ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of DEVELOPMENT RESEARCH

International Journal of Development Research
Vol. 5, Issue, 08, pp. 5317-5320, August, 2015

Full Length Research Article

MALARIA IN PREGNANCY AND ITS ASSOCIATION WITH ABO BLOOD GROUP AND HAEMOGLOBIN GENOTYPE

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ARTICLE INFO

Article History:
Received 19th May, 2015
Received in revised form
08th June, 2015
Accepted 09th July, 2015
Published online 31th August, 2015

Key words:
Prevalence,
Malaria, pregnancy,
ABO blood group,
Hemoglobin genotype.

ABSTRACT

The prevalence of malaria among 250 pregnant women attending antenatal clinic in Agbonchia Health Centre Eleme, Rivers State, Nigeria was investigated using thick and thin blood film stained with Giemsa. The stained slides were examined microscopically for malaria parasites. The prevalence of malaria among the pregnant women was 40.6% and the prevalence among 50 non-pregnant women used as control was 16.0%. The prevalence of malaria by age group among the pregnant women was 36.1-46.8% from age 20-40yrs. The prevalence of malaria by haemoglobin genotype are HbAA 42.4% and HbAS 34.6%, statistically at $p<0.05$ there is no significant difference in the prevalence of malaria between HbAA and HbAS, but there is significant difference in the prevalence of severe malaria between HbAA and HbAS. The prevalence of malaria among ABO blood groups showed that blood group O had the highest prevalence 44.4% but minimal episode of severe malaria compared with the other blood groups. Blood group O and HbAS protects against severe malaria in pregnancy.

INTRODUCTION

Malaria is a life threatening parasitic infection transmitted by female anopheles mosquitoes. The world Health Organization documented 219 million cases of malaria in 2010 and the disease killed between 660,000 to1.2 million people globally (Nayyar *et al.,* 2012). About 125 million pregnant women are at risk of infection with malaria each year in sub Sahara Africa. Malaria infection during pregnancy is a serious public health problem in tropical and sub-tropical regions of the world (Nosteri *et al;* 1991). Pregnant women are more susceptible to *Plasmodium* infection, with more episodes of clinical malaria, increased prevalence and parasite density during episode (McGregor, 1984). They are at higher risk of malaria infection as pregnancy reduces their immunity to infection (Shulman and Dorman, 2001). Malaria is one of the major cause of mental, fatal and neo-natal mortality worldwide (Ballamy, 2004). The high rate of malaria transmission predisposes pregnant women to premature delivery, low birth weight and stillbirth (FMH, 2005a). Miscarriage, preterm birth and stillbirth are common in pregnant women infected with malaria (Feresus *et al*; 2004). Malaria infection in malaria endemic areas are associated with about 20,000 newborn death, 10,000 maternal deaths, 8-14% of low birth weight babies and 3.8% of all infant deaths (WHO, 2006). Blood group O and haemoglobin genotype HbAS had been shown to confer protection against malaria and severe malaria (Otajevwo and Enbulele, 2014, Hailu and Kebede, 2013, Zerihum *et al*., 2010, Deepa *et al*., 2011). Principally, the impact of malaria infection in pregnancy is due to the presence of the parasites in the placenta which is the major cause of anemia. The objectives of this study were: to determine the prevalence rate of malaria among pregnant women attending antenatal in Agbonchia clinic, Eleme. The prevalence of malaria by age group among the pregnant women, and the protective role of haemoglobin genotype HbAS and blood group O in pregnancy.

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MATERIALS AND METHODS

Study Area: This study was carried out among pregnant women attending antenatal at Agbonchia Health Centre in Eleme Local Government Area of Rivers State, Nigeria. Eleme Local Government covers an area of about 138km^2 and a population of 200,884. It is located between latitude $4^0$45S and $4^0$50N, longitudes $7^0$05E and 7.105W. Agbonchia is in the

fresh water zone, with characteristic rainforest vegetation of the Niger Delta Area. It is the most populated town in Eleme and the people are predominantly farmers.

Subjects: A total of 250 pregnant women attending antenatal in Agbonchia Health Centre, Eleme from May – August, 2014 were selected randomly without prior knowledge of their clinical or family history. The women were of varying age ranging from 20 – 40 years; whereas 50 non-pregnant women were used as control.

Collection of samples: Venous bloods were collected by phlebotomy with the aid of tourniquet applied on the upper arm of the subject to enable location of vein. Ethanol (70%) was used to clean the site of collection, allowed to dry and blood collected with hypodermic syringe directly into EDTA bottle. This was gently mixed properly to avoid coagulation.

Preparation of blood smear: The preparation of thick and thin blood smears were done according to (Monica Cheesbrough, 2002).

Thin smear: Thin blood smear were allowed to air dry for 10 minutes and fixed with methanol by dipping the thin smear carefully in methanol for 5 seconds (to avoid methanol touching the thick smear). This was allowed to dry and Giemsa stain was applied for 8 – 10 minutes. This was rinsed with distilled water and allowed to dry.

Thick smear: The thick smears were air dried for about 30 minutes (not fixed in methanol) but dipped in water to de-haemoglobinize. This was allowed to dry and stained by the same procedure as the thin smear. When the smears were dry, immersion oil was applied and they were viewed with X100 under the microscope (Cheesbrough, 2002).

Microscopic examination: The back of each air dried blood film slides were carefully cleaned with cotton wool and examined using 10x and 40x firstly for cell morphology and detection of malaria schizonts, trophozoites, and gametocytes and 100x objective for *Plasmodium spp.*

$$\text{Parasite per ml} = \frac{\text{Parasite count x 500}}{\text{Set range of WBC (500)}}$$

Determination of Blood Genotypes of Subjects

Haemolystate of each blood sample was prepared by washing the EDTA blood 3 times with normal saline. Distilled water was added to the test tube containing the blood; this was allowed for 5 minutes for complete haemolysis before use.
Haemoglobin genotype separation was carried out using electrophoresis method as described by (Cheesbrough, 2002).

Determination of blood group: The blood groups of subjects were determined by rapid tile grouping method. Autisera A, B, AB, and antisera D were reacted with the EDTA anticoagulated blood for the determination of blood groups. For the Rhesus negative factor, each blood was spun in a centrifuge and observed for agglutination both macroscopically and microscopically.

Statistical analysis: Chi-square was used to analyze data.

RESULTS

Out of 250 pregnant women examined for malaria parasiteamia, 102 (40.8%) were infected with malaria parasites. The 50 non pregnant women used as control 8(16.0%) were positive as shown in Table 1.

Table 1. Prevalence of malaria among pregnant/non pregnant women

Subjects	Number examined	Number positively/ percentage
Pregnant women	250	102 (40.8)
Non pregnant women	50	8 (16.0)

Numbers in parenthesis = Percentages

The pregnant women were group into 4 groups at 6 years (yrs) interval. Ages 20 – 25 yrs 71, 31(43.6%) were positive, ages 25 – 30 yrs, 83, 30(36.1%) positive, ages 31 – 35 yrs, 64, 26(40.6%) were positive, ages 36 – 40 yrs, 32, 15(46.8%) had malaria respectively, Table 2.

Table 2. Prevalence of malaria among pregnant women by age group

Age groups (yrs)	No sample	Numbers positive/ percentages
20 – 25	71	31 (43.6)
26 – 30	83	30 (36.1)
31 – 35	64	26 (40.6)
36 – 40	32	15 (46.80
Total	250	102 (40.8)

Number in parenthesis = Percentages

Among the 250 pregnant women, 198 (79.2%) subjects were genotype AA, whereas 52 (20.8%) were genotype AS. Among genotype AA, 84(42.4%) had malaria, while AS 18(34.5) were positive for malaria parasites. Among the AA blood genotype, 18(7.2%) suffered severe malaria, while genotype AS 2(0.80%) had severe malaria.

Table 3. Prevalence of malaria and severe malaria by haemoglobin-genotype among pregnant women

HB-Genotype	No sampled	No positive	Severe malaria in group	Overall Severe malaria
AA	198 (79.2)	54 (42.4)	18 (9.1)	18(7.2)
AS	52 (20.8)	18 (34.6)	2 (3.8)	2(0.8)
Total	250	102 (40.8)	20 (8.0)	20(8.0)

Number in parenthesis = Percentages

From the subjects examined, 51 were blood group A, 18(35.3%) had malaria; 30 were blood group B, 10(33.3%) were positive, 7 subjects were blood group AB, 2(28.6%) are infected, whereas 162 were blood group O, 72(44.4%) had malaria parasitemia respectively. The prevalence of severe malaria among ABO blood group were, blood group A, 10(19.6%), blood group B, 5(16.7%), blood group AB, (0.00) and blood group O, 2(1.2%) had severe malaria and the overall prevalence of severe malaria were, blood group A, 10(4.0%), B, 5(2.0%), AB, (0.0) and O, 2(0.8%) respectively.

Table 4. Prevalence of malaria and severe malaria by ABO blood group among pregnant women

ABO-Group	No. sampled	No. positive	Severe malaria in each group	Overall severe malaria
A	51 (20.4)	18 (35.3)	10(19.6)	10(4.0)
B	30 (12.0)	10 (33.3)	5(16.7)	5(2.0)
AB	7 (2.8)	2 (28.6)	0 (0.0)	0(0.0)
O	162(64.8)	72(44.4)	2(1.2)	2(0.8)
Total	250	102 (40.8)	17 (6.8)	17(6.8)

Numbers in parenthesis=Percentages

DISCUSSION

The pregnant women examined were 250 and the prevalence of malaria parasitemia among them was 40.8%; while the prevalence among 50 non-pregnant women used as control was 16.0%. Other workers investigating the prevalence of malaria in pregnancy had recorded different rates of malaria prevalence among pregnant women from different zones. In Southwest Nigeria, the prevalence observed are from 36.5-72% among pregnant women (Adefioye, 2007, Anorlu *et al*., 2001, Okwa, 2003). In Owerri Southeast Nigeria, a prevalence of 11.1% was observed (Ogbusu *et al.*, 2008), whereas (Uneke *et al*., 2007) observed a prevalence of 16%. In Southsouth Nigeria, (Wagu *et al*., 2013) had a prevalence of 26%, while (Wagbatsoma and Omoike, 2008) recorded a prevalence 21.2%. In Yola Southeast of Adamawa Nigeria, (Chessed *et al*; 2008) observed a prevalence of 56.3% among pregnant women.

Wagu *et al.,* (2013) in Port Harcourt, Rivers State, Nigeria, had a prevalence of 26%. This prevalence was low compared with that obtained in this study (40.8%). The might be due to the effect of the Rivers State Government, Rollback Malaria Programme. In 2009, biolavicide was used in Port Harcourt metropolis to control mosquito larvae by preventing their maturing into full fledged mosquitoes. Insecticide treated nets were distributed free to the inhabitants of Port Harcourt. Malaria drugs were administered free to malaria infected individuals. The Rollback Malaria Programme was not effective in the rural communities such as Agbonchia, where this study was conducted. This may have accounted for the difference in prevalence observed in Port Harcourt metropolis and Agbonchia, both in Rivers State. The prevalence obtained in this study is similar to the prevalence observed in some other part of Nigeria, among pregnant women.

The prevalence of malaria among pregnant women was 40.8%, while the non pregnant women had a prevalence rate of 16.0%. Statistical analysis at $P<0.05$ showed significant difference in the prevalence rates of malaria among pregnant and non-pregnant women. Pregnancy may predispose to malaria because of the lowered immunity associated with pregnancy. The risk associated with malaria in Sub-Sahara Africa among pregnant women is well known, as declared in 2005 by African Heads of States; that 60% of pregnant women in Sub- Saharan Africa are at the risk of malaria (USAID, 2005). The prevalence of malaria parasitemia among pregnant women by age groups showed that, the age group 36 – 40 yrs had the highest prevalence rate 46.8%. The high prevalence of *Plasmodium falciparum* infection among age groups 20 – 40 years may be attributed to frequent childbirth which may cause low haemoglobin level, coupled with reduced immunity known to be associated with pregnancy; the level of poverty prevalent in the society and malnutrition may aid in reducing the immune status of some pregnant. Although, at p<0-05 there was no significant difference in the prevalence of malaria among the age groups. The result obtained agrees with the findings of (Adefioye *et al*., 2007). The prevalence of malaria parasitemia among pregnant women by haemoglobin genotypes are HbAA 42.4% and HbAS 34.6% and the prevalence of severe malaria are AA 7.2% and AS 0.8% respectively. In a related study, (Yahaya *et al*., 2013) observed prevalence of HbAA 42.3% and HbAS 32.5% in Idah, Kogi State, Nigeria. Uneke *et al*., (2007) had malaria prevalence of HbAA 17.9% and HbAS 12.5% parasitemia. Hamoglobin HbAA was more prevalent as compared to HbAS as shown in Table 3. This was also observed by (Nwafor and Bamigo, 2001., Akhigbe *et al*., 2009). Statistically, there was significant difference at $P<0.05$ in the prevalence of severe malaria among pregnant women by blood genotype.

The potential protective effect of sickle cell haemoglobin, HbAS against malaria is because sickle cell causes a defect in haemoglobin molecule of the blood by distorting it into curved shape (sickle). Due to this phenomenon, there is ineffective intake or release of oxygen. The infestation with malaria parasites causes the red cell to sickle the more and hence are removed from circulation when the malaria parasite had not completed their life cycle in the blood cells (Modiano *et al*; 2001; Akhigbe *et al*; 2011; Uzoegwu and Onwurah, 2003). From results obtained, blood group O is associated with high prevalence of malaria (44.4%) compared to other blood groups, but very low episode of severe malaria. This finding is similar to those obtained by (Fisher and Boone, 1998; Adam *et al*., 2007; Amoo *et al*., 2008).

Statistical analysis $p>0.05$ did not show significant difference in the prevalence of malaria among ABO blood groups but there was significant difference in prevalence of severe malaria between blood group O and blood group A and B respectively. Blood group O confers significant protection to severe *Plasmodium falciparum* malaria in pregnancy, compared to non 0 blood groups. Reduced resetting observed in O group offers protection to *P. falciparum* infection (Row *et al.,* 2007; Zarihun *et al.,* 2011). Statistical analysis at $p< 0.05$ showed significant difference between the rate of malaria infestation and severe malaria in blood group O subjects. Rosetting parasites and resetting are risk factors associated with severe malaria in pregnant women with non O blood group.

Conclusions: The prevalence of malaria among pregnant and non-pregnant women was significant, predicting that pregnancy may predispose to malaria. Haemoglobin genotype HbAS and blood group O offers some protection against *P. falciparum* severe malaria in pregnant women, irrespective of their reduced immune status.

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