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Changes in levels of antioxidant markers and status of some enzyme activities among *Falciparum* Malaria Patients in Yemen

Abstract

Clinical data indicate that oxidative stress and activities may play important roles in *Plasmodium falciparum*. The aim of this study was to assess the erythrocyte antioxidants and evaluate enzyme activities as marker for Plasmodium falciparum malaria patients. The diagnosis of malaria was confirmed by thick and thin film with Giemsa staining of malaria parasite. Ninety consenting individuals, sixty infected patients and thirty uninfected subjects comprising both sexes were randomly selected and age range between 18 to 48 years. The levels of Erythrocytic superoxide dismutase eSOD, serum ceruloplasmin (CP), vitamin C, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were determined. Low levels of essential antioxidants such as eSOD and vitamin C in the patients have been found to be associated with an increased risk of *Plasmodium falciparum* malaria. Low levels of antioxidants observed in the *falciparum* malaria patients may be due to their increased utilization to free radical scavenger. High level of CP observed in the *Plasmodium falciparum* malaria patients may be of powerful potency against oxidative stress. Furthermore, lowered levels of antioxidants especially of vitamin C in malaria infection suggest that antioxidant may play a major role in the treatment of malaria infection. It was detected that serum levels of ALP and LDH were significantly higher in *Plasmodium falciparum* malaria patients group, compared with the healthy group. Higher levels of LDH and ALP in the *Plasmodium falciparum* malaria patients may be used in the diagnosis and treatment and monitoring of patients with malaria. Results also suggest that the rise in LDH activity may be as a result of the cellular anoxia of liver rather than other tissues in malaria patients.

Keywords: *Plasmodium falciparum*, antioxidants, ceruloplasmin, superoxide dismutase, enzymeactivities

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Ali A Al Ezzi,[1] Mohamed B Al Salahy,[2] Bushra H Shnawa,[3] Gamal H Abed,[2] Ahmad M Mandour[4]

[1]Faculty of Science and Education, Aden University, Yemen
[2]Faculty of Science, Assiut University, Egypt
[3]Faculty of Science, Soran University, Iraq
[4]Faculty of Medicine, Assiut University, Egypt

Correspondence: Ali A Al Ezzi, Department of Biology, Faculty of Science and Education, University of Aden, Yemen, Email dr.aliizy@gmail.com

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Abbreviations: ESOD, eerythrocytic superoxide; SOD, eerythrocytic superoxide; CP, ceruloplasmin; ALP, alkaline phosphatase LDH, lactate dehydrogenase; ROS, reactive oxygen species

Introduction

Malaria continues to be a major public health issue. In 2015, an estimated 214 million malaria cases occurred, leading to almost 438,000 deaths.[1] Malaria is one of the most common diseases in Republic of Yemen. Malaria cases in the country are registered all over the year, and a majority of the Yemeni population (65%) is exposed to malaria transmission, with 43% being at high-risk of acquiring the infection[2] *P. falciparum*, the most dangerous species is the major *Plasmodium* species in Yemen with only minimal cases caused *by P. vivax*.[3]

The parasitic infections such as malaria in host organisms often lead to changes in hepatic, erythrocyte activity and oxidative stress condition which are a disturbance in the balance between the productions of reactive oxygen species (ROS) and antioxidant defense.[4] It is also known that erythrocytes are equipped with antioxidant enzymes that could protect them against damage.[5] Adapting to the oxidative stress exerted by the host immune response against malaria infection, *P. falciparum* has developed an elaborate reduction-oxidation (redox) system to maintain adequate antioxidant defense throughout its complex life cycle.[6]

Enzymes biocatalysts are involved in all chemical transformation reactions in the body. It may even decrease the amount of free energy needed to activate a specific reaction for the body function. An enzyme may favor the production of only one of the products, where, more than one product is possible in a reaction. Enzyme activities can be affected by molecules produced by the attack of any disease such as malaria, as well as by any dysfunction in metabolic pathways.[7]

The combination of acute hepatocellular injury and red cell haemolysis induced by the invading merozoites may account for the increase in serum ALP and LDH activity during this infection. Therefore a serum ALP and LDH activities is a potentially valuable enzymatic marker of acute malarial infection.[8] Antioxidants are compounds that are involved in effective scavenging of free radicals and in suppressing the actions of reactive oxygen substances. Antioxidant barriers are extensively distributed and include both enzymatic and non-enzymatic systems. The most important enzymatic antioxidants are superoxide dismutase, glutathione peroxidase and catalase. Non-enzymatic factors that may function as antioxidants are reduced glutathione, vitamin C, vitamin E, β-carotene, ceruloplasmin and bilirubin.[9] These enzymatic and non-enzymatic antioxidant systems are necessary for sustaining life by maintaining a delicate intracellular redox balance and minimizing undesirable cellular damage caused by ROS.[10] Therefore, we aimed to estimate antioxidants and enzyme activities such as levels in serum of patients with *Plasmodium falciparum* malaria to compare the ones in the healthy subjects in order to estimate the possible relationship between these parameters.

Materials and methods

Selection of subjects

This study was carried out between October 2014 to March 2015 in the endemic area in Abbs districts. Patients were selected from the Malaria Center in Abbs Rural Hospital, in Hajjah Governorate, Yemen. The study population consisted of 60 patients, who were reported ill with fever (temperature >37.5°C), headache, vomiting, chills, diarrhea, and other clinical signs and symptoms of malaria as previously documented.[11] Patients included both sex adults between the ages of 18 and 48 years. Thirty (30) healthy subjects who were symptomatic and negative for *P. falciparum* in their peripheral blood were used as control individuals.

Collection hemolysate and preparation of erythrocyte

An aliquot of 5ml of venous blood samples were collected randomly from malaria patients and normal healthy subjects. 1ml of the blood was slowly ejected into EDTA containing tubes for malaria parasite tests blood, while the rest was left for about 30 min to coagulate. Samples were centrifuged at 1500 rpm for 15 minutes. Serum was transferred into Bijou bottle and stored frozen until required for the estimation of CP, Vit. C, ALP, LDH. Erythrocytes were washed three times in cold phosphate-buffered saline, followed by re-centrifugation and removal of the supernatant fluid and the buffy coats. The crude red cells were lysed in nine volumes of ice-cold distilled water to prepare a 10% erythrocyte hemolysate and used for the estimation of superoxide dismutase (SOD).

Estimation of ceruloplasmin (CP)

CP was estimated according to the method of Houchin.[12]: In Cuvette format: add 0.5 ml of 0.1% p-phenylene-diamine reagent, 100 ml serum was added and mix, leave at 37 oC for 15 minutes, Then 1.25 ml sodium azide was added and OD is measured at 525 nm; Calculate CP activity = 150 X OD525 nm mg/dl.

Estimation of ascorbate (Vitamin C)

Serum total ascorbate was estimated by the 2, 4-dinitrophenylhydrazine (DNPH) and method reported by McCormick and Green.[13]

Estimation of superoxide dismutase (SOD)

SOD activity was determined based on its ability to inhibit the autoxidation of epinephrine in alkaline conditions as described by Misra & Fridovich[14] Sigma SOD enzyme was used as standard. OD was measured at 480 nm. The hemoglobin content of the erythrocytes was determined in hemolysate using hemoglobin kit (SPECTRUM) by spectrophotometry.

Estimation alkaline phosphatase (ALP) lactate dehydrogenase (LDH): ALP estimated by Roche/ Hitachi –U.S. Acobasc systems. LDH estimated by kits commercial.

Statistical Analysis

Data was entered into program of Microsoft Excel and statistic was applied. The data were expressed as mean ± SE. The results were analyzed statistically using column statistics and one t- tests. Correlation among the investigated parameters was tested by curves and regression using linear regression to test departure from linearity with runs test. These analyses were carried out using computer statistics Prism 3.0 Package (Graph and Software, Inc, San Diego, USA). The minimum level of statistical significance was set at P< 0.05, 0.01.

Ethical considerations

Ethical approval was given by the Hospital Management and Center of Malaria in Abs area.

Results

The patients with *P. falciparum* infection showed marked increase of the levels of CP ($p<0.001$) as compared to healthy control subjects (Table 1). The lysate SOD levels were decreased significantly ($p<0.0001$) as compared to healthy control subjects (Table 1 & Figure 1). Serum vitamin C was decreased significantly ($p<0.0001$) as compared to healthy subjects (Table 1 & Figure 1). In contrast, Serum ALP and LDH levels of patients with *Plasmodium falciparum* malaria were significantly higher ($p<0.0001$) than the healthy control subjects group (Table 2 & Figure 2).

Table 1 Antioxidants of *falciparum* malarial infected and healthy subjects

	Patients with malaria	Healthy subjects
Parameters	N=60	N=30
CP mg/dl	89.97±3.42**	70.69±1.37
SOD U/min g Hb	7.66±0.32***	19.64±0.50
Vit. C mg/dl	0.41±0.01***	0.83±0.07

**= p<0.001, ***= p<0.0001.

Table 2 Antioxidants of *falciparum* malarial infected and healthy subjects.y

	Patients with malaria	Healthy subjects
Parameters	N=60	N=30
(ALP) U/L	151.4±8.85**	108.2±6.80
(LDH) U/L	253.1±3.92***	165.7±6.51

**= p<0.001, ***= p<0.0001

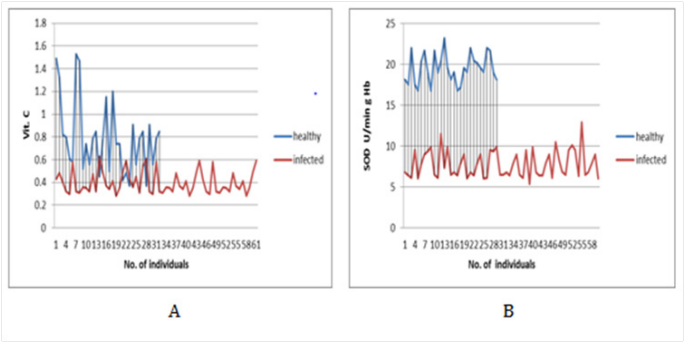

Figure 1 Effect of *P. falciparum* malaria infection on (A) Vit. C and (B) SOD in patients.

Discussion

We have found in our study highly significant decrease in erythrocyte antioxidants, superoxide dismutase (SOD). The mean erythrocyte concentration of SOD was significantly lower in patients than healthy subjects. Several studies reported reduction of SOD activities of erythrocytes in patients with malaria,[15-18] which confirms results obtained. This affirms its role as an antioxidant, where levels decreased in an effort to offset the oxidant stress. SOD upgrades endothelial cell damage triggered by adherent parasitized RBCs, underscoring their probable therapeutic benefit as endothelial cell protectors[19] Pabon et al.[20], have reported increase in SOD and glutathione peroxidase activity in patients with non-complicated malaria.

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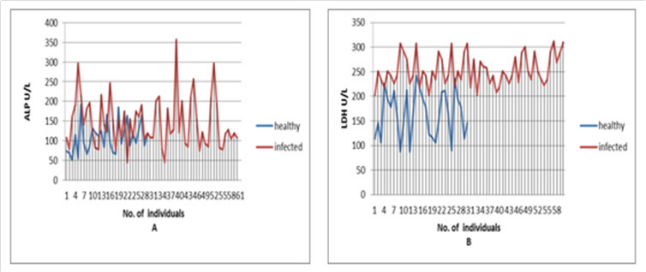

Figure 2 Effect of *P. falciparum* malaria infection on (A) ALP and (B) LDH in patients.

In our findings the levels of CP in *Plasmodium falciparum* malaria are significantly increased as compared to the normal healthy controls. Das et al.[21], have reported high levels of CP in both symptomatic and asymptomatic malaria. Kamble et al.[22] have reported high levels of CP in both *Plasmodium falciparum* malaria and *Plasmodium vivax* malaria. The significant rise in serum CP of malaria infected patients may stimulate liver in spite of its pathogenic case, due to malaria infection, to synthesize an excess of CP. The rise of serum CP during malarial infection may prevent Fenton reaction where the Fe+++ without formation of hydroxyl free redicals. This result suggests that in malarial patients, CP was activated to contract the production of hydroxyl free radicals. Furthermore, the level of vitamin C was significantly decreased in malaria subjects when compared with the healthy subject control ($P<0.0001$). This finding is similar to previous studies.[23] The decrease in antioxidant Vitamin C in the patient groups might be due to counteract increase oxidative stress during acute phase. Supporting to this interpretation the present data showed negative significant correlation between LPO and vitamin C, and this may explain how important vitamin C is in face of damage resulting from oxidative stress situations.

The liver damage may have been caused by the free radicals generated by the *P. falciparum* parasite. The levels of hydroxyl and peroxide radicals induced by *P. falciparum* parasites may be responsible for the changes in the enzyme levels.[24] The result of this study shows also significant increase in the serum ALP activity in malaria.[25] patients. Our results are consistent with other studies which reported that majority of the patients show elevation in serum activities of ALP indicating liver damage as described by several studies.[26-28] The observed elevation in serum ALP activity is an indication that the hepatic stage of the parasite's life cycle occur in its human host and is accompanied by significant perturbation in the hepatocytes membrane leading to leakage of this enzyme out of the liver cells. The increased serum ALP activity among the patients indicates that the liver stage of *Plasmodium falciparum* malaria infection is accompanied by a perturbation of the host hepatocytes pathways and damage to hepatocytes membrane leading to leakage of this enzyme out of the liver cells.[8]

Also, this elevation of ALP may be indicator of cholestasis as causative effect of malaria infection. This is consistent with a previous study,[29] which linked between the serum elevation of ALP activity and cholestasis. Various authors have reported close relationship between incidence of severe malaria and liver damage characterized by jaundice.[30,31] LDH activity is present abundantly in tissues (liver, RBC) which get infected by malaria parasite during completion of asexual cycle. So, raised LDH level may be considered as an evidence for *P. falciparum* infection. Being rich sources of LDH, the acute liver injury and red blood cell destruction will be followed by the release of LDH into the circulation. This finding has important implications because it highlights the potential of using serum LDH activity as an index in the monitoring of acute *P. falciparum* malaria infection,[32] particularly when all other possible causes of increased serum LDH levels have been observed.[8] In our study, LDH was increased in *P. falciparum* affected patients as compared to healthy subjects. The increased LDH concentrations among patients with malaria were reported by several authors.[8,33,34] The observed increase in serum LDH activity in this study may be responsible for acute *P. falciparum* malaria infections during the attack of the liver and RBC cells by sporozoites. The parasite may exert anoxia (Hypoxia) in hepatic tissue which may cause the significant rise in LDH. This interpretation was based on the authors who associated between the rise in LDH and hypoxia.[35,36]

Conflicts of interest

There is no conflict of interest.

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