International Journal of Research in Medical Sciences
Tyagi AG et al. Int J Res Med Sci. 2017 Apr;5(4):1649-1654
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20171281

Original Research Article

Study of antioxidant status in malaria patients

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Received: 18 January 2017
Accepted: 03 March 2017

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ABSTRACT

Background: Oxidative stress plays an important role in the development of metabolic changes in malaria patients. During infection RBCs are exposed to continual oxidative stress. The univalent reduction of oxygen results in a series of cytotoxic oxygen species such as O2-, H2O2, OH•. Objective was to determine the level of oxidative stress in patients suffering from malaria.
Methods: The present study was conducted on 551 malaria patients and 211 age-sex matched controls, in department of Biochemistry, C U Shah Medical College, Surendranagar, Gujarat from April 2012 to May 2013. In stage-I, day-1 malaria patient's v/s control group, In stage-II, day-3 v/s day-1 after anti-malarial treatment and in Stage-III day-3 v/s day-1 after anti-malarial + antioxidant treatment.
Results: The mean erythrocytic activity of SOD, CAT, GST were decreased (0.71±0.25EU, 9.9±2.4µmol/sec, and 11.7±3.9 U/gmHb% respectively), mean level of GSH and MDA were increased (42.1±6.06gm/Hb%, 10.9±2.83 respectively) significantly (P<0.001) as compared to control group. In the follow up study with anti-malarial treatment the mean levels of erythrocytic GSH and MDA (28.7±7.54gm/Hb% and 8.08±1.95nM/L) decreased significantly (P<0.001 and P<0.01 respectively), whereas mean activity of erythrocytic enzymes like SOD, CAT and GST (0.99±0.15 EU, 15.8±2.68µmol/sec and 22.5±5U/gmHb%) were increased significantly (P<0.001) as compared to day-1.
Conclusions: Erythrocytic antioxidant enzymes, GSH and MDA may be considered to be reliable biochemical markers for diagnostic and therapeutic potential in malaria.

Keywords: Glutathione peroxidase, Malondialdehyde, Malaria, Plasmodium falciparum, Superoxide dismutase

INTRODUCTION

Malaria is one of the oldest diseases in the World. In the 18th century people of ancient Italy associated Malaria with bad air, 'Mala-aria' from which the name malaria is derived. It was formerly referred to as ague or marsh fever in English. Malaria probably influenced, to a great extent, human populations and human history. Malaria affects worldwide more than 300 million people, of which 1-2 million die every year. New drugs and treatment strategies are needed to face the rapidly increasing problem of drug resistance. During a malaria infection both host and parasites are under oxidative stress. Increased production levels of ROS (reactive oxygen species) are produced by activated neutrophils in the host and during degradation of hemoglobin in the parasite. The effect of ROS in malaria can be both beneficial and pathological, depending on the amount and place of production. Enhanced ROS production after the administration of pro-oxidants, which is directed against the intra-erythrocytic parasite, inhibits the infection both

in-vitro and in-vivo. The reactive oxygen species (ROS) are one class of effector implicated in insect innate immunity. ROS are multifunctional molecules involved in host defence, mitogenesis, hormone biosynthesis, apoptosis, necrosis, and gene expression.[1]

The importance of ROS in immune response was first described in phagocytic cells through ROS production by NADPH oxidases (NOX) followed by pathogen killing.[2] To date, six human homologues of the NOX protein family (Nox-1, Nox-3, Nox-4, Nox-5, Duox-1 and Duox-2) have been identified in various nonphagocytic cells.[1] Homologues of some of these proteins were also discovered in nematodes, fruit flies, green plants, fungi and slime moulds.[3] The dual oxidases (DUOXs) are important in hormone production, extracellular matrix production and host defence.[4] ROS producing DUOX proteins were described in Drosophila melanogaster and Anopheles gambiae after pathogen challenges.[2,5-8] In *A. gambiae*, DUOX proteins, together with a peroxidase, are responsible for preventing a strong immune activation by producing a dityrosine network, which decreases gut permeability to immune elicitors.[9] This mucous protection may prevent the deleterious effect of the immune response to the host itself and to commensal gut bacteria. In spite of ROS being beneficial for parasite clearance, they are potentially toxic to the host itself. For this reason, the lifespan of these molecules must undergo a fine-tuned regulation, which is accomplished through the action of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, as well as the control of ROS generation. SODs transform superoxide ($O_2^{-\bullet}$) into hydrogen peroxide (H_2O_2) and catalase detoxifies H_2O_2 into water and oxygen. Other molecules as vitamin C and uric acid are also antioxidant components utilized by the organisms to neutralize deleterious effects of high levels of ROS.[10] However, ROS are also involved in pathological changes in host tissue like damage of the vascular endothelial lining during a malaria infection. Pro oxidant support the host defence against the parasite when working in or near the infected cell but potentially causes vascular damage when working on or near the vascular lining. Antioxidants potentially counteract these agents.

Treatment with antioxidant, and chelators of metals prevent their catalytic function in the generation of ROS.[11] Oxidative damage is the major factor leading to the consequences of various diseases and wide range of tissue injuries which causes damage to various organs. The generation of reactive oxygen species (ROS) leads to rapid peroxidation and formation of reactive products which causes severe damage to the cell molecules and structure. Hence oxidative stress has a functional duality due to its involvement in tissue damage. Biochemical studies by blood level determination of oxidative stress markers and antioxidant enzymes become very essential as it reflect the severity of disease. This study was aimed to determine the level of oxidative stress in response to oxidative enzymes (catalase, superoxide dismutase and glutathione-s-transferase), reduced glutathione (GSH) and lipid peroxidation product malondialdehyde (MDA) in patients suffering from malaria. Present study provides useful information for protection, diagnosis and monitoring treatment. So, blood levels of antioxidant enzyme have been studied for their importance in the patients of malaria.

METHODS

The present comparative study was conducted on patients suffering from malaria and admitted in the department of Medicine, C. U. Shah Medical College and Hospital, Surendranagar, Gujarat, India from April 2012 to May 2013. Age of the patient ranged from 13-82 years. Two hundred eleven, age-and sex-matched healthy subjects were selected as control. The entire patients selected in the study were from middle-socio economic group.

Inclusion criteria

The patient on the bases of clinical symptoms similar to malaria like fever, rigors and headache were selected for the study. Clinical history of each patient was taken regarding detail history of fever and its duration, type, intensity and mode of subsidence. The selected patients were sent for the hematological investigation and the diagnosis was confirmed. The diagnosis of malaria was done by peripheral blood smear examination. Routine general hematological profile including hemoglobin, total erythrocyte count (TRBC), total leukocyte count (TLC) and differential leukocyte count (DLC) were carried out. Patients having blood transfusion, gastrointestinal and renal symptoms, tuberculosis, meningitis, epilepsy and anti-malarial chemoprophylaxis were excluded from the study group.

Exclusion criteria

Patients having gastrointestinal and renal symptoms or past history of similar illness, patients who received blood transfusion or underwent surgical operation or had past history of any disease like tuberculosis, meningitis, epilepsy and patients who were on anti-malarial chemoprophylaxis were excluded from the study group.

Design of study

This study included 551 patients suffering from malaria as a study group and 211 age-sex-matched healthy people serve as a control group.

Stage-I: Whole study group v/s control group.

Stage-II: Out of 551 patients selected for the whole study group, 220 subjects got admitted and were treated for anti-malarial drug for three days. The result obtained on day-3 was compared with the result obtained at the time of admission (day-1)

Stage-III: Out of 551 day-1 patients, 109 patients were followed up after anti-malarial + antioxidant therapy (Contents: β-carotene, Vitamin C and Vitamin E, minerals like copper, manganese, zinc and selenium) for 3 days. The results obtained on day-3 were compared with the results obtained in day-1.

Sample collection

Blood sample were collected in Tri sodium salt of EDTA vial at the time of admission (Day-1) and on third day (Day-3). The samples were analyzed within 24 hours of sample collection.

Methods of investigation

The Erythrocytic reduced glutathione (GSH) is measured by Beutler method, catalase (CAT) is measured by Sinha method, superoxide dismutase (SOD) is measured by Mishra and Fridorich method, glutathione-s-transferase (GST) is measured by Habig method and plasma malondialdehyde (MDA) is estimated by Buege method.[12-16]

Statistical analysis

The Statistical analysis was done using statistical Graph Pad-Quick calculation. Descriptive statics were used to describe the data i.e. Mean and SD and data were analysed stastically using paired sample‘t’ test. Difference in levels were considered to be significant when P<0.05. All parameters levels were represented as Mean±SD.

RESULTS

In the stage-I comparative study, activity of CAT, SOD, and GST decreased significantly (P<0.001) whereas levels of GSH and MDA increased significantly (P<0.001) in whole study group as compared to that of control group.

Table 1: comparative study of antioxidant enzymes and MDA in control and whole study group patients (stage-I).

Parameter	Group	No.	Mean	S.D.	Range	‘P’ value
Reduced glutathione (gm /Hb %)	Control	211	17.9	8.3	9.3-24.8	<0.001
	Study	551	42.1	6.06	28-56	
Catalase (CAT) (µmol/sec)	Control	211	27.3	5.7	16.6-37.9	<0.001
	Study	551	9.9	2.4	4.5-15.8	
Superoxide dismutase (SOD) (EU)	Control	211	1.49	0.18	1.24-1.81	<0.001
	Study	551	0.71	0.25	0.1-1.04	
Glutathione-S-transferase (GST) (U/gmHb%)	Control	211	32.2	6.47	25-45.8	<0.001
	Study	511	11.7	3.9	6.2-20.8	
Malondialdehyde (MDA) (nM/L)	Control	211	4.36	1.14	2.3-7.0	<0.001
	Study	551	10.9	2.83	7.5-19.3	

Table 2: Comparative study of antioxidant enzymes and MDA in follow up patients [at admission (day-1) and before discharge (day-3) stage-II].

Parameter	Group	No.	Mean	S.D.	Range	‘P’ value
Reduced glutathione (gm /Hb %)	Day-1	220	42.9	6.03	28-56	<0.001
	Day-3	220	28.7	7.54	20-49	
Catalase (CAT) (µmol/sec)	Day-1	220	9.89	2.33	4.5-15.8	<0.001
	Day-3	220	15.8	2.68	10-20	
Superoxide dismutase (SOD) (EU)	Day-1	220	0.65	0.26	0.1-1.04	<0.001
	Day-3	220	0.99	0.15	0.57-1.1	
Glutathione-S-transferase (GST) (U/gm Hb%)	Day-1	220	11.5	3.80	6.2-20.8	<0.001
	Day-3	220	22.5	5.00	10.2-31	
Malondialdehyde (MDA) (nM/L)	Day-1	220	10.75	2.77	7.5-19.2	<0.01
	Day-3	220	8.08	1.95	6.8-14.75	

In the stage-II comparative study, activity of CAT, SOD, and GST increased significantly (P<0.001) whereas levels of GSH and MDA decreased significantly (P<0.001 and P<0.01, respectively) after anti-malarial treatment (day-3) as compared to those before treatment (day-1) in follow up patient of malaria. In the stage-III comparative study, activity of CAT, SOD, and GST were increased significantly (P<0.001) whereas levels of GSH

and MDA decreased significantly (P<0.001) after anti-malarial + antioxidant treatment (day-3) as compared to those before treatment (day-1) in follow up patient of malaria.

Table 3: Comparative study of antioxidant enzymes and MDA in follow up patients [at admission (day-1) and before discharge (day-3) after antioxidant therapy, stage-III].

Parameter	Group	No.	Mean	S.D.	Range	'P' value
Reduced glutathione (gm/Hb %)	Day-1	109	40.8	5.7	28-56	<0.001
	Day-3	109	22.0	6.0	11-37	
Catalase (CAT) (µmol/sec)	Day-1	109	9.78	2.26	5.5-15.8	<0.001
	Day-3	109	24.6	4.43	18-35	
Superoxide dismutase (SOD) (EU)	Day-1	109	0.72	0.23	0.19-1.04	<0.001
	Day-3	109	1.28	0.24	0.9-1.7	
Glutathione-S-transferase (GST) (U/gmHb%)	Day-1	109	11.8	3.90	6.2-20.8	<0.001
	Day-3	109	31.3	6.92	20-46	
Malondialdehyde (MDA) (nM/L)	Day-1	109	10.9	2.85	7.5-19.2	<0.001
	Day-3	109	6.2	1.49	3.0-9.5	

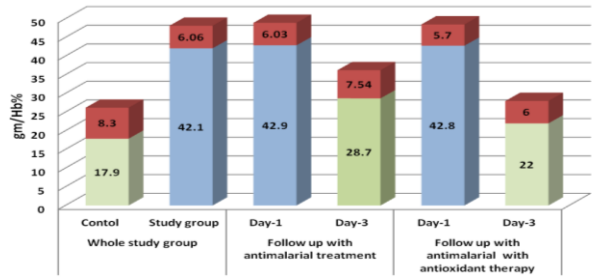

Figure 1: Comparative study of reduced glutathione (GSH) in malaria patients.

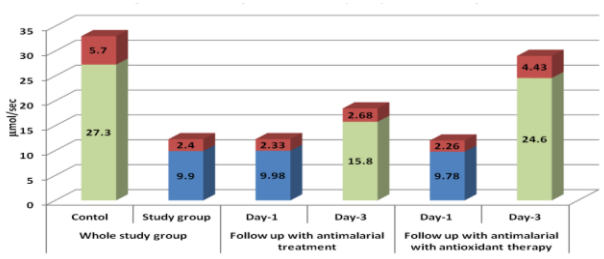

Figure 2: Comparative study of catalase (CAT) in malaria patients.

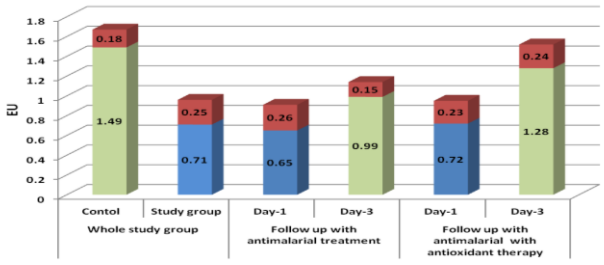

Figure 3: Comparative study of superoxide dismutase (SOD) in malaria patients.

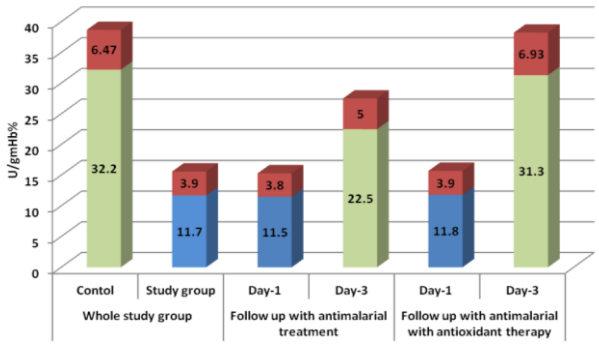

Figure 4: Comparative study of glutathione-S-transferase in malaria patients.

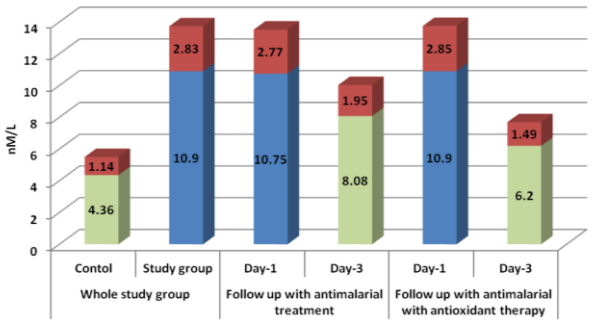

Figure 5: Comparative study of malondialdehyde (MDA) in malaria patients.

DISCUSSION

Oxidative stress plays an important role in the development of metabolic changes in malaria patients. During infection RBCs are exposed to continual oxidative stress. The univalent reduction of oxygen results in a series of cytotoxic oxygen species such as O_2^-,

H_2O_2, $OH^{\bullet}$. These highly reactive species can cause a wide spectrum of cell damage including lipid peroxidation, inactivation of enzymes, alteration of intracellular oxidation-reduction state and damage to DNA.

Many protective mechanisms have evolved in the cells to minimize the toxic effects of the free radicals. These are by antioxidants like GSH and enzymes like CAT, GST and SOD, which catalyses the dismutation of superoxide anions (O_2^-) and H_2O_2 to H_2O at expense of GSH.[17] Reduced glutathione (GSH) plays an important role in protecting cells against oxidative stress and toxic agents. A decrease in its utilization leads to an increase of cellular GSH and total intracellular-SH groups and expose the RBCs to the risk of oxidative stress. The increase of –SH groups in oxidatively stressed RBCs is due to the decrease GST activity of erythrocytes. A longer conjugation with –SH groups of toxic agents due to the decrease in GST activity may lead to their accumulation in the erythrocytes.[18]

A fall in the erythrocytic SOD and CAT in malarial patients indicated that there was a decreased utilization of reduction potential in detoxication of ROS in the patients. This increased vulnerability of the erythrocyte to cellular damage. In the present study, erythrocytic activity of SOD, CAT, GST were decrease and GSH levels increase significantly ($P<0.001$) as compared to control group (Table 1, Figure 1, 2, 3 and 4), higher level of MDA as index of lipid peroxidation compared to their control. In the follow up study with anti-malarial treatment the levels of erythrocytic GSH and MDA decreased significantly ($P<0.001$ and $P<0.01$ respectively), whereas erythrocytic enzymes like SOD, CAT and GST were increased significantly ($P<0.001$) as compared to day-1 (Table 2, Figure 1, 2, 3, 4 and 5).

This could be due to the parasitic clearance leading to decrease in ROS generation by the parasite. These biochemical parameters can provide reliable data for the efficacy of drug. In follow up patients, treated anti-malarial therapy supplemented with antioxidant therapy, showed marked improvement in all the biochemical parameters, which came either very close to or within their respective normal range as compared to control. The level of erythrocytic SOD, CAT and GST marked increase ($P<0.001$) (Table 3, Figure 2, 3 and 4) and GSH and MDA shows significantly decrease ($P<0.001$) as compared to day-1 (Table 3, Figure 1 and 5). It was found that when antioxidant supplementation was given along with anti-malarial treatment patients shows good recovery indicating good prognosis.

CONCLUSION

In view of present finding, we suggest that erythrocytic antioxidant enzymes, GSH and MDA may be considered to be reliable biochemical markers and possess promising rational for diagnostic and therapeutic potential in malaria.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Tyagi AG, Tyagi RA, Choudhary PR, Shekhawat JS. Study of antioxidant status in malaria patients. Int J Res Med Sci 2017;5:1649-54.