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Full Length Research Paper

Effect of blood group and demographic characteristics on malaria infection, oxidative stress and haemoglobin levels in South Western Nigeria

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Malaria infection generates oxidative stress which has serious effect on the haemoglobin (Hb) level of the infected individuals. This work studied the effect of blood group, age and gender on prevalence of malaria, oxidative stress and Hb level. 5 ml of blood samples were collected into EDTA bottle from 120 volunteered adult males and non-pregnant females. The plasma was separated and used to quantify oxidative stress by measuring malonaldehyde (MDA) and superoxide dismutase (SOD) levels using colorimetric method, while haematological parameter and malaria parasite screening was done using a whole blood. The parasite density and MDA levels were significantly higher ($P < 0.05$) in individuals with blood group A than those with blood group B and O. SOD and Hb levels were lower in those with blood group A than those with blood group B and O. The parasite density and MDA levels were significantly higher in age group 18 - 21 years than other age groups but SOD was higher ($P < 0.05$) in age group 22 - 25 and 26 - 30 than age group 18 - 21. The parasite density and MDA were higher ($P < 0.05$) in males than females in this study. The findings indicate that gender, sex and blood group have impact on malaria infection and oxidative stress.

Key words: Malaria, blood grouping, gender, oxidative stress, haemoglobin, age.

INTRODUCTION

Malaria still remains one of the unconquered diseases in the world today. It is common in the tropics, especially in the south of Sahara (WHO, 1997). Malaria causes about 350 - 500 million infections in humans and it is responsible for approximately 1.3 - 3 million deaths annually (Snow et al., 2005). In Africa, mortality remains high because there is limited access to treatment (Weather et al., 2002). Children and pregnant women living in malaria endemic areas are at risk of varying degrees of malaria morbidity and mortality (Falade et al., 2008). Between 25 and 39% of deaths in children <5 years old has been attributed to malaria infection (Macete et al., 2006). It is estimated that 200 children in Africa die of malaria every hour of each day all year round (Jepsen, 2000) while at least 24 million pregnancies are threatened by malaria infection each year (Shane, 2004).

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Different methods such as intermittent presumptive treatment (IPT) and insecticide-treated bed net (ITN) have been adopted to reduce the prevalence of malaria infection among pregnant women and children (Falade et al., 2007). In addition, an individual living in malaria endemic areas also have the tendency to develop immunity against malaria infection. Thus, in *Plasmodium falciparum* endemic areas, protective immunity against malaria infection is acquired slowly after a large number of infections and its maintenance requires a sustained exposure to infected mosquito (Akanbi et al., 2009). The level of immunity against malaria has also been related to age of the individuals living in malaria endemic areas (Akanbi et al., 2006). Some individuals have the benefits of genetically controlled protection mechanisms against malaria, such as blood group determinants, abnormal haemoglobin and red blood cell enzymes deficiency. It has been reported that severe malaria occurs more frequently in individuals with non-O blood group (Fischer and Boone, 1998); even gender can also affect the

Table 1. Effect of gender on malaria parasites, MDA, SOD and Hb levels

Gender	n1	n2	Parasite density /µl	MDA (nmol/ml)	SOD (µmolmg^{-}protein)	Hb (g/dl)
Male	68	67	724.7 ± 20	1.97 ± 0.3	22.9 ± .1	13.1 ± 2
Female	52	52	616.9 ± 19	1.58 ± 0.1	21.9 ± 1.61	0.2 ± 1

n1 represent the total number study according to their gender; n2 represent the total number positive according to their gender; values are expressed as mean ± S.D.

prevalence of malaria infection (Mande et al., 1984).

Various studies have also established that malaria infection is accompanied by increased production of reactive oxygen species (ROS) which indicates the environment for oxidative stress (Akanbi et al., 2009). Malaria parasite is sensitive to oxidative stress, and the level of oxidative stress is influenced by the severity of malaria infection as measured from plasma parameters (Farombi et al., 2003; Sibmooh et al., 2004). Though oxidative stress destroys malaria parasites, but may also render host tissues such as erythrocytes more vulnerable to oxidative damage and thereby resulting to anaemia in malaria infected individuals (Egwunyenga et al., 2004*).*

Unfortunately, most of these studies on prevalence of malaria infection have focused on children and pregnant women, neglecting adult males and non-pregnant females who are equally exposed to mosquito vectors. Therefore, this study reports the role of gender, age and blood groups on prevalence of malaria infection, oxidative stress and haemoglobin (Hb) levels in adult males and non-pregnant female in south western Nigeria.

MATERIALS AND METHODS

The study was carried out in Akungba-Akoko, Ondo state Nigeria, at the peak of the rainy season (August to October 2006) among one hundred and twenty (68 males and 52 non-pregnant females) individuals who gave informed consent. However, patients who had received blood transfusion within the last two months before the study were excluded from the study. Questionnaires were used to obtain information on demographic variables such as: age, sex and antimalaria drugs previously used, and last episode of malaria was also collected from all the participants. Study participants were grouped into three age groups. The youngest participant was 18 years old while the oldest was 30 years old. In grouping participants, a five-years age interval was used (18 – 21, 22 – 25 and 26 - 30 years).

Blood collection

Five milliliters of blood was collected by venipuncture from each participating individual. 2 ml of blood was immediately transferred into a bottle containing Ethylenediamine tetra acetic acid (EDTA) to determine haematological parameters, while the remaining 3 ml of blood was transferred into a serum bottle and used to obtain serum for determination of oxidant and antioxidant levels. Patients who have been transfused within the last two months before the study were excluded from the study. Those who were malaria positive were treated accordingly. The study was reviewed and approved by the Local Institution Review Board.

Parasitological study

Thick and thin peripheral blood films were made on the same slide from each sample. The thin film was carefully fixed with methanol and all the slides were flooded with Giemsa's stain diluted 1 in 10 with buffer pH 7.2. The slide was stained for 20 min and examined microscopically for the presence and load of parasites. For the positive slides, the number of malaria parasite counted per 200 white blood cells was recorded and used to calculate parasite density assuming 8000 leucocytes/µl of blood.

Determination of Hb level

Hb level was determined by colorimetric method using Drabkin's solution as described by Watson-Williams et al. (1965).

Determination of malondialdehyde (MDA)

MDA in serum was assessed by measuring the thiobarbituric acid reactive substances (TBARS) and expressed in term of MDA formed per mg protein according to the procedure described by Rice-Evans et al. (1986).

Determination of superoxide dismutase (SOD)

SOD level was measured by the method described by Marklund and Marklund (1974).

Determination of ABO blood group

Three spots of blood from each subject were made on the white plain tile and a drop of each antiserum A, B and D was applied to each spot respectively. The mixture was further stirred with a plastic stirrer and rocked for some time. Signs of agglutination were observed showing red pigment. Antisera D were used to determine the Rhesus factor.

RESULTS

Table 1 shows that, of the 120 adults studied, 68 were males while 52 were females. 99% were malaria positive and 67 and 52 of male and female, respectively, were malaria positive. The mean parasite density was significantly higher ($P < 0.05$) in males (724.7 ± 20/µl) than in females (616.9 ± 19/µl). The mean MDA and Hb levels were also significantly higher ($P < 0.05$) in males than in the females. Table 2 summarizes the effect of age on parasite densities, MDA, SOD and Hb levels. Parasite density was significantly higher ($P < 0.05$) among those

Table 2. Effect of age on parasite density, MDA, SOD and Hb levels.

Age (years)	n1	n2	Parasite density(/µl)	MDA (nmol/ml)	SOD (µmolmg^{-}protein)	Hb (g/dl)
18-21	34	33	774±20	2.35±0.5	187.5±10.1	10.3±1.2
22-25	56	56	671±18	1.84±0.1	284.6±20.1	11.1±1.0
26-30	30	30	632±17	1.37±0.2	196.8±17.2	11.0±1.1
Total	120	119				

n1 represent the total number study per age group; n2 represent the total number positive per age group; values are expressed as mean± S.D.

Table 3. Effect of blood groups on parasite density, MDA, SOD and haemoglobin levels.

Blood groups	n1	n2	Parasite density/µl	MDA (nmol/ml)	SOD (µmolmg^{-} protein)	Hb (g/dl)
A	20	20	814±20.3	1.92±0.1	19.4±12.3	11.0±0.2
B	22	22	754±18.2	1.88±0.3	35.9±16.1	11.2±0.1
O	78	77	634±14.5	1.89±0.2	21.7±12.5	13.5±1.1
Total	120	119				

n1 represents the total number study according to the blood group; n2 represents the total number positive according to the blood group; values are expressed as mean± S.D.

within the age range 18 - 21 years than all other age groups. The same pattern was observed for MDA levels which was significantly higher ($P < 0.05$) in age group 18 - 21 (2.35 ± 0.5) than age group 26 - 30 (1.37 ± 0.2) and age group 22 - 25 years (1.84 ± 0.1). However, this pattern was different for SOD levels. SOD level was significantly higher in age group 22 - 25 years than other age groups. There were no significant differences in Hb levels among all the age groups (Table 2). Table 3 shows that the parasite density was significantly higher ($P < 0.05$) in those with blood group A than other blood groups and it was least in those with blood group O. MDA level was marginally higher in those with blood group A (1.92 ± 0.1) than those with blood group B and O (1.88 ± 1.3 and 1.89 ± 0.2, respectively), while SOD was significantly higher ($P < 0.05$) in those with blood group B than those with blood group A and O. The Hb levels were significantly higher ($P < 0.05$) among those with blood group O than those with blood group A and B.

DISCUSSION

Malaria has been a major selective force on the human population resulting in emergence of several erythrocyte polymorphisms which confer resistance to severe malaria (Himiedan et al., 2004). This study has further confirmed that blood groups have an influence on malaria parasite density. The parasite density was higher in individuals with blood group A and B than those in the blood group O. This agrees with the previous studies where it has been reported that patients with blood group A have greater risk for severe malaria with a trend for a protective effect of blood group O (Fisher et al., 1998; Lell et al., 1999). The mechanism that are involved in rendering individuals with blood group A vulnerable to malaria may be related to the rosette formation by the *P. falciparum* with those with blood group A. It has been confirmed that some strains of *P. falciparum* preferentially trigger rosette formation depending on the red blood cell group (Barragan et al., 2005*).* The high frequency of those with blood group O in this study may also suggests that those with Blood group O may have a selective advantage over other blood groups. This also occurred in the study carried out by Lell et al. (1999). The increase in MDA and decrease in SOD level among those with blood group A suggests that there is oxidative environment and stress in this blood group as compared with other blood groups. This could be because of the higher malaria parasite density which is associated with this blood group in this study as compared with other blood groups. Previous study have confirmed that MDA levels may reflect the severity of a disease process (Das et al., 1993), and those with malaria parasite has been shown to have a higher MDA level (Egwunyenga et al., 2004). The high Hb level among those with blood group O could be associated with very low malaria parasite density that is found among people with blood group O as compared to other blood groups. Haemoglobin is the major food material of malaria parasite (Kulkarni et al., 2003). The increase in malaria parasites present causes a serious reduction in the haemoglobin level, as malaria parasite depends on haemoglobin for its food.

The highest mean parasite density was found among those within the age range 18 - 21 years while it was least among those within the age range 26 - 30 years. This could be due to the number of exposure to mosquito bites among people within the age range of 26 - 30 years

as it has been reported that the number of exposure to mosquito bite increases with age. The number of exposure to mosquito bites by individuals had been confirmed to increase the level of immunity against malaria infection (Hommel, 1991), and this increases with age. The MDA level was significantly higher among age group 18 - 21 years than other age groups, while SOD level was least among age group 18 - 21 than other age groups. The increase in MDA and decrease in SOD level among age group 18 - 21 years suggests that there is an oxidative environment and stress in this age group when compared with other age groups. This could be because of the high level of the malaria parasite density observed in the age group 18 - 21 years. There were no differences in Hb levels of all the age groups.

The mean parasite density was higher in male than in female. This could be due to the fact that males expose their bodies more than females when the weather is hot and thus increases their chances of being bitten by the mosquito. Other studies have shown that the genetic and humoral factors could be attributed to the higher immunity level to malaria and a variety of other parasitic diseases in female than in male (Mandel et al., 1984). The high MDA level in male could be because of the high parasite density that was observed in male. The SOD was marginally higher in male than in female, while Hb level was significantly higher in male than in female.

This study shows that blood grouping, age and gender have a serious effect on the parasite density. It was also discovered that the level of oxidative stress increases with increase in parasite density.

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