Full Length Research Paper

Glutathione peroxidase (GPx) activity in platelets of patients with malaria parasitaemia caused by Plasmodium falciparum

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Abbreviations

DIC, Disseminated intravascular coagulation; EDTA, ethylene diamine tetra acetic acid; GPx, glutathione peroxidase; K2 (EDTA), ethylene diamine tetra acetic acid dipotassium salts; MDA, malondialdehyde; NTCP, non-thrombocytopenic patients; OS, oxidative stress, RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; SPSS, statistical package for social sciences, TCP, thrombocytopenic patients.

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Traditionally Plasmodium falciparum has been considered to cause severe malaria while Plasmodium vivax is known to cause benign malaria. However many recent studies have shown that P. vivax is also responsible for many cases of severe malaria. Acute malaria is often associated with mild or moderate thrombocytopenia in malaria endemic areas. This study was carried out to assay the activity of the enzyme glutathione peroxidase in platelets of patients with thrombocytopenia mediated by P. falciparum malaria. It was found that the activity of the enzyme glutathione peroxidases (GPx) was positively correlated with thrombocyte count (r = 0.602; P < 0.001) and there was a significant difference (P > 0.001) in the activity of glutathione peroxidase (GPx) among the studied groups; 1979 ± 70.49 U/L (Mean ± S.D), 384 ± 49.46 U/L and 112 ± 4.23 U/L for the control group, malaria non thrombocytopenic group and malaria thrombocytopenic group respectively. It was concluded that the activity of glutathione peroxidase (GPx) is positively correlated with thrombocyte count and inversely related to the severity of infection of malaria caused by P. falciparum.

Key words: Plasmodium falciparum, glutathione peroxidase (GPx), thrombocytopenia, oxidative stress (OS).

INTRODUCTION

Malaria is a major health problem in the tropical and temperate regions of the world which poses a significant burden on health expenditure (Srikanth et al., 2012; Jairajpuri et al., 2014). It is caused by obligate intra-erythrocytic protozoa of the genus plasmodium. Humans can be infected with one (or more) of the following five species: Plasmodium falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi. Plasmodia are primarily transmitted by the bite of an infected female anopheles mosquito, but infections can also occur through exposure to infected blood products (transfusion malaria) and by congenital transmission. Among the five species, P. falciparum is the predominant one in Sudan, and is responsible for most of malaria-related morbidity and
mortality (Greenwood et al., 2005).

Traditionally *P. falciparum* has been considered to cause severe malaria while *P. vivax* is known to cause benign malaria. However many recent studies have shown that *P. vivax* is also responsible for many cases of severe malaria (Singh et al., 2014).

Acute malaria is often associated with mild or moderate thrombocytopenia in non-immune adults and children in malaria endemic areas and is sensitive but non-specific indicator of infection with malaria parasites. Profound thrombocytopenia is unusual, and thrombocytopenia is rarely associated with hemorrhagic manifestations or a component of disseminated intravascular coagulation either in non-immune adults or children in endemic areas (Kelton et al., 1983), (Wickramasinghe and Abdalla, 2000) and severe thrombocytopenia should alert one to consider a possibility of malaria (Joshi et al., 2014).

Reactive oxygen (ROS) or nitrogen species (RNS) are considered to play diverse roles in many aspects of physiological and pathological events (Akaike and Maeda, 2000). Oxidative stress can be measured in biological fluids by analysis of endogenous products of lipid peroxidation such as malondialdehyde (MDA) or by measurement of enzymes involved in antioxidant mechanisms.

Glutathione peroxidase (GPx) is an important antioxidant enzyme that catalyzes the reduction of organic and inorganic hydroperoxides to water in oxygen-consuming organisms, using glutathione as an electron donor (Kang et al., 2014).

This study was carried out to assay the activity of the enzyme glutathione peroxidase in platelets of patients with thrombocytopenia mediated by *P. falciparum* malaria.

**MATERIALS AND METHODS**

Forty patients with malaria participated in this study; twelve were male and twenty eight female, their ages ranged between (2- 75 years). They were admitted to Sennar Teaching Hospital (Sudan). The patients were confirmed to have malaria through blood film examination and ten healthy individuals from Sennar area were randomly selected as control group.

Blood samples were collected by finger puncture technique to prepare thick and thin blood film (BF) for malaria investigation. Before any treatment, 5 ml of venous blood were collected and transferred into Ethylene Diamine Tetra Acetic acid dipotassium salts (K$_2$(EDTA)) test tubes for the assay of the activity of platelet enzyme glutathione peroxidases. Blood samples were transported, using a preserving container that contains dried ices, to the laboratory where the investigations were carried out immediately.

Platelets were prepared and lysed according to the method adopted by Araujo et al. (2008). 2 ml of blood were centrifuged at 500 rpm for 5 min to obtain platelet-rich plasma (PRP). The platelets were washed three times by centrifugation at 2,000 rpm for 10 min. After each centrifugation, the supernatant was decanted and discarded, and the platelets pellets were resuspended into 500 μL of a sodium chloride solution (0.89%) and immediately kept in refrigerator at 4ºC until the biochemical assays were performed. Platelet-poor plasma was separated from the remaining blood after the PRP separation by centrifugation at 3,500 rpm for 10 min.

The glutathione peroxidase enzyme activity was measured according to the method adopted by Paglia and Valentine (1967).

**Statistical analysis**

Results of this study were statistically analyzed using (SPSS) program. Significant differences between groups were assessed by one-way ANOVA and t-test. Correlation matrix was done and the r values were obtained with level of significance.

**RESULTS**

Blood film examination indicated that the 40 patients admitted to Sennar Teaching Hospital were infected with the parasite *P. falciparum*. The distribution of respondents according to thrombocyte count (> 150.000) revealed that 10 (25%) of the malaria patients were thrombocytopenic and 30 (75%) were without thrombocytopenia.

Results presented in Table 1 and Figures 1 and 2) indicate that there was a significant difference (P > 0.001) in the activity of glutathione peroxidase (GPx) among the studied groups, the highest enzyme activity was observed in the control group (1979 ± 70.49 U/L) followed by malaria non thrombocytopenic patients (384 ± 49.46 U/L) and the least was the malaria thrombocytopenic group (112 ± 4.23 U/L).

Results presented in Figure 3 indicated that the activity of the enzyme glutathione peroxidases (GPx) was positively correlated with thrombocyte count (r = 0.602; P < 0.001).

**Discussion**

The suggested mechanism of thrombocytopenia may be through peripheral destruction (Ladhani et al., 2002), excessive removal of platelets by splenic pooling as well as platelet consumption by the process of disseminated intravascular coagulation (DIC) (Maina et al., 2010). The relative thrombocytopenia may also be due to a shortened life span of the platelets (Malik et al., 2010). Antiplatelet antibodies have also been implicated in the
Table 1. Levels of glutathione peroxidase and thrombocytes count (x10^3) in the studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Malaria TCP</th>
<th>Malaria NTCP</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione peroxidase (U/L)</td>
<td>112.0 ± 4.23*</td>
<td>384.0 ± 49.46</td>
<td>1979 ± 70.49</td>
<td>0.001</td>
</tr>
<tr>
<td>Thrombocytes count X10^3 cell/cmm</td>
<td>70.7 ± 5.164*</td>
<td>283.2 ± 27.60</td>
<td>270.4 ± 31.97</td>
<td>0.001</td>
</tr>
</tbody>
</table>

All data are expressed as mean ± SD.
*Significant difference as compared with control group (Significance at level p < 0.05).

Figure 1. Activity of glutathione peroxidase in platelets in males and females of Non-thrombocytopenic, thrombocytopenic malaria patients and healthy control group.

Figure 2. The activity of the platelets GPx in different groups of age of Non-Thrombocytopenic, Thrombocytopenic malaria patients and healthy control group.
Figure 3. Correlation between blood platelet count and platelet glutathione peroxidase (GPx) \( (r = -0.60; p < 0.001) \) in Non-thrombocytopenic, thrombocytopenic malaria patients and healthy control group.

pathogenesis of thrombocytopenia (Lathia and Joshi, 2004).

Presence of thrombocytopenia in a patient with acute febrile illness in the tropics increases the possibility of malaria. This may be used in addition to the clinical and microscopic parameters to heighten the suspicion of this disease and prompt initiation of the treatment (Gupta et al., 2013). Jairajpuri et al. (2014) found that low platelet count is a characteristic finding of malarial infection and thrombocytopenia may be more common than anemia in acute malaria infection. In their study, thrombocytopenia (platelet < 100,000/mm\(^3\)) emerged as a strong predictor of malaria, an observation of many studies which they confirmed. This is in concordance with our study.

Glutathione peroxidase (GPx) is a selenium-dependent and lipid peroxide-scavenging enzyme that effectively reduces lipid peroxides with the concomitant oxidation of glutathione. Its activity can be altered under oxidative stress (OS) conditions (Gutteridge, 1995). When pro-oxidants increase or antioxidants fall, oxidative stress ensues that leads to excessive molecular damage and tissue injury (Januel et al., 2006).

Bilgin et al. (2012) assessed lipid peroxidation by measuring MDA, an end product of fatty acid peroxidation. They found that the difference of MDA levels were significantly higher in male and female patients with vivax malaria than in healthy controls \( (P < 0.001) \). On the other hand, superoxide dismutase (SOD) and GPx activities were found to be significantly lower in male and female patients with vivax malaria than in healthy controls \( (P < 0.05) \). This may indicate oxidative stress as a mediator of tissue damage concurrent with \textit{P. vivax} infection.

Our results revealed that the activity of the platelets enzyme glutathione peroxidase (GPx) was very low in thrombocytopenic malaria patients (TCP) compared to malaria non-thrombocytopenic (NTCP) and control subjects. These results agreed with the previously mentioned study and that obtained by Araujo et al. (2008) who found increased activity of GPx in platelets of NTCP compared to TCP. Moreover, they suggested that malondialdehyde (MDA) and GPx are important markers of platelet OS in malaria caused by \textit{P. vivax} and could be implicated in the mechanisms of malaria-induced thrombocytopenia.

Conclusions

Our results suggest that the activity of glutathione peroxidase (GPx) is positively correlated with
thrombocyte count and hence inversely related to the severity of infection of malaria caused by *P. falciparum*.

**REFERENCES**


