Glutathione Peroxidase and Superoxide Dismutase in Children with High and Low Malaria Parasitaemia

A.O. Oluboyo†, E.F. Onodjohwo†, B.O. Oluboyo†, H. C. Ukaegbu‡

ABSTRACT

Background: Young children are especially vulnerable to severe malaria and oxidative stress resulting from the effects of the malaria which may contribute to greater population of malaria deaths.

Objectives: To evaluate selected antioxidant enzymes (serum glutathione peroxidase and superoxide dismutase) levels and determine their relationship with other parameters in malaria infested subjects.

Study design: The study was designed to evaluate selected antioxidants in children (both males and females) between the ages of 6 months to 15 years old who were tested positive for Plasmodium falciparum malaria, either presenting with severe malaria or uncomplicated malaria but without cerebral malaria.

Methods: A total of ninety (90) children were recruited; 30 had severe malaria, 40 had uncomplicated malaria and 20 were control subjects. Blood samples were collected for the detection of malaria parasites, malaria parasite density, packed cell volume (PCV), haemoglobin and total white cell counts (WBC) using conventional methods. Glutathione peroxidase and superoxide dismutase were analyzed using Enzyme immunoassay technique.

Results: Severe malaria showed significant decrease (p < 0.05) in PCV, Haemoglobin, Glutathione peroxidase and Superoxide dismutase compared with uncomplicated and control subjects. There was significant increase (p > 0.05) in total white cell counts and malaria parasite density in those who had severe malaria. Glutathione peroxidase and Superoxide dismutase showed significant positive correlation in both severe and complicated malaria but not with other parameters.

Conclusion: There was significant decrease in the levels of Glutathione peroxidase and superoxide dismutase in high parasitaemia. The significantly low antioxidant enzyme levels in severe malaria subjects are a pointer to the fact that these antioxidants are used up in combating the effects of oxidative stress as a result of the malaria.

KEY WORDS

glutathione peroxidase, superoxide dismutase, children, malaria

INTRODUCTION

People of all ages and gender are affected by Malaria, but the most vulnerable are young children, pregnant women, HIV/AIDS patients among others. Young children are especially vulnerable to severe malaria and form a greater population of malaria deaths worldwide. In 2016, World Health Organization (WHO) estimated a total of 445,000 deaths from malaria and estimated 216 million cases of malaria globally compared to the 211 million deaths and 446,000 cases of malaria in 2015. It has been shown that severe malaria may develop in the incidence of malaria infection, if anti-malarial treatment is not given on time, particularly in Plasmodium falciparum malaria.

In humans, P. falciparum metabolizes hemoglobin resulting in the formation of reactive oxygen species (ROS) such as superoxide anions (O2−), hydrogen peroxide (H2O2) and hydroxyl radicals (HO), which are the main cause of oxidative stress. Constant generation of reactive oxygen species leads to damage of biomolecules such as lipids, proteins and DNA, by peroxidation, oxidation and damage to nucleic acids. These reactions alter intrinsic cell properties such as cell fluidity, ion transport, protein cross-linking, inhibition of protein synthesis, DNA damage, ultimately resulting in cell death. In other to protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize reactive oxygen species (ROS). Oxidative stress occurs when there is an imbalance between these antioxidants and pro-oxidant in favor of pro-oxidant. Oxidative stress in malaria may be caused via two major mechanisms. One of the mechanisms may be as a result of degradation of host haemoglobin by the malaria parasite, which reproduces in the erythrocytes. The other mechanism involves the immune response of the host, which activates a cascade that culminates with the release of free radicals by activated macrophages to tackle the parasite. During the course of malarial infection, the host immune system is activated thereby causing release of ROS resulting in the host cell coming under oxidative stress and one of the consequences of this is the development of malarial anemia. Besides host production of ROS in response to infection, the malaria parasite itself is capable of producing free radicals, which in turn interfere with the biochemistry of red blood cells (RBC) and may facilitate the internalization of the parasite in hepatocytes and RBC. Glutathione peroxidase and Superoxide dismutase are two powerful means of detoxifying reactive oxygen species. Therefore, the study aimed to evaluate serum glutathione peroxidase

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and superoxide dismutase (antioxidant enzymes) levels and determine their relationship with other parameters in malaria infected subjects.

MATERIALS AND METHODS

The study investigated children (both male and female) between the ages of 6 months to 15 years who were admitted at the Federal Teaching Hospital, Ido-Ekiti, Ekiti State for malaria parasitemia (high parasitaemia) but without cerebral malaria. Ethical approval was also collected from Federal Medical Center, Ido-Ekiti, Ekiti State. Out of the 90 children recruited as subjects, 30 were severe malaria subjects, 40 were uncomplicated malaria subjects, and 20 were control subjects.

Detection of Malaria Parasite Using Thick Blood Film

Principle: The alcohol based Romanowsky stain which requires dilution before use, stains the chromatin of malaria parasites (at the trophozoites stage) and gives satisfactory results depending on the length of the staining time.

Thick blood film stained with Giemsa stain was used to determine the presence of Plasmodium species in the blood of the participants.

Thin Blood Film

Principle: Leishman stain is a two part Romanowsky stain consisting of a mixture of eosin (an acidic stain) and methylene blue (a basic stain) in alcohol. Eosin stains the basic part of the cell (cytoplasm) giving it a blue colouration while methylene blue stains the acidic content of the cell (the nucleus) giving it a red colouration.

This thin blood film stained with Leishman stain was used to identify the presence of Plasmodium falciparum in the blood of the participants.

Estimation of Packed Cell Volume

Principle: Red blood cells in a capillary tube are separated in a haematocrit centrifuge at 3500 revolution per minute (RPM) for 5 minutes with the aid of centrifugal force. The percentage of red blood cell in the sample is then read with the aid of haematocrit reader.

White Blood Cell Counts

Principle: Whole blood is diluted 1:20 in a weak acid solution (Turks solution) which lyses the non-nucleated red blood cells leaving only the white blood cells and nucleated red blood cells to be counted microscopically using an improved Neubauer ruled counting chamber. The number of white blood cells per litre of blood is calculated.

Malaria Parasite Density

Principle: The malaria parasite density gives information on the severity of malaria infection and on the response to treatment. It is performed in relation to both white blood cells count and parasites count on a particular field of the slide.

The number of malaria parasites per 250 or 500 white blood cells were determined and expressed per microlitre of blood.

Glutathione Peroxidase Estimation

Principle: The principle is based on the double antibody sandwich enzyme linked immunosorbent assay (ELISA) technology. Specific purified glutathione peroxidase pre-coated onto the microwell plates reacts with native antigen in serum and enzyme labeled secondary antibody to form antigen - antibody - enzyme-labelled antibody complex. After attaining equilibrium, unbound antigen and secondary antibody are washed. The density of colour produced after addition of chromogen is directly proportional to the concentration of Glutathione peroxidase present in the sample.

Table 1. Selected haematological parameters, glutathione peroxidase and superoxide dismutase in severe, uncomplicated malaria and control subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Severe Malaria</th>
<th>Uncomplicated Malaria</th>
<th>Control Malaria</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>25.93 ± 6.08</td>
<td>29.6 ± 3.83</td>
<td>38.87 ± 5.42</td>
<td>52.43 ± 3.83</td>
<td>0.000*</td>
</tr>
<tr>
<td>WBC (x 10^9/L)</td>
<td>12.63 ± 3.32</td>
<td>7.76 ± 2.49</td>
<td>8.11 ± 2.49</td>
<td>35.847 ± 2.49</td>
<td>0.000*</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>8.71 ± 2.08</td>
<td>10.19 ± 3.17</td>
<td>13.5 ± 4.87</td>
<td>48.377 ± 4.87</td>
<td>0.000*</td>
</tr>
<tr>
<td>Glutathione Peroxidase (pg/ml)</td>
<td>31.41 ± 5.5</td>
<td>55.68 ± 10.19</td>
<td>58.3 ± 15.78</td>
<td>496.407 ± 15.78</td>
<td>0.000*</td>
</tr>
<tr>
<td>Glutathione Dismutase (ng/ml)</td>
<td>24.47 ± 5.42</td>
<td>50.64 ± 12.06</td>
<td>116.83 ± 225.10</td>
<td>0.000*</td>
<td></td>
</tr>
</tbody>
</table>

Key: * = significant at P < 0.05

Table 2. Relationship between parameters in severe malaria subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Glutathione Peroxidase</th>
<th>Superoxide Dismutase</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>-0.013</td>
<td>-0.024</td>
</tr>
<tr>
<td>WBC (x 10^9/L)</td>
<td>0.148</td>
<td>0.088</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>-0.015</td>
<td>-0.031</td>
</tr>
<tr>
<td>MP Density (x 10^3/μl)</td>
<td>0.123</td>
<td>0.085</td>
</tr>
<tr>
<td>Glutathione Peroxidase (pg/ml)</td>
<td>1.000</td>
<td>0.959</td>
</tr>
<tr>
<td>Superoxide Dismutase (ng/ml)</td>
<td>0.959</td>
<td>1.000**</td>
</tr>
</tbody>
</table>

Key: ** correlation is significant at the 0.01 level

Table 3. Relationship between parameters in uncomplicated malaria subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Glutathione Peroxidase</th>
<th>Superoxide Dismutase</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>-0.013</td>
<td>-0.024</td>
</tr>
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<td>0.085</td>
</tr>
<tr>
<td>Glutathione Peroxidase (pg/ml)</td>
<td>1.000</td>
<td>0.959</td>
</tr>
<tr>
<td>Superoxide Dismutase (ng/ml)</td>
<td>0.959</td>
<td>1.000**</td>
</tr>
</tbody>
</table>

Key: ** correlation is significant at the 0.01 level
Superoxide Dismutase Estimation

Principle: The principle is based on the double antibody sandwich enzyme linked immunosorbent assay (ELISA) technology. Specific purified superoxide dismutase pre-coated onto the microwell plates reacts with native antigen in serum and enzyme-labeled secondary antibody to form antigen - antibody - enzyme-labelled antibody complex. After attaining equilibrium, unbound antigen and secondary antibody are washed. The density of colour produced after addition of chromogen is directly proportional to the concentration of Superoxide dismutase present in the sample.

Statistical Analysis

Results obtained were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 20.0. All parameters were expressed as mean ± standard deviation (SD). One way analysis of variance (ANOVA) was performed and a value of P < 0.05 was considered significant. Correlation analysis was also performed to assess the relationship between the parameters.

RESULTS

Table 1 shows that there were significant decrease (P < 0.05) in PCV, Haemoglobin, Glutathione peroxidase and Superoxide dismutase in malaria subjects compared with control subjects. WBC showed significant increase in severe malaria compared with uncomplicated malaria and control.

Table 2 shows that Glutathione peroxidase and Superoxide dismutase were significantly correlated at P < 0.01. PCV, WBC, Haemoglobin and Malaria parasite density showed no significant correlation with Glutathione peroxidase and Superoxide dismutase in severe malaria subjects.

Table 3 shows that Glutathione peroxidase and Superoxide dismutase were significantly correlated at P < 0.01. PCV, WBC, Haemoglobin and Malaria parasite density showed no significant correlation with Glutathione peroxidase and Superoxide dismutase in uncomplicated malaria subjects.

Figure 1 shows that malaria parasite density is significantly elevated in the severe malaria compared to uncomplicated malaria.

Figure 2 shows that Glutathione peroxidase levels were higher in females than males in the severe and uncomplicated malaria. Control showed significant increase in glutathione peroxidase levels in males compared with females.

Figure 3 shows that Superoxide dismutase levels were higher in females than males in the severe and uncomplicated malaria. Control showed significant increase in Superoxide dismutase levels in males compared with females.

DISCUSSION

Previous reports have indicated Plasmodium falciparum as a cause of oxidative stress in malaria infected patients, due to the degradation of...
haemoglobin, which leads to the production of free radicals\textsuperscript{1,2,10}. It has also been reported that free radicals are also produced by cells of the host immune system, in response to infection by \textit{Plasmodium falciparum}. The Reactive Oxygen Species produced are mopped up by the action of antioxidant enzymes; superoxide dismutase, glutathione peroxidase and catalase\textsuperscript{1,2,23,50}. This study was carried out to evaluate Glutathione peroxidase and Superoxide dismutase in subjects with severe malaria (high parasitaemia) and uncomplicated malaria (low parasitaemia) compared with apparently healthy subjects (control).

PCV was estimated in all the subjects as a marker for anaemia which is usually associated with malaria infection as a result of destruction of erythrocytes at the end of the parasites erythrocyte phase of its lifecycle. Results obtained from this study showed significant difference in PCV among the three groups. The PCV in control subjects was significantly higher than the values obtained in uncomplicated malaria and severe malaria subjects. This is in line with similar reports where increase in PCV was obtained in severe malaria infection\textsuperscript{10,26}. There was no significant correlation between PCV, Glutathione peroxidase and Superoxide dismutase in both severe and uncomplicated malaria. White blood cell count was estimated in all the subjects as a way of measuring the malaria parasite density in the malaria subjects and the determination of levels of high or low parasitaemia. The results showed higher parasitaemia in severe malaria compared with uncomplicated malaria. The mean WBC count for the severe malaria was significantly higher than that of the control and uncomplicated malaria subjects. This is in accordance with previous works which can be attributed to the high levels of the malaria parasite in the severe malaria subjects, prompting leucocytosis in defense against the infection\textsuperscript{19,20}. Haemoglobin was estimated in all the subjects as a measure of determining the extent to which erythrocytes have been destroyed in different levels of parasitaemia as a result of infection with the \textit{Plasmodium falciparum}. The results obtained from this study showed significant variation in haemoglobin among the three groups. The mean haemoglobin of the control group was significantly higher than the severe and uncomplicated malaria groups, with the severe malaria group having the lowest haemoglobin. This can be attributed to higher malaria parasite density in severe malaria subjects, and the resultant destruction of red blood cells by the parasites. This is in accordance with previous reports which showed low level of haemoglobin during malaria infection which could predispose the subjects to developing anaemia\textsuperscript{19,20}.

In this study, levels of Glutathione peroxidase and Superoxide dismutase were found to be significantly higher in control subjects compared with uncomplicated and severe malaria with severe malaria group having the lowest levels of glutathione peroxidase and superoxide dismutase. These findings are similar to a study where enzyme antioxidant levels in severe malaria subjects were observed to be significantly reduced in comparison to uncomplicated malaria and control\textsuperscript{11}. The significance of low enzyme antioxidant levels in severe malaria subjects serves as evidence of the utilization of the antioxidants in offsetting the reactive oxygen species generated during malaria infection\textsuperscript{11,18}. This implies that there is a higher oxidative stress in subjects with higher parasitaemia. There was significant positive correlation between glutathione peroxidase and superoxide dismutase in both severe and uncomplicated malaria groups. There were no significant correlations between glutathione peroxidase, superoxide dismutase and PCV, WBC and Haemoglobin. Based on gender, Glutathione peroxidase and Superoxide dismutase were compared among the severe, uncomplicated and control groups. The female subjects had higher levels of glutathione peroxidase and superoxide dismutase in the severe and uncomplicated malaria groups. The findings in this study are partly in line with a report where results obtained showed that superoxide dismutase levels were higher in female than male pediatric subjects and glutathione peroxidase levels were higher in males than females\textsuperscript{25,52}. The larger population of female subjects involved in this study may be a reason for this variation. In the control group however, the male subjects had higher levels of the antioxidants.

**CONCLUSION**

The results obtained from this study showed significant decrease in the antioxidant levels in the malaria subjects compared with control. The significantly low antioxidant enzyme levels in severe malaria subjects are a pointer to the fact that these antioxidants are utilized in the neutralization of the damaging consequences of over-production of ROS. This study further confirms the opinion that high parasitaemia induces consequent risk factors such as anaemia among others in severe malaria.

**RECOMMENDATION**

Based on the findings from this study, we recommend that in addition to the routine drug therapy, foods and fruits high in antioxidants should be prescribed for the treatment of malaria in children and in adults.

**ACKNOWLEDGEMENT**

We acknowledge the staff members of Department of Chemical Pathology and Haematology units, Federal Teaching Hospital, Ido-Ekiti, Ekiti State, Nigeria for assisting in sample collection.

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