PREVALENCE AND SUSCEPTIBILITY OF MALARIA PARASITES INFECTION IN ASSOCIATION WITH BLOOD GROUP AND HAEMOGLOBIN GENOTYPE POLYMORPHISM IN PREGNANCY

*1Ebadan, M.I., 2Obodo B. N., 3Amiegheme, F. E., 4Uwaifo, F., 5Omigie, B. E., 2Iyevhobu L.K., 2Umassor, A. C. and 2Ayeki, G.E

1 Quality Assurance Clinia-Lancet Laboratories Victoria Island Lagos, Nigeria. 2Department of Medical Laboratory Science, College of Medicine, Ambrose Alibi University, Ekpoma, Edo State. 3Department of Nursing Science, School of Basic Medical Sciences, College of Medicine, UNIBEN, Edo State, Nigeria. 4Eromosele Clinic Ekpoma, Edo State. Department of Chemical Pathology, Irrua Specialist Teaching Hospital (ISTH), Edo state Nigeria.

*Corresponding author: maxee4j@yahoo.com

ABSTRACT

This study determined the prevalence of malaria infection among 146 pregnant women attending antenatal clinics in Ekpoma and its environs, in relation to blood groups and haemoglobin genotypes. They comprised 62, 40 and 44 pregnant women in their first, second and third trimesters respectively. Venous blood for the determination of haemoglobin genotypes and microscopic examination of malaria parasites, was collected via the median cubital vein. Malaria parasites were examined using both thin and thick blood films stained via the Giemsa method. Blood group ‘ABO’ was determined using commercially prepared anti sera, while their genotypes were determined using standard Haemoglobin Electrophoretic method. The results showed that 64 (44%) of the pregnant women were positive to malaria parasites, while 82 (56%) were negative to malaria parasites. Those in categories A Rhesus ‘D’ (26; 54%), B Rhesus D (11; 39%) and O Rhesus D (27; 42%), were infected with malaria parasite, while no prevalence of malaria parasites was recorded in the AB blood group category. Pregnant women with Hb-AA genotype had a higher malaria prevalence of 39 (42%), while those with Hb-AS genotype had malaria prevalence of 46% (n=25). These results revealed a varying relationship between malaria infection and blood group/genotype polymorphism.

Key Words: Malaria, Genotype, Pregnancy, Blood group, Susceptibility

INTRODUCTION

Malaria poses an enormous public health burden and remains an endemic challenge in Nigeria, with about 588 million people at risk (Snow et al., 2005; WHO 2008). It places a huge burden on human life, and has been reported to be a key health problem affecting developing countries. Also, it is a mosquito-borne infections disease of humans caused by eukaryotic of the genus Plasmodium (WHO, 2000). Five species of Plasmodium can infect humans and is transmitted by infected female anopheles’ mosquitoes. The species include Plasmodium falciparum, Plasmidium vivax, Plasmodium ovale and Plasmodium malaria (Singh et al., 2004; Sutherland and Hallet, 2009).

The protection of pregnant women living in malaria-endemic countries has been of particular interest to many National Malaria Control Programs because of their reduced immunity. However, most cases of malaria in pregnancy in areas of stable malaria transmission are asymptomatic (Anorlu et al., 2002; Mockenhaupt et al., 2002). This is attributed to anti-disease immunity acquired during previous exposures which protects against clinical malaria (Staalsoe et al., 2004). The principal impact of malaria infection is due to the presence of parasites in the placenta causing maternal anemia (potentially responsible for maternal death when severe) and low birth weight (LBW) (Newman et al., 2003; Rogerson and Boeuf, 2007).

Furthermore, it is important to note that genetic factors play a key role in determining resistance/susceptibility to parasitic and infectious disease. Genetic markers such as haemoglobin genotypes (AA, AS, AC, SS, CC and SC), and ABO blood groups have been associated...
in with various disease conditions including malaria
(Sakallioglu and Sakallioglu, 2007). Host genetic and
environmental factors may be important in the genesis
of diseases. ABO blood groups are one set of
agglutinogens (antigens), which genetically determine
the carbohydrate molecules carried on the surface of
the red blood cells. ABO blood groups have shown
association with various non-infectious diseases (Umit
et al., 2008) and infectious diseases (Jeffery and
Kenneth, 2005; Dey and Cederbaum, 2006).

A broad range of available evidence suggests that the
origin, distribution and relative proportion of ABO
blood groups in humans may have been directly
influenced by selective genetic pressure from
*Plasmodium falciparum* infection (Christine and
Cserti, 2007). Clinical reports of ABO blood groups
and *Plasmodium falciparum* infection, reveals a
correlation between disease severity and ABO groups
(Gayathri et al., 2013).

Since malaria has re-emerged as a major problem past
years, it would be useful to know if there is any
relationship between genotype, blood group and
malaria parasites infection. However, the correlation
of severity of malarial infection to the patient’s blood
group and genotype has been of recent interest in the
quest for the answers to the factors influencing clinical
course of the disease and management. The
observation by Miller et al., (1975) that human
erthrocytes lacking the Duffy blood group antigens
are refractory to invasion by *Plasmodium vivax*
parasites indicate the usefulness of studying the
association of blood group with malaria.

ABO and malaria have both been studied for over 100
years, and there are numerous literatures on the effects
of ABO blood group on various forms of malaria from
multiple countries, many coming to contradictory
conclusions covered in some recent reviews reported
Remarkably, until recently, there has been no clear
answer to the crucial and obvious question: does ABO
blood group affect susceptibility to life-threatening
relationship between malaria infection and Hb
genotypes in Nigeria. This indicated that there were
differences in susceptibility to malaria among
individuals with haemoglobin genotypes A and AS.
The study also indicated that blood group O
individuals were more susceptible to malaria, than
other blood groups A, AB and B, while blood group
AB were least infected with malaria. However, based on the contradictory results obtained
on the influence of genetic factors (genotype and
blood group) in the prevalence of malaria parasites
infection among pregnant women; This study is
therefore set to correlate the blood groups and
genotype of pregnant women’s susceptible to malaria
parasites and to understand the differential host
susceptibility that will promote diagnosis and
management of pregnant women.

**MATERIALS AND METHODS**

**Area of Study:** This study was carried out in Ekpoma,
The Headquarter of Esan West Local Government area
of Edo State. It is located at latitude 6° 45’N and
longitude 6° 08’E. It is moderately populated with the
peoples’ occupation being farming and trading. The
main sources of water in the locality are rainfall and
well. The well is augmented by irrigation scheme
provided by the Government for public use. University
is situated in this region. It is usually cold at night and
very hot during the day. It also has undulating
topography (World Gazetteer, 2007).

**Study Population:** The subjects used in this study
were pregnant women attending antenatal clinic who
were recruited from Faith dome hospital, Eguavon
hospital Irukpen and Eseohe Medical hospital
Ekpoma. Ethical permission was obtained from the
management of the hospitals and informed consent
was sought from the subjects before sample collection.
A total of one hundred and forty-six (146) pregnant
women were recruited for this study; which comprised
of sixty-two (62), forty (40) and forty-four (44) for
first, second and third trimesters respectively. Socio-
demographic profile such as age and duration of
pregnancy were obtained. The age range of the
subjects used in this study was between 19-40 years.
Pregnant women who have not been on anti-malaria
medications for a period of three months obtained
through verbal question were recruited for this study
while pregnant women who have been on anti-malaria
medications within period of three months were
excluded for the study.

**Sample collection:** Venous blood was collected via
the median cubital vein. The area was tied with a
tourniquet, cleaned with cotton wool moistened with
methylated spirit and allowed to dry, and using a
sterile needle and syringe, venous blood was collected.
The blood samples obtained were placed in the EDTA
capillary bottle with identifier using a unique identification
number, trimester, age and name of the pregnant
woman) which were immediately taken to the

**Study Population:** The subjects used in this study
were pregnant women attending antenatal clinic who
were recruited from Faith dome hospital, Eguavon
hospital Irukpen and Eseohe Medical hospital
Ekpoma. Ethical permission was obtained from the
management of the hospitals and informed consent
was sought from the subjects before sample collection.
A total of one hundred and forty-six (146) pregnant
women were recruited for this study; which comprised
of sixty-two (62), forty (40) and forty-four (44) for
first, second and third trimesters respectively. Socio-
demographic profile such as age and duration of
pregnancy were obtained. The age range of the
subjects used in this study was between 19-40 years.
Pregnant women who have not been on anti-malaria
medications for a period of three months obtained
through verbal question were recruited for this study
while pregnant women who have been on anti-malaria
medications within period of three months were
excluded for the study.

**Sample collection:** Venous blood was collected via
the median cubital vein. The area was tied with a
tourniquet, cleaned with cotton wool moistened with
methylated spirit and allowed to dry, and using a
sterile needle and syringe, venous blood was collected.
The blood samples obtained were placed in the EDTA
capillary bottle with identifier using a unique identification
number, trimester, age and name of the pregnant
woman) which were immediately taken to the
laboratory for analysis with standard Laboratory methods and operating procedures (SOP).

**Sample Analysis:** The blood samples obtained were observed macroscopically for lysis and cloth formation. Microscopic examination of the blood samples obtained from pregnant women were done using prepared and stained thick and thin blood film. The blood samples were stained using 10% Giemsa stain and both blood films were examined microscopically using Oil Immersion Objective Lens (X100 objectives). The thick blood film was examined first in order to detect the presence of malaria parasite. This was followed by the examination of the thin blood film for identification of the *Plasmodium species* present. Blood group determination was determined using commercially prepared anti sera-Anti A, B, AB and anti D). The genotypes of the pregnant women were determined using standard Haemoglobin Electrophoresis method (Cheesbrough, 2000).

**Data Analysis:** The results were presented in tables. The percentage prevalence was calculated in each case. Comparative analysis of the result was done using two tailed Chi-test at p≤0.05 level of significant and 95% confidence interval.

**RESULTS**

The results showed that out of one hundred and forty-six (146), pregnant women, 64 (44%) of the pregnant women were positive to malaria parasites infection, while 82 (56%) was negative to malaria parasites infection (Table 1).

Table 2 shows the prevalence of malaria parasites infection in pregnancy according to trimesters. Sixty-two (62) pregnant women in their first trimester were screened, 29 (47%) was positive to malaria parasites test while 33 (53%) was negative. Also, forty (40) pregnant women in their second trimester were screened, 15 (38%) was positive to malaria parasite infection while 25 (62%) was negative while in third trimester, forty-four (44) pregnant women were screened 20 (45%) was positive to malaria parasites while 24 (56%) was negative. There was no significant difference among them (X²=0.916, df= 2, P-value =0.632 P>0.05).

Table 3 shows the prevalence of malaria parasites infection in pregnant women in relation to age. It was observed that 1(33%) of the pregnant women within ≤ 19 years were infected with malaria parasite while 2(67%) were not infected. Also, 6 (38%) pregnant women within age 20-24 years were infected while 10(67%) were not infected, 14(42%) of pregnant women within 25-29 years were infected, 27 (47%) for 30-34 years and 16 (47%) for 35-39 years were infected with malaria parasites. Also, pregnant women within 40 years above had no prevalence of malaria parasites infection from the two (2) pregnant women examined. The prevalence of malaria parasitic infection in relation to age of pregnant women had no statistical significant difference (X²=3.357, df= 5, P-value =0.64; P>0.05).

Table 4 shows the prevalence of malaria parasites infection in pregnant women in relation to blood groups. Out of 48 blood group A Rhesus ‘D’ pregnant women examined 26(54%) were infected with malaria parasite while 11(39%) of pregnant within blood group B Rhesus ‘D’ were also infected with malaria parasite. Also, six (6) pregnant women within blood group AB Rhesus ‘D’ examined had no prevalence of malaria parasite infection. Furthermore, 64 blood group O Rhesus ‘D’ pregnant women were examined and 27(42%) were infected with malaria parasite. The prevalence of malaria parasitic infection in relation to blood group among pregnant women was statistically significant (P<0.05) (X²=7.07, df= 3, P-value=0.04).

Table 5 shows the prevalence of malaria parasites infection in relation to genotype polymorphism. This study showed that pregnant women with Hb-AA genotype had a higher malaria prevalence of 39 (42%) while 53 (58%) had no malaria parasitic infection. Also pregnant women with Hb-AS genotype had malaria prevalence of 25 (46%) while 26 (54%) had no malaria parasitic infection. There was no prevalence of malaria infection in pregnant women with in Hb-SC and Hb-SS genotypes. The prevalence of malaria infection in relation to genotype was not statistically significant ((X²=0.211, df=1, P-value =0.645; P>0.05).
Table 1: Prevalence of Malaria parasite infection in Pregnant Women attending Antenatal Clinic

<table>
<thead>
<tr>
<th>SUBJECTS</th>
<th>Examined</th>
<th>Infected (%)</th>
<th>Not Infected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant Women</td>
<td>146</td>
<td>64 (44%)</td>
<td>82 (56)</td>
</tr>
</tbody>
</table>

Table 2: Prevalence of Malaria Parasite infection in Pregnancy according to Trimester

<table>
<thead>
<tr>
<th>TRIMESTERS</th>
<th>Examined</th>
<th>Infected (%)</th>
<th>Not Infected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIRST</td>
<td>62</td>
<td>29 (47)</td>
<td>33 (53)</td>
</tr>
<tr>
<td>SECOND</td>
<td>40</td>
<td>15 (38)</td>
<td>25 (62)</td>
</tr>
<tr>
<td>THIRD</td>
<td>44</td>
<td>20 (45)</td>
<td>24 (55)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>146</td>
<td>64(44)</td>
<td>82(56)</td>
</tr>
</tbody>
</table>

Table 3: Prevalence of Malaria parasites infection in Pregnancy in relation to Age

<table>
<thead>
<tr>
<th>AGE(years)</th>
<th>Examined</th>
<th>Infected (%)</th>
<th>Not Infected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥19</td>
<td>3</td>
<td>1(33)</td>
<td>2(67)</td>
</tr>
<tr>
<td>20 – 24</td>
<td>16</td>
<td>6(38)</td>
<td>10(62)</td>
</tr>
<tr>
<td>25 – 29</td>
<td>33</td>
<td>14(42)</td>
<td>19(58)</td>
</tr>
<tr>
<td>30 – 34</td>
<td>58</td>
<td>27(47)</td>
<td>31(53)</td>
</tr>
<tr>
<td>35 – 39</td>
<td>34</td>
<td>16(47)</td>
<td>18(53)</td>
</tr>
<tr>
<td>≥40</td>
<td>2</td>
<td>0(0)</td>
<td>2(100)</td>
</tr>
<tr>
<td>Total</td>
<td>146</td>
<td>64(44)</td>
<td>82(56)</td>
</tr>
</tbody>
</table>

Table 4: Prevalence and Susceptibility of Malaria parasites infection in Pregnancy in relation to ‘ABO’ Blood Group

<table>
<thead>
<tr>
<th>BLOOD GROUP</th>
<th>Examined</th>
<th>Infected (%)</th>
<th>Not Infected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>48</td>
<td>26(54)</td>
<td>22(46)</td>
</tr>
<tr>
<td>B</td>
<td>28</td>
<td>11(39)</td>
<td>17(61)</td>
</tr>
<tr>
<td>AB</td>
<td>6</td>
<td>0(0)</td>
<td>6(100)</td>
</tr>
<tr>
<td>O</td>
<td>64</td>
<td>27(42)</td>
<td>37(58)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>146</td>
<td>64(44)</td>
<td>82(56)</td>
</tr>
</tbody>
</table>

Table 5: Prevalence and Susceptibility of Malaria parasites infection in Pregnancy in relation to genotype polymorphism

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>Examined</th>
<th>Infected (%)</th>
<th>Not Infected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb-AA</td>
<td>92</td>
<td>39 (42)</td>
<td>53 (58)</td>
</tr>
<tr>
<td>Hb-AS</td>
<td>54</td>
<td>25 (46)</td>
<td>29 (54)</td>
</tr>
<tr>
<td>Hb-SC</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Hb-SS</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
</tbody>
</table>

Ebadan et al., IJCR 2017; 6(2): 2 – 8
DISCUSSION

The findings that 44% (n=64) of the pregnant women were infected with malaria parasite, is in line with the 45% prevalence rate reported by Mvondo et al., (1992) in pregnant women. However, the report of this study is not in agreement with the high prevalence rates (74% and 66%) reported by Akinboroye et al., (2008) and Aribodor et al., (2007) respectively. According to Onwere et al., (2008), they stated that predisposition of the immune system to infections could be attributed to climatic factor such as raining season. In fact, this study was carried out within the rainy season and the rainfall was higher and longer; given rise to much surface water. More so, Uneke, (2007) and Ekwunife et al., (2011), had reported a high rate of malaria infection during rainy seasons.

Furthermore, this study showed that there was no significant difference in the prevalence rate of malaria parasites infection among the pregnant women based on trimesters. Women at their early phase of pregnancy (first trimester), had a high prevalence of 47% (n=29) than those in the second trimester 38% (n=15) and 45% (20) in the third trimester. This correlated with the study conducted by Brabin, (1983) in western Kenya where the prevalence rate of malaria infection was highest at 13 – 16 weeks’ gestation (1st trimester), with similar number of recoveries in the 2nd and 3rd trimesters. The loss of immunity in early pregnancy was equivalent to a decrease in the rate of recovery from infection. The recovery seen in the late pregnancy suggests that the women maintained satisfactory immune response to malaria infection; re-acquiring their pre-pregnancy immune status at about the time of delivery (Saute et al., 2002). This observation could also be as a result of constant intermittent preventive treatment (IPT) given to pregnant women during antenatal care visit, which usually commence during the second trimester.

The findings of this study also showed an association between the prevalence of malaria parasite among pregnant women and age. There was high prevalence in age group 30-34, which does not agree with the report by Dicko et al., (2003) who reported that adolescents within the age range of 25-29 years and young pregnant adults within the age range of ≤ 19, 20-24 and 25-29 years, are more susceptible to malaria than older pregnant women, due continuous development of malaria immunity in older women. While age group 35-39 had a high prevalence of 47%. However, the prevalence of malaria parasites in pregnant women with respect to age was not statistically significant (P>0.05) due to variation in number of pregnant women in different age groups.

The prevalence of malaria parasite in relation to ABO blood group was 42% (n=27) with pregnant women of blood ‘O’; 54% (n=26) for blood group ‘A’; 39% (n=11) for blood group ‘B’; and no recorded prevalence for blood group ‘AB’. The prevalence of malaria parasite infection in relation to blood group among pregnant women was statistically significant (P<0.05). The prevalence of malaria among ‘ABO’ blood groups women was higher among the ‘A’ group followed by the ‘O’ group. The high prevalence number recorded among ‘A’ and ‘O’ blood groups may be due to the fact that more people in this group were sampled. Findings from studies evaluating the relationship between malaria and ‘ABO’ blood group are contradictory (Uneke, 2007). However, the high infection rates observed among all blood groups suggest that they are all susceptible to malaria. In fact, there is evidence that the ‘ABO’ histo-blood group is not correlated to the incidence of malaria (Fischer and Boone, 1998; Ekwunife et al., 2011), but it has been linked as a co-receptor in parasite and vascular cyto adherence, absent in blood group ‘AB’ with higher rosette rates among non-group O compared to group O erythrocytes (Cserti and Dzik, 2007).

The prevalence of malaria parasites infection in relation to genotype polymorphism, revealed [Hb-AA] genotype to have the high prevalence of malaria parasite (Plasmodium falciparum) infection of 39 (42%), genotype [Hb-AS] had prevalence of 25 (46%) while genotype [Hb-SC] and [Hb-SS] was not encountered in this study. The prevalence of malaria infection in this study in relation to genotype had no statistical significant difference among the genotypes with malaria parasitaemia (P>0.05). Malaria parasites infection has shown to be consistently higher in individuals with [Hb-AA] genotype compared to those with [Hb-AS] (Eteng, 2002). Resistance to malaria infection has been found to be associated with certain genetic factors. The haemoglobin [Hb-S] is known to interfere with the growth and replication of Plasmodium falciparum (Akinboroye et al., 2008). People with [Hb-AA] genotype are more susceptible to malaria because their red blood cells are conducive for the growth and development of Plasmodium falciparum (Williams et al., 2005). This report from this study is in agreement with article published by Akinbode et al., (2009). In ‘Malaria and genetic polymorphism of haemoglobin genotypes’ Who’s
study revealed that there was relationship between malaria infection and Hb genotypes and also indicated that there were differences in susceptibility to malaria among Hb genotypes AA, AS and SS individuals.

This research therefore, revealed a high prevalence of malaria parasite infection among pregnant women in first trimester, to have a high infection than other trimester of pregnancy and also indicated that pregnant women at age 34-39 to be more susceptible to infection with blood group O Rhesus ‘D’ pregnant women common and high among the subject with high malaria parasites infection. The prevalence and relation of malaria parasite infection to haemoglobin genotype polymorphism show genotype Hb-AA to be more susceptible to malaria parasites infection than other genotype [Hb-AS], with no subject encountered with [Hb-SC] and [Hb-SS].

The findings of this study, promote knowledge and assessment of red blood cells’ genotypic property in susceptibility to parasitic infections in pregnancy. It is recommended that pregnant women should protect themselves from malaria parasites infection through exposure prophylaxis by avoiding being bitten by mosquitoes. This can be achieved by wearing clothing, use of insecticides and repellents, limiting outdoor activities at night, keeping their surroundings clean, using and keeping mosquito nets in good repairs and above all, they should endeavour to report clinical symptoms for early diagnosis and treatment of cases for proper management.

REFERENCES


