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▣ BRIEF COMMUNICATION

Oxidative Stress in Vivax Malaria

Ramazan Bilgin[1,*], Mustafa S. Yalcin[1], Guzide Yucebilgic[1], Ismail S. Koltas[2] and Süleyman Yazar[3]

[1]University of Cukurova, Arts & Science Faculty, Chemistry Department (Biochemistry Division), Adana, Turkey; [2]University of Cukurova, Medicine Faculty, Parasitology Department, Adana,Turkey; [3]Erciyes University, Medicine Faculty, Parasitology Department, Kayseri, Turkey

Abstract: Malaria is still a leading cause of morbidity and mortality. The increase in lipid peroxidation reported in malaria infection and antioxidant status may be a useful marker of oxidative stress during malaria infection. The aim of this study was to investigate the role of antioxidant enzymes against toxic reactive oxygen species in patients infected with *Plasmodium vivax* and healthy controls. Malondialdehyde levels, superoxide dismutase, and glutathione peroxidase activities were determined in 91 *P. vivax* patients and compared with 52 controls. Malondialdehyde levels, superoxide dismutase, and glutathione peroxidase activities were 8.07±2.29 nM/ml, 2.69±0.33 U/ml, and 49.6±3.2 U/g Hb in the patient group and 2.72±0.50 nM/ml, 3.71±0.47 U/ml, and 62.3±4.3 U/g Hb in the control group, respectively. Malondialdehyde levels were found statistically significant in patients with vivax malaria higher than in healthy controls ($P<0.001$). On the other hand, superoxide dismutase and glutathione peroxidase activities were found to be significantly lower in vivax malaria patients than in controls ($P<0.05$). There was an increase in oxidative stress in vivax malaria. The results suggested that antioxidant defense mechanisms may play an important role in the pathogenesis of *P. vivax.*

Key words: *Plasmodium vivax*, malondialdehyde, superoxide dismutase, glutathione peroxidase, malaria

Plasmodium vivax is the most common human malaria parasite of the 4 species affecting humans. Currently, this infection is endemic in many countries of Asia, South Pacific, North Africa, Middle East, and South and Central America [1]. Malaria is a major health problem in the tropics with 300-500 million cases occurring annually, and an estimated 1.1-2.7 million deaths each year as a result of severe malaria [2,3]. Vivax malaria has been endemic in Turkey for a long time. *Anopheles sacharovi* was known to be the vector capable of transmitting *P. vivax* in Turkey. The prevalence of malaria in Turkey followed a decreased pattern between 1950 and 1975 compared to values exceeding 140,000 cases per year despite the lower population of the country before 1950. A sudden increase was observed at the end of the 1970s, reaching values over annual 100,000 cases. In 1957, the Turkish government, in collaboration with the World Health Organization and UNICEF, introduced a malarial eradication project that involved active and passive case detection combined with chloroquine-primaquine treatment of identified patients with malaria. Improved socioeconomic conditions and governmental malaria control activities succeeded to control the disease, although 2 peaks were observed in the mid-1980s and mid-1990s with a resulting 10,224 cases reported at the end of 2002 [4].

Oxidative stress is an important clinical and pathobiochemical factor as well as an effective therapeutic principle in malaria [5]. Oxidative stress is associated with generation of reactive oxygen species that are responsible for the damage of a variety of cellular components. The prevention of such biological damage can be achieved by dismutation of superoxide to H_2O_2 which in turn is removed by catalase (CAT) and glutathione peroxidase (GPX). Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, which is the most abundant malondialdehyde (MDA), superoxide dismutase (SOD), and GPX. Therefore, measurement of MDA, SOD, and GPX is widely used as an indicator of lipid peroxidation. Increased levels of lipid peroxidation products have been associated with a variety of diseases in both humans and model systems [6-11]. In this study, we determined the levels of MDA, SOD, and GPX activities in patients infected with *P. vivax* and the control group.

We assayed levels of MDA, SOD, and GPX activities in 91

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* Corresponding author (rbilgin@cu.edu.tr)

subjects aged between 15-46 years (47 males and 44 females). Blood samples for the control group were obtained from healthy subjects. All subjects fasted after midnight before blood collection in the next morning. Total 91 patients and 52 controls were examined in this study. The study was approved by the Committee for Ethics of Medical Experiment on Human Subjects, Faculty of Medicine, Cukurova University, Adana, Turkey. The mean age of the patient group, which consisted of 47 males and 44 females, was 28.6 ± 9.8 years and 29.3 ± 9.4 years, respectively. The mean age of the control group, which included 27 males and 25 females, was 29.1 ± 9.9 years and 28.9 ± 9.2 years, respectively. Lipid peroxidation was assessed by measuring MDA, an end product of fatty acid peroxidation. according to the method of Ohkawa et al. [12]. SOD activity was determined according to the method of Sun et al. [13]. GPX activity was determined according to the method of Beutler [14].

Statistical analysis was performed with SPSS software package (Version 11.0 for Windows). The data were expressed as the mean ± SD. For comparison of the 2 groups of continuous variables, independent sample *t*-test was used. A probability value of $P < 0.05$ indicated a statistically significant difference.

Table 1 shows the MDA levels, SOD, and GPX activities of patients infected with *P. vivax* and the control group. 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, SOD and GPX activities were found to be significantly lower in male and female patients with vivax malaria than in healthy controls ($P < 0.05$; Table 1).

Malarial infection is associated with an increased production of reactive oxygen species by phagocytic cells. Changes due to toxic metabolites of the host and parasite may render the erythrocytes more vulnerable to damage. Several explanations have been suggested that involve depressed erythropoiesis, impaired structural integrity of erythrocytes because of lack of certain essential metabolites, and reduced ability of erythrocytes to protect themselves from the oxidative stress induced by malarial parasites leading to increased lipid peroxidation [15-18]. SOD and GPX are intracellular and antioxidant defense mechanisms to cope with increased oxidant stress. SOD and GPX eliminate superoxide anions and hydroperoxides that may oxidize cellular substrates, and they prevent free radical chain reactions [18]. GPX is the key enzyme in the glutathione redox cycle responsible for the reduction of hydroperoxides. GPX has an absolute requirement for reduced glutathione as a co-substrate that undergoes inactivation by hydroxyl radicals and superoxide anion [19]. It has been stated that GPX is the preferential pathway for the elimination of low concentartions of hydrogen peroxide [20]. Decreased GPX activity has been found in patients with vivax malaria. Bhatttacharya et al. [21] detected decreased erythrocyte GPX activity to which our results agreed well. An increase in lipid peroxidation was reported in human *Plasmodium falciparum* malaria [22]. On the other hand, MDA levels were investigated in vivax malaria. Yazar et al. [23] found the relationship between *P. vivax* and MDA, which is a well-established mechanism of cellular injury in humans, which is used as an indicator of oxidative stress in cells and tissues. Acute non-complicated *P. falciparum* or *P. vivax* malaria resulted in high oxidative stress. This resulted from lipid peroxidation rather than from a reduced total antioxidant status. Therefore, it was proposed that MDA/total antioxidant status index was a useful marker of oxidative stress during malaria infection [24].

Table 1. MDA levels, SOD, and GPX activities of patients infected with *Plasmodium vivax* and control group

Parameters	No. subjects	Age (years)	MDA levels (nM/ml)	SOD activity (U/ml)	GPX activity (U/g Hb)
Patients					
Female	44	29.3 ± 9.4	8.31 ± 2.16	2.67 ± 0.35	47.5 ± 3.5
Male	47	28.6 ± 9.8	7.83 ± 2.42	2.71 ± 0.31	51.7 ± 2.9
Total	91	29.4 ± 9.3	8.07 ± 2.29	2.69 ± 0.33	49.6 ± 3.2
Controls					
Female	25	28.9 ± 9.2	2.79 ± 0.47	3.69 ± 0.49	60.2 ± 4.2
Male	27	29.1 ± 9.9	2.64 ± 0.52	3.73 ± 0.45	64.4 ± 4.4
Total	52	28.5 ± 9.7	2.72 ± 0.50	3.71 ± 0.47	62.3 ± 4.3
			$P < 0.001$	$P < 0.05$	$P < 0.05$

The data are expressed as mean ± SD.

The detoxification of reactive oxygen species (ROS) is a challenge for erythrocytes infected with *Plasmodium*. As a result of the high metabolic rate of rapidly multiplying parasites, large quantities of toxic redox-active by-products are generated. Central to the generation of oxidative stress is the degradation of host hemoglobin by the parasite. Hemoglobin represents the major source of amino acids for *Plasmodium*, but its degradation in an acidic food vacuole results in the production of toxic free heme (ferri/ferroprotoporphyrin IX; FP) and ROS. During malaria infection, especially, changes in the splenic structure can result in asymptomatic enlargement or complications, such as hematoma formation, rupture, hypersplenism, ectopic spleen, torsion, or cyst formation. It was mentioned that massive splenic enlargement may cause an abnormal immunological response in malaria. Spontaneous distruption of the spleen

is one of the important complication of *P. vivax* infection. The ability to properly diagnose and manage these complications is important. Spleen-conserving procedures should be the standard whenever possible especially in patients with a high likelihood of future exposure to malaria [25].

The results of our study suggested that one of the main reasons for high MDA levels in patients with *P. vivax* malaria could be a decreased activity of the defense system protecting tissues from free radical damage. These results may indicate oxidative stress as a mediator of tissue damage concurrent with *P. vivax* infection.

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