



Study of Malondialdehyde Level and Glutathione Peroxidase Activity in Patients Suffering from Malaria

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Authors' contributions

This work was carried out in collaboration among all authors. Author AVN designed the study, author NJP performed the statistical analysis, author SAP wrote the protocol and wrote the first draft of the manuscript. Author SP managed the analyses of the study. Authors URS and KUS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Malaria is one of the most common Parasitic infection prevalent worldwide especially in India, South Asia and Africa. About 250 million cases and approximately One million deaths of malaria reported per year worldwide. Oxidative stress (O.S.) has been implicated as possible mediator of thrombocytopenia in malarial patients. All eukaryotic cells, specially immune effector cells generate reactive oxygen species (ROS) and reactive nitrogen species (RNS) as a mean to combat invading microbes i.e. via the 'Oxidative burst', which increases the oxidative burden on the microbe to lethal levels. An excess of ROS such as superoxide anions, hydrogen peroxide (H₂O₂), hydroxyl radicals and /or RNS, such as nitric oxide (NO) and peroxy nitrite (ONOO-) creates a potentially dangerous situation known as oxidative or nitrosative stress respectively.

Aim: Present study aims to study the status of serum Malondialdehyde and Glutathione Peroxidase activity in hemolysate among the patients with Malaria.

Materials and Methods: This is cross-sectional observational study on 200 non-treated malaria patients, compared with 100 normal individuals. Out of total 200 malaria patients 96 were

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plasmodium (P) vivax & 104 were P falciparum diagnosed cases.

Results: Mean MDA level in the P. Vivax malaria cases was 12.29 ± 0.32 micromole/L which was found to be higher compared to the controls with mean MDA level is 6.55 ± 0.24 micromole/L, whereas the mean MDA level in P. Falciparum malaria cases was 13.5 ± 0.18 micromole/L which was higher compared to the controls with mean MDA level of 6.55 ± 0.24 micromole/L.

Conclusion: The present study on malaria explains the role of oxidative stress in the pathophysiology of malaria which is a multifactorial phenomenon and represents an important aspect of the intricate and complex host- parasite relationship. Oxidative stress is aggravated by reduced effectiveness of the antioxidant defence system; hence it is advised to provide antioxidant supplements through diet that can reduce the disease severity and risk of death during infection.

Keywords: Malaria; oxidative stress; malondialdehyde; glutathione peroxidase.

1. INTRODUCTION

Malaria is one of the most common Parasitic infection prevalent worldwide especially in India, South Asia and Africa. Every year there are about 250 million cases of Malaria reported and approximately One million deaths, mostly in children under five years of age [1].

Malaria is usually associated with various degree of reduced blood count. Though the anaemia is haemolytic in nature, the hemopoietic response is reduced as evidenced by disproportionate reticulocyte count, reduced platelet and WBC count this indicate some problem with hematopoiesis. Thrombocytopenia is a frequent finding in malaria especially with falciparum [2].

Oxidative stress (O.S.) has been implicated as possible mediator of thrombocytopenia in malarial patients. It has been demonstrated that it also has important functions in the structural and functional alterations of platelets in malaria [3].

Clinical illness is caused by the erythrocytic stage of parasite. The release of mature Schizonts i.e., merozoites from erythrocytes, triggers a host immune response. The cytokines, reactive oxygen intermediates and other cellular products are released during the immune response which plays a prominent role in pathogenesis and are probably responsible for the fever, chills, sweats, weakness and other systemic symptoms associated with malaria.

All eukaryotic cells, especially immune effector cells generate reactive oxygen species (ROS) and reactive nitrogen species (RNS) as a mean to combat invading microbes i.e., via the ' Oxidative burst', which increases the oxidative burden on the microbe to lethal levels [4].

An excess of ROS such as superoxide anions, hydrogen peroxide (H_2O_2), hydroxyl radicals and /or RNS, such as nitric oxide (NO) and peroxynitrite (ONOO-) creates a potentially dangerous situation known as oxidative or nitrosative stress respectively. The generation of ROS such as hydroxyl radicals (OH) in the liver is the main reason for induction of oxidative stress and apoptosis of parasites [5].

Oxidative stress is an imbalance between the systemic manifestation of reactive oxygen species and a biological systems ability to readily detoxify the reactive intermediates or to repair the resulting damage.

1.1 Aim

Present study aims to study the status of serum Malondialdehyde and Glutathione Peroxidase activity in hemolysate among the patients with Malaria.

1.2 Objectives

To evaluate the level of Malondialdehyde in Serum and Glutathione Peroxidase Activity in the hemolysate of the samples of the patients infected with P. Vivax & P. Falciparum malaria separately and

1. To compare it with control group.
2. To compare the level of MDA and GPX in P. Vivax & P. Falciparum malaria separately.

2. MATERIALS AND METHODS

This is cross-sectional observational study on 200 non-treated malaria patients, compared with 100 normal individuals. Out of total 200 malaria patients 96 were plasmodium vivax & 104 were Pl. falciparum diagnosed cases.

4 ml of venous blood samples were collected from each participant in metal-free plastic tubes i.e. vacutainer tubes 2 ml of the above blood sample was transferred to EDTA bulb and allowed to stand at 37⁰ C for 15 min., then centrifuged at 3000 rpm for 15 minutes and the supernatant plasma was aspirated.

The sediment packed red cells were washed twice with 5 ml of saline. About 1 ml of RBC suspension obtained was washed with equal volume of hemolysing reagent (2.7 mM EDTA and 0.7 mM β – mercaptoethanol) to hemolyse the RBCs within the sample. The diluted RBC suspension was then centrifuged and hemolysate obtained was collected in vial. The hemolysate was stored in a refrigerator immediately at 4⁰ C avoiding exposure to light. This hemolysate was used for the estimation of the antioxidant enzyme Glutathione Peroxidase activity.

The remaining 2 ml of the blood sample in the vacutainer was transferred to plain bulb and allowed to clot at 37⁰ C for 15 min. Then the sample was centrifuged and supernatant serum was transferred into an eppendorf tube and then the sample was stored in at -20⁰ C. This serum was further used for the biochemical analysis of MDA.

Serum Malondialdehyde which is produced during lipid peroxidation usually measured as thiobarbituric acid reducing substances or lipid peroxides [6]. First 40% trichloroacetic acid (TCA) is added to the serum samples [7]. Addition of acid is necessary to precipitate proteins and release the MDA bound to the amino groups of proteins and other amino compounds [8].

The serum MDA under high temperature (90-100⁰ C) and in the presence of acid medium reacts with freshly prepared thiobarbituric acid. The reaction yields a light pink MDA-TBA adduct product of 2 mol of TBA plus 1 mol of MDA. The

coloured complex can be extracted into organic solvents such as butanol [9]. The absorbance of supernatant is measured spectrophotometrically at 532 nm against a blank [10]. 1,1,1,1 Tetramethoxypropane (TMOP) is used as standard [6] and the result were expressed as micromole/L.

Glutathione peroxidase tests were performed by the method adopted by Elhassan et al. [11]. The method used to determine glutathione peroxidase activity is called the coupled test procedure in which there is regeneration of the product enzymatically by glutathione reductase and the observation of the decrease in NADPH concentration at 340 nm. This rate of decrease in absorbance at 340 nm is directly proportional to the glutathione peroxidase activity in the sample [12,13].

2.1 Statistical Analysis

Unpaired student's t-test was applied for the comparison of MDA levels between two test groups P. Vivax and P. Falciparum and similarly GPx activity between test groups P. Vivax and P. Falciparum. Pearson's Correlation analysis was performed to determine the degree of correlation between the two parameters MDA & GSH-Px.

3. RESULTS

Mean MDA level in the P. Vivax malaria cases was 12.29 \pm 0.32 micromole/L which was found to be higher compared to the controls with mean MDA 6.55 \pm 0.24 micromole/L, whereas the mean MDA level in P. Falciparum malaria cases was 13.5 \pm 0.18 micromole/L which was higher compared to the controls with mean MDA of 6.55 \pm 0.24 micromole/L (Table 1 & 2).

t-test value in the P. Vivax (test group 1) is 13.9 and p-value \leq 0.001, while in P. Falciparum (test group 2) is 19.85 with p-value < 0.001 which is highly significant.

Table 1. MDA levels in control and test groups

Parameter	Control	P. Vivax (test group 1)	P. Falciparum (test group 2)
MDA Mean \pm SE	6.55 \pm 0.24	12.55 \pm 0.32	13.5 \pm 0.18
t-test	-----	3.27	
p-value		< 0.1	

Data presented as micromole/L MDA = Malondialdehyde SE = Standard Error

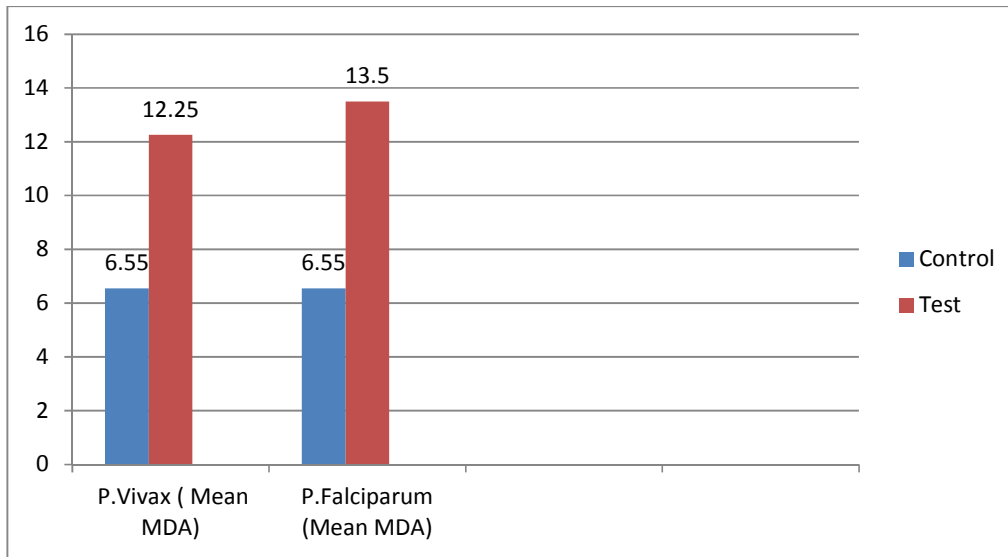


Fig. 1. Comparison of mean MDA level in P. Vivax, P. Falciparum malaria and control subjects

Table 2. Gpx activity in control and test

Parameter	Control	P. Vivax (test group 1)	P. Falciparum (test group 2)
Gpx activity Mean \pm SE	195.82 \pm 8.46	48.56 \pm 5.81	50.21 \pm 5.58
t-test	-----	0.19	
p value		≥ 0.1	

Data presented as IU/L, Gpx = Glutathione peroxidase, SE = Standard Error

Table 3. Correlation between MDA (nmol/L) & GSH-Px (IU/L) in P. Vivax

P. Vivax	Glutathione peroxidase activity (IU/ml)	r
MDA (nmol/L)	Correlation coefficient	-0.41
p - value		≥ 0.05

Table 4. Correlation between MDA (nmol/L) & GSH-Px (IU/L) in P. falciparum

P. falciparum	Glutathione peroxidase activity (IU/ml)	r
MDA (nmol/L)	Correlation coefficient	-0.44
p - value		≥ 0.05

r = correlation coefficient

t-test value in the P. vivax (test group 1) among control and subjects is 11.9 with p-value ≤ 0.001 , and P. Falciparum (group 2) is 12.23 with p-value < 0.001 which is highly significant.

There is negative correlation between the level of MDA and activity of Gpx in P. Vivax test groups. This explains that there is simultaneous

decrease in the activity of Gpx and increase in the serum level of MDA.

There is negative correlation between the serum levels of MDA & GPx activity in P. Falciparum.

That is GPx activity decreases within the infected cells. It induces lipid peroxidation which results in

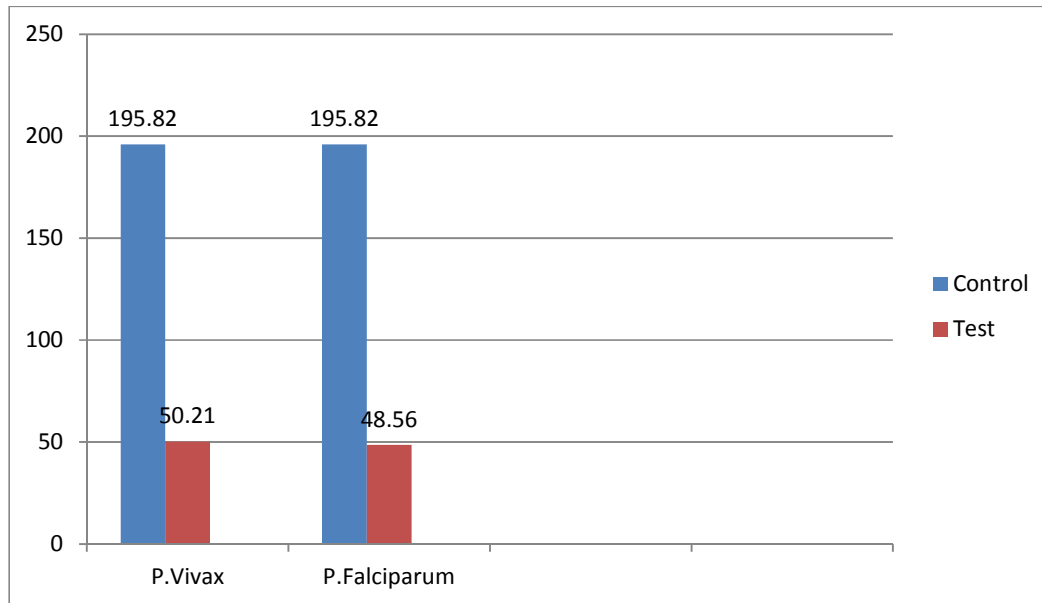


Fig. 2. Comparison of mean GPx activity in P. Vivax and P. Falciparum malaria

increased production of lipid peroxidant MDA & increased levels of MDA is observed with the host cells.

4. DISCUSSION

Many studies have shown that increased oxidative stress is present in malaria [14-22]. Consistent with this view, the main objective of this study was to provide the evidence that there is presence of oxidative stress in P. Vivax and P. Falciparum malaria depicted by increased lipid peroxidation (MDA levels) which causes an imbalance in the antioxidant enzyme activities (Glutathione Peroxidase) and also that there is correlation between the two parameters.

It is known that malarial infection is accompanied by increased production of reactive oxygen species (ROS) and malarial parasites are sensitive to oxidative damage [4,5].

Extreme production of ROS such as superoxide anions (O_2^-), Hydrogen Peroxide (H_2O_2) and Hydroxyl radicals (OH^\cdot) leading to intensified oxidative damage at the level of lipid peroxidation. Mammalian cells possess enzymatic antioxidant defences to cope with oxygen free radicals these are Superoxide dismutase (SOD), Catalase (CAT) and Glutathione (GSH) Peroxidase [16,23].

Oxidative stress is assessed by measuring MDA, a marker of lipid peroxidation and glutathione

peroxidase, an antioxidant enzyme. There is increase oxidative stress observed among patients with severe malaria marked by rise in lipid peroxidation marker i.e. MDA. This finding is in accordance with observations made by several studies [7,16,13]. There is significant increase in MDA levels in P. Falciparum than in P. Vivax was also observed in our study.

GSH Peroxidase (GSH-Px) catalyses the reduction of lipid peroxides at the expense of Glutathione. H_2O_2 is also being inactivated by GSH-Px resulting in increased consumption of GSH [24].

5. CONCLUSION

The present study on malaria explains that the role of oxidative stress in the pathophysiology of malaria is a multifactorial phenomenon and represents an important aspect of the intricate and complex host- parasite relationship. Oxidative stress is therefore aggravated by reduced effectiveness of the antioxidant defence system; hence it is advised to provide antioxidant supplements through diet that can reduce the disease severity and risk of death during infection.

CONSENT

Written consent obtained from all participants in the language they understand.

ETHICAL APPROVAL

Ethical approval was obtained from Institutional Ethics Committee of the BYL Nair Hospital, Mumbai.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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