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Effect of Age on Prevalence of Malaria and Oxidative Stress in Children

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Objective: To determine the effect of age on prevalence of malaria infection and oxidative stress in children.
Study Design: One hundred and seventy children within the age range 0-5 years were recruited into this study from Specialist hospital, Ikare, Ondo state, Nigeria. The children were divided into three age range groups. The first group was within age range 0-1 year, the second group was within age range 2-3 years and the last group was within age range 4-5 years. The study considered those who were malaria positive only.
Methodology: Five milliliters of blood was collected by venipuncture from each child and the blood was immediately transferred into ethylene diamine tetraacetic acid (EDTA) bottle. The plasma was separated from the whole blood and was used to determine the triglyceride, total plasma protein, malondialdehyde (MDA), superoxide dismutase (SOD) and reduced glutathione (GSH) levels. The whole blood was screened for the presence of malaria parasite.
Results: The prevalence of malaria infection was significantly higher in the group within the age range 2-3 years than in other age ranges studied, but the parasite density was significantly higher ($P<0.05$) in the group within the age range 4-5 years than other age ranges studied. The parasite

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density was significantly lower in the group within the age range 0-1 year than those within the age ranges 2-3 years and 4-5 years. There was a significant increase in total protein, SOD and GSH levels in the children within the age range 0-1 year as compared with other age groups studied. The triglyceride and MDA levels were significantly higher in age group 2-3 and 4-5 years than in the children within the age range 0-1 year.

Conclusion: This study showed that the older children were more susceptible to malaria infection and oxidative stress as compared to those within the age range 0-1year, therefore, more attention should be given to these age groups.

Keywords: Children; malaria parasite; oxidative stress; lipid profile; protein level.

1. INTRODUCTION

Malaria is the most deadly vector borne disease which constitutes a significant public health problem in over 100 countries with well over 500 thousand deaths annually [1]. About half of the world's population lives where the infection is endemic [2], though it is considered to be eradicated in the developed countries but there is still an occurrence of imported malaria cases from the immigrants who travelled from malaria endemic areas [3,4], therefore malaria still remains the most important parasitic disease that afflicts man in the world which needs the utmost attention [5]. Children and pregnant women have been reported to be most vulnerable to malaria attack [6]. In 2009 malaria was estimated to attack about 225 million people globally, while it was responsible for the death of about 781,000 people in the same year, accounting for 2.23% of death worldwide [7], but as a result of concerted efforts malaria death has been reduced to 655,000 people in 2010 [8]. Children are the most affected, especially those within the age range of 6 months to 5 years [9]. An average child under 5 years of age suffers at least two serious episode of malaria attack in a year, while those above 5 years suffers at least one episode [2]. About 90% of death is recorded as a result of malaria infection in children. Children living in malaria endemic areas are at increased risk of death due to malaria as a result of some complications with the features such as impaired consciousness, jaundice, respiratory distress, and hypoglyceamia which are common in chronic malaria [10]. Cerebral malaria in children which is often caused by *P. falciparum* has been reported to have a mortality rate of 25% even with the best treatment [11]. Early diagnosis and immediate treatment of malaria infection with the appropriate drugs is important and this prompt action will save the life of the infected child [11]. Five main species of *Plasmodium* are responsible for the transmission of malaria infection, but most of the complications that occur during and after malaria infection are as a result of untreated *P. falciparum* [12].

Apart from some basic complications that occurred during infection with *P. falciparum,* changes in lipoprotein level during acute malaria infection, especially *P. falciparum* infection has also been reported [12]. Lipoproteins are very essential component of the body system, which are involved in the transportation of some particles such as cholesterol and triglyceride to membrane and some organs in the body [13], but their levels vary in the body. The abnormalities in the metabolism of lipid and lipoproteins are associated mostly with both infectious and non infectious diseases [14]. The increased total cholesterol level in the body has been associated with the several diseases in which malaria infection is one of them. The changes in the level of lipoproteins during malaria infection have been reported to be associated with the modification in the oxidation of lipoproteins, and the level of modification is related to the severity of the malaria infection [15]. The importance of protein in the body cannot be overemphasized, especially during the immune response to various infections in the body. Reduction in the level of total protein in the body can affect the defense mechanism of the body, but it has been reported that there is always a reduction in protein level during autoimmune disease and various chronic infections [16].

Oxidative stress is another common phenomenon during malaria infection. It is believed that oxidative stress is one of the means by which the body can resist the parasite from being developed through the production of reactive oxygen species (ROS) and this could also be responsible for the destruction of cell in the host body [17]. ROS has been reported to be an armory used by immune system to destroy the parasite in the body [14]. Drastic increase in malondialdehyde (MDA) and decrease in superoxide dismutase (SOD) and reduced

gluthathione (GSH) levels have been reported in malaria infected individuals, which is an indication of the oxidative stress environment [18]. Though all these parameters have been studied in the adults infected with malaria parasite but there is no enough information about the effect of age on prevalence of malaria infection, plasma protein and oxidative stress in children, therefore this study investigated the effect of age on malaria infection and oxidative stress in children living in Akoko areas of Ondo state, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Population

The study was carried out at specialist hospital, Ikare, Ondo State, Nigeria. One hundred and seventy children within the age range of 0-5 years who were ill with (Temperature>37ºC), fever, diarrhoea, headache, vomiting, and some other malaria symptom were recruited for this study. The details of the study were explained to the mother or guardian of the children and the verbal consent was sought for and obtained from them. Questionnaires were distributed among the parents who gave their consent purposely to get some information about their children such as; age, sex, haemoglobin genotype, blood group, last episode of malaria, and drugs previously given to them. The children who were critically ill and those that have been transfused about two months before the commencement of this study were excluded from the study. Those with serious symptoms of malaria infection were treated immediately by the medical practitioner.

2.2 Blood Collection

Five milliliters of blood was collected by venipuncture from each child by the trained technologists under the supervision of the medical doctors, and the blood was immediately transferred into ethylene diamine tetraacetic acid (EDTA) bottle. The plasma was later separated into the plain bottles and different biochemical parameters (triglyceride, plasma protein, MDA, SOD and GSH) levels were determined from the plasma. The whole blood was screened for the presence of malaria parasite.

2.3 Malaria Parasite Screening

Thick film of the whole blood was prepared on the slide to screen for the presence of the malaria parasite. The film was allowed to dry on the slide before it was stained with 10% Giemsa stain. The stain was left for twenty minutes after which it was washed away from the slide and slide was mounted on the light microscope for the screening of the malaria parasite. A slide was regarded as negative when no parasite was seen after screening minimum of 200 fields. For the positive slide the number of parasite counted per 200 white blood cells was used to calculate parasite density on the basis of 8000 leucocyte/µl of blood as described by Nwagwu et al. [19].

2.4 Determination of Biochemical Parameters

2.4.1 Determination of triglyceride level and Protein level

Total triglyceride level was measured by the Tietz [20] method as described in the manual of Randox total triglyceride kit. Total protein was determined using Biuret method as described by Peter et al. [21].

2.4.2 Determination of Plasma MDA, GSH, and SOD level

MDA, GSH, and SOD were used as oxidative indicators.

2.4.2.1 Determination of plasma MDA

Lipid peroxidation in plasma was assayed by measuring the thiobarbituric acid reactive substances which is expressed in MDA formed per mg protein as illustrated by Vashney and Kale [22]. 0.2 ml of plasma was diluted with 0.2 ml of distilled water to make 0.4 ml. The solution was mixed with 1.6 ml of 0.1 M Tris KCl buffer, and this was added to 0.5 ml of 10% TCA. 0.5 ml of 52 mM TBA was finally added and the solution was placed in water bath for 45 minutes at 80°C and was later cooled in ice and centrifuged at room temperature for 10 minutes at 3,000 rpm. The absorbance of the clear supernatant was measured by spectrophotometer against reference blank of distilled water at 532 nm.

2.4.2.2 Determination of plasma GSH level

GSH level was determined by the method described by Jollow et al. [23]. 0.2 ml of plasma was added to 1.8 ml of distilled water to make 2 ml of plasma solution. The solution was deproteinated by addition of an equal volume (2 ml) of 4% sulfosalicyclic acid. The solution was

centrifuged at 8,000 rpm for 15 minutes at room temperature. 0.5 ml of supernatant was added to 4.5 ml of Ellman reagent and it was incubated at room temperature for 30 minutes and the absorbance was read at 412 nm with spectrophotometer against the blank. The blank was prepared with 8 ml of 0.1 m phosphate buffer, 2 ml of diluted precipitating solution.

2.4.2.3 Determination of plasma SOD level

SOD activities were determined by the method demonstrated by Misra and Fridovich, [24]. 0.2 ml of plasma was diluted in 1.8 ml of distilled water to make 2ml of plasma solution. 2.5 ml of 0.05 M phosphate buffer was added to 0.2 ml sample dilution. The reaction started immediately when 0.3 ml of freshly prepared 0.03 mm of adrenaline was added to the solution and this was mixed thoroughly. The increase in absorbance was read in spectrophotometer at 480 nm at every 30 seconds for 150 seconds and average of the absorbance for each sample was recorded.

2.5 Statistical Analysis

The differences among groups were analyzed by the one-way analysis of variance (ANOVA). Proportions were compared using the chi-square, while the means were compared using the *t*-test. Inter-group comparisons were done using Duncan's Multiple Range Test (DMRT) with 95% confidence intervals. The SPSS 15.0, SPSS Inc., Chicago, Illinois, USA, was used for this analysis. The results were expressed as mean ± standard deviation (SD). The level of significance was estimated at P <0 .05.

3. RESULTS

Among the children studied, the prevalence of malaria infection was higher in the group within the age range 2-3 years than in the age ranges 0-1 and 4-5 years. The parasite density was significantly higher (P<0.05) in the group within the age range 4-5 years when compared with other age ranges studied. The parasite density was significantly lower (P<0.05) in the group within the age range 0-1 years than those within the age ranges 2-3 years and 4-5 years as shown in Table 1.

The protein level was significantly higher in the age group 0-1 years than in other age groups studied. The level of protein (16.70±2.60) was significantly reduced in the age group 4-5 years when compared with age groups 0-1 year and 2-3 years (27.06±2.05 and 22.90±1.50 respectively) (Table 2). Triglyceride was significantly lower in age group 0-1 year (77.67±3.50) when compared with the triglyceride level in children within the age ranges of 2-3 years and 4-5 years (100.19±3.90 and 99.54±2.90 respectively). SOD level and GSH level were significantly reduced (p<0.05) in the children within the age range of 4-5 years than in those within the age ranges 0-1 and 2-3 years (Table 2). The levels of SOD and GSH were significantly higher (p<0.05) in the children within the age range 0-1 year when compared with other age ranges studied. The MDA level was significantly reduced (1.20±0.00) in the children within the age range of 0-1 year when compared with the children within the age ranges of 2-3 years (3.10±0.20) and 4-5 years (4.00±0.40) as shown in Table 2.

Table 1. Prevalence of malaria infection in children and the mean parasite density

Age (Years)	n	Number infected (%)	Parasite density/µl
0 - 1	60	43(71. 6)	2799±60b
2 - 3	61	54 (88.5)	2909±51b
4 – 5	49	36 (73.5)	7022±29a

**'n' stands for number of children who participated in the study; **The values of parasite density is a mean ±SD; **Means that carried different superscript letter along the column are statistically different at P<0.05, while those that carried the same superscript letter are not statistically different at P<0.05 using Duncan's multiple range test*

4. DISCUSSION

Children under five years have been reported to be highly susceptible to malaria infection, possibly, as a result of the low level of immunity to malaria infection in them [19]. The level of immunity against malaria infection is determined by the number of exposure to infected mosquito bite [19]. Among the three groups studied, the prevalence of malaria infection and the parasite density were lower in the group within the age range 0-1 year when compared with other age groups. This result is similar to a study conducted in Cameroun by Nkuo-Akenji et al. [25]. The prevalence of malaria infection was highest in the group within the age range 2-3 years than in any other groups studied, but the level of the parasite density was highest in the group within the age range 4-5 years.

Table 2. Effect of age on protein, triglyceride, sod, mda, and gsh level in malaria positive children

Age (years)	Proteins(g/dl)	Triglyceride (mg/dL)	SOD (nmol/L)	MDA (µmol/mL)	GSH(Ul/L)
0-1	27.06±2.05a	77.67±3.50b	3.73±0.50a	1.20±0.00b	79.24±10.40a
2-3	22.90±1.50b	100.19±3.90a	2.19±0.40b	3.10±0.20bc	56.20±09.30bc
4-5	16.70±2.60bc	99.54±2.90a	0.94±0.15bc	4.00±0.40a	21.03±7.80b

** Values are represented as Mean±SD; **Means that carried different superscript letter along the column are statistically different at P<0.05; while those that carried the same superscript letter are not statistically different at P<0.05 using Duncan's multiple range test*

The reduction in prevalence of malaria infection and low parasite density in the age group range 0-1 year could be as a result of the acquisition of maternal immunity against malaria parasite conferred by immunoglobulin G (IgG), which is acquired by the foetus through placental from the mother, mostly during the third trimester of pregnancy, which is still functional at this age group [26]. Acquired maternal immunity reduces as the baby continues to advance in age and tends to build up their own immunity, hence the younger children enjoy the protection more than the older baby and this could have been responsible for the high prevalence of malaria infection and parasite density among the children within the age ranges 2-3 and 4-5 years. This shows that the level of maternal immunity against malaria infection in children reduces as they grow old until they are able to develop their own immunity against malaria, and therefore the older children are more prone to the infection.

The oxidative stress is well pronounced in malaria infected children in this study, as it was reported in the adults, especially among pregnant women [15]. The unnecessary increase in MDA level and decrease in SOD and GSH levels are the indications of oxidative stress environment in an individual. The significant increase in MDA level and decrease in SOD and GSH levels in children within the age ranges 2-3 years and 4-5 years showed that there was oxidative stress in these two age groups. This increase in the MDA level and decrease in GSH and SOD level were associated with increase in parasitaemia found in those two groups. The decrease in the level of GSH and SOD may be attributed to increase utilization of the host's plasma antioxidants by malaria parasites to counter the oxidative damages imposed by the immune cell [27]. The reduction in the MDA level and increase in the SOD and GSH levels in the groups within the age ranges 0-1 year and 2-3 years when compared with group in the age range 4-5 years shows that those within the age range 4-5 years were more prone to oxidative stress compared with other age ranges studied. The increase in MDA level in this age groups could be as a result of their response to malaria infection which could lead to activation of natural immunity, that is capable of generating large amount of reactive oxygen species which might be responsible for imbalance in the oxidant and antioxidant level in the body of the host, that eventually lead to the destruction of parasite [5]. *In vitro* study has demonstrated the ability of oxidative stress to promote the killing of parasites [28].

There was a significant increase in the plasma protein level in the group within the age range 0-1 year when compared with the other age groups studied. This increase could be a function of the low level of parasite density in this age group as compared with other age ranges with higher parasite density. This agrees with the previous study, which reported a significant decrease in the protein level in malaria positive children [29]. The significant reduction in the protein level in the group within the age range 4-5 years when compared to those within the age ranges 0-1 and 2-3 years was associated with significant increase in the malaria parasite density which was recorded in this group when compared with the other age groups studied.

The level of triglyceride has been reported to be lower in malaria infected individuals when compared with non infected counterpart [15]. The level of triglyceride was significantly reduced in the group within the age range of 0-1 year when compared with those in the age groups 2-3 and 4-5 years. Those in the age group 2-3 years had higher triglyceride as compared with other age groups studied. This result is contrary to what have been reported in the adults where those with higher level of malaria parasite density usually have low level of triglyceride [15], therefore there is a need for further study to ascertain what could have been responsible for

the low level of triglyceride in children within the age range 0-1 year which had low parasite density.

5. CONCLUSION

The older children (age range 2-5 years) were more susceptible to malaria infection and oxidative stress than those within the age range 0-1 year. Therefore there is a need for special attention on these age range in malaria endemic areas.

ETHICAL APPROVAL

This study was reviewed and approved by the local Institution Review committee.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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