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

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

[1] Quality Assurance Clina-Lancet Laboratories Victoria Island Lagos, Nigeria.[2]Department of Medical Laboratory Science, College of Medicine, Ambrose Alli University, Ekpoma, Edo State. [3]Department of Nursing Science, School of Basic Medical Sciences, College of Medicine, UNIBEN, Edo State, Nigeria. [4]Eromosele 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

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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, Plasmodium 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

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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 erythrocytes 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 by Cserti and Dzik (2007) and Uneke, (2007). Remarkably, until recently, there has been no clear answer to the crucial and obvious question: does ABO blood group affect susceptibility to life-threatening malaria? But Akinboye *et al.,* (2009) revealed 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^{o} 45^{l}N and longitude 6^{o} 08^{l}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 Gazetter, 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 bottle with identifier using a unique identification (number, trimester, age and name of the pregnant woman) which were immediately taken to the

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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 $p \leq 0.05$ 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 (X^2cal=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 (X^2cal=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) (X^2cal=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 ((X^2cal=0.211, df=1, P-value =0.645; P>0.05).

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Table 1: Prevalence of Malaria parasites infection in Pregnant Women attending Antenatal Clinic

SUBJECTS	Examined	Infected (%)	Not Infected (%)
Pregnant Women	146	64 (44%)	82 (56)

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

TRIMESTERS	Examined	Infected (%)	Not Infected (%)
FIRST	62	29 (47)	33 (53)
SECOND	40	15(38)	25(62)
THIRD	44	20(45)	24(55)
TOTAL	146	64(44)	82(56)

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

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

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

BLOOD GROUP	Examined	Infected (%)	Not Infected (%)
A	48	26(54)	22(46)
B	28	11(39)	17(61)
AB	6	0(0)	6(100)
O	64	27(42)	37(58)
TOTAL	146	64(44)	82(56)

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

GENOTYPE	Examined	Infected (%)	Not Infected (%)
Hb-AA	92	39 (42)	53(58)
Hb-AS	54	25 (46)	29(54)
Hb-SC	0(0)	0(0)	0(0)
Hb-SS	0(0)	0(0)	0(0)

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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 and bushes around living homes that can support the breeding of mosquitoes. 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 3rdtrimesters. 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 falciparium*) 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 heamoglobin [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 Akinboye *et al.*, (2009). In 'Malaria and genetic polymorphism of haemoglobin genotypes'' Who's

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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.

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