpo ( Ebonyi) were selected. In these locations existing health structures (Private and public) were used. These included St. Michaels Hospital, (Umuahia) General Hospital Okigwe, Devine Maternity Home, (Okigwe) Mater Misericordiae Afikpo.

**ETHICAL APPROVAL**

Prior to the commencement of the study, an approval was sought from the Chief Medical Directors of the public clinics and directors/matrons of the private clinics/maternity by writing, explaining the objectives of the study and seeking co-operation. A total of 844 randomly selected pregnant women who were in the 2nd stage of labour were involved after proper explanation of their involvement in the study and their consent received. This is made up of 301 from Okigwe, 287 from Afikpo and 256 from Umuahia. Obstetrics and gynaecological information relating to each participant were collected from their record. Only the qualifies midwives were used in the collection of certain aspects of the data after they have been taught with specific examples.

**Sample collection and laboratory examination**

Peripheral blood samples were collected by finger puncture using lancet. Thick smears were made, labelled and allowed to dry under room temperature. Placental blood smears were made by making an incision on the maternal side of the placenta after delivery and collecting few drops of blood to prepare thick smears. These were labelled and allowed to dry under room temperature. Sample slides were transported to the microbiology laboratory of Federal College of Agriculture Ishiagu for examination.

**Placental tissue collection**

After delivery the placenta, was collected, placed maternal side up and cleaned with sterile normal saline. A paracentric area was incised and a biopsy specimen of about 2.5cm² was cut out and placed in a specimen bottle containing fixative (10% neutral buffered formaline), corked and transported in batches to Abia State University Teaching Hospital Aba for histological procedures.

In the histopathology laboratory the biopsy specimens were processed. This involved dehydration by immersing the tissues in increasing grades of alcohol. Clearing was done using cedar-wood oil and rinsed with zylene.

Embedding was done with paraffine wax in an electric oven. This was followed by casting in a metal filled with molten wax, sectioning was done with microtome (Baired and Tatlock Model) set at 6 microns thickness. Sections were then fixed on clean slides and laid on hot plates flooded with distilled water. Staining was done using Harris haematoxylin staining technique (1900 AD) and counterstained with eosin. After dehydration in graded alcohol clearing was done with Xylol. Mounting of the
prepared slides under cover slip was done using Canada Balsam. Microscopic examination was done to detect malaria parasites as black dots.

### DATA ANALYSIS

The data were analyzed using the statistical program SPSS for windows (version 17.0) while distribution of infection according to parity was analyzed using chi-square statistics.

### RESULTS

A total of 844 pregnant women were involved in the study. From each participant, three samples were collected and consequently a grand total of 2532 samples were collected and examined. Of this number, 844 were peripheral blood samples, 844 were placental blood samples while 844 were placental histology. Examination of peripheral blood samples revealed that 134 (15.9%) of the women were infected with malaria parasites while placental blood smear identified 210 (24.9%) of them to be infected. Placental histology yielded higher infection prevalence having identified 512 (60.7%) of the same women to be infected. (Table 1a).

Similar trend was observed in the three locations. Placental histology identified the highest infection rates of 66.5%, 58.2% and 56.6% respectively in Okigwe, Afikpo and Umuahia followed by placental blood smear. The least infections were revealed in peripheral blood smear which recorded 17.3%, 16.7% and 13.3% in Okigwe, Afikpo and Umuahia respectively. The least infections were revealed in peripheral blood smear which recorded 17.3%, 16.7% and 13.3% in Okigwe, Afikpo and Umuahia respectively. The least infections were revealed in peripheral blood smear which recorded 17.3%, 16.7% and 13.3% in Okigwe, Afikpo and Umuahia respectively. The least infections were revealed in peripheral blood smear which recorded 17.3%, 16.7% and 13.3% in Okigwe, Afikpo and Umuahia respectively.

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### DISCUSSION

About 24 million women that become pregnant yearly in malaria endemic areas of Africa are at increased risk of contacting the infection especially with *Plasmodium falciparum* and suffer its related complications (Steketec et al, 1996.) South eastern Nigeria is endemic for malaria and it does not appear that pregnant women in this area are protected from the observed impact of malaria in other endemic areas of Africa. Diagnosis of malaria involves the use of various diagnostic tools to identify presence of parasites in various tissues and organs. We used three tools for comparison; peripheral blood, placental blood and placental histology.

Placental histology proved more sensitive than the others in detecting presence of malaria parasites followed by placental blood microscopy. Peripheral blood microscopy yielded the least sensitivity (Table 1). This result was in agreement with the work of Mockenhaupt et al (2002) and Steketec et al (1996). Even though peripheral blood microscopy has the reputation of being simple and can be used in small laboratories that can be found in rural communities, it has in this work failed to identify 73.8% of malaria infected pregnant women and this is a source of worry as this represents the number exposed to possible consequences of malaria in pregnancy. However Rogerson et al (2003) believes that this method identifies mainly women at highest risk.

Although placental blood microscopy and placental histology yielded higher sensitivity. They may not be useful in clinical diagnosis being accessible only on delivery. The use of histological tool in the study of malaria in pregnancy has however revealed a significant proportion of pregnant women with malaria related morbidity who are often neglected in any program of prevention, control or treatment especially when asymptomatic. Placental blood microscopy prepared with blood from the incised portion of the placenta yielded low sensitivity compared with placental histology which

<table>
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<tr>
<th>Table 1(a): Malaria prevalence by the three diagnostic methods</th>
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<tr>
<td><strong>No Examined</strong></td>
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<tr>
<td>Peripheral Blood Smear</td>
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<tr>
<td>Placental Blood Smear</td>
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<tr>
<td>Placental Histology</td>
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<tr>
<td><strong>Total</strong></td>
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aggress with the work of Rogerson et al (2003). This points to the possibility of retention of parasitized erythrocytes which sequester in the inter-villus spaces and trophoblastic villi through adhesion to host receptors such as chondroitin sulphate A (CSAs) and Hyaluronic Acid (HA) (Beeson et al, 2000). Sequestration not only leads to interference of parasites and macrophages with oxygen and nutrient supply to the foetus but also leads to diverse placental pathologies that are consequential to both maternal and foetal health. (Singer et al, 2004). High prevalence rate by placental histology justifies the current global drive to control malaria in pregnancy through intermittent preventive treatment (IPT) and use of insecticide treated nets (ITNs). Consequently there is the need for various Governments and other stakeholders in the region to scale up actions to ensure success in the attainment of vision 20 – 2020 on maternal and child health in this regard.

Parity related placental infection rate reveals that primigravid women were more infected which is in agreement with the work of Mbanefo et al (2010) in Anambra State Nigeria. Infection rate was least among the multigravid women although differences were not statistically significant (P< 0.05). Multigravid women mount immune responses earlier (at 12th week) than primigravid women and consequently resist malaria infection better. The revelations made in this study have however provided an advocacy for:

The expansion of our primary healthcare system to permeate our rural communities where health delivery systems are lacking.

Provision of enough drugs for IPT in line with national treatment policy for pregnant women at subsidized rate in all PHCs.

A more sensitive and cheaper means of identifying malaria in the peripheral blood is urgently needed as every medical examination in this part of the world includes those for malaria parasite.

REFERENCES


Okoko BJ, Enwere G, Ota MOC (2003). The Epidemiology and

Table 1: Malaria Infection in the Locations by the three Diagnostic Methods

<table>
<thead>
<tr>
<th>Diagnostic Method</th>
<th>Okigwe (n = 301) No +ve (%)</th>
<th>Afikpo (n = 287) No +ve (%)</th>
<th>Umuahia (n = 256) No +ve (%)</th>
<th>Total (n = 844) No +ve (%)</th>
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<tbody>
<tr>
<td>Peripheral blood</td>
<td>52 (17.3)</td>
<td>48 (16.7)</td>
<td>34 (13.3)</td>
<td>134 (15.9)</td>
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<tr>
<td>Placental blood</td>
<td>81 (26.9)</td>
<td>71 (24.7)</td>
<td>58 (22.7)</td>
<td>210 (24.9)</td>
</tr>
<tr>
<td>Placental histology</td>
<td>200 (66.5)</td>
<td>167 (58.2)</td>
<td>145 (56.6)</td>
<td>512 (60.7)</td>
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Table 2: Provide table title
consequences of maternal malaria: a review of immunological basis.


