SECONDARY DEFENSE ANTIOXIDANT STATUS OF VITAMIN C, VITAMIN E AND GSH IN MALARIA, CAUSED BY PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX.

TARIQ JAVEED, GHULAM MUSTAFA, IKRAMULLAH KHAN* AND MUHAMMAD KHALID KHAN

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gomal University, D.I. Khan, Pakistan

ABSTRACT

Present investigations focused on the antioxidant defense in malaria caused by plasmodium Falciparum and plasmodium Vivax the mean ±SEM values of Vitamin C, Vitamin E and GSH very highly significantly decreased as compared with normal individuals in both malaria species which cause malaria disease. The antioxidant levels in female were decreased very significantly due to decreased levels of antioxidant as compared with the male patients.

The results are shown as mean ±SME, the antioxidant levels in malarial patients was compared with normal individuals, in both genders. The antioxidant levels of vitamin C, Vitamin E and glutathione decreased in malaria caused by both species, a much greater decrease in patients infected by Plasmodium Vivax. P <001 was considered significant. The decrease of antioxidant levels was higher in female patients as compared with male patients.

Antioxidant supplements like Vitamin C, Vitamin E and GHS may be used with anti malarial therapy, as a preventive measure because malaria affects the secondary antioxidant defense system of the body.

Keyword: Plasmodium Falciparum, Plasmodium Vivax, Antioxidant status, Secondary defense system.

INTRODUCTION

Malaria is a parasitic disease caused by a unicellular protozoan plasmodium (Krotoski *et al.*, 1982), leading to damage of red blood cells and vital organs of the body.

Malaria is the World most important parasitic infection (Meis *et al.*, 1983). Plasmodium parasite can infect humans, the most serious forms of the disease are caused by Plasmodium Falciparum and Plasmodium Vivax but the other related species can also affect human (Medis *et al*., 2001).

Malaria parasites infects a human body, first in the liver and then in the bloodstream. First, sporozoites enter the bloodstream, and migrate to the liver. They infect liver cells (hepatocytes), where they multiply into merozoites, these rupture the liver cells, and escape back into the bloodstream. Then, the merozoites infect red blood cells (erythrocytes), where they develop into a ring forms, the trophozoites(a feeding stage), converted into a multinucleated schizonts (a reproduction stage), and merozoites again. The merozoites rupture the blood cells and return to the bloodstream to infect more red blood cells. Only the ring forms circulate in the bloodstream; the other red blood cells stick (adhere) to the walls (endothelium) of small blood vessels (venules), preventing the infected red blood cells from traveling to the spleen and being destroyed (Bledsoe, 2005, Sturm *et al*., 2006).

Plasmodium Falciparum is the most dangerous among other infections in human being the highest rates of complications and mortality have been reported. The terophozoites of Plasmodium Falciparum develops inside the red blood cells followed by adhering to endothelial cells in blood vessels, thus evading clearance in the spleen (Cholera *et al*., 2008).

The enlargement of the spleen is the most common complication of malaria caused by plasmodium Vivax. Malaria produced by plasmodium Vivax relapses often occurs months to years after treatment because some of the parasites can become dormant in the liver (Trampuz *et al.*, 2003, Mockenhaupt *et al.*, 2004).

The oxidant stress originates due to destruction of red cells, which cause imbalance between the generation of reactive oxygen species and the antioxidant defense system (Bornefont-Rousselot, 2000, Medis *et al.*, 2001).

Total antioxidant capacity is a parameter characterizing the sum of the activities of antioxidant present in the material studied. TAC most frequently studied in blood plasma may be a marker of the antioxidant status of the body (Bartosz, 2003).

**Corresponding author:* e-mail: ikramdik@gmail.com

The aim of this study was to compare the antioxidant status of secondary defense system affected by malaria caused by Plasmodium Falciparum and Plasmodium Vivax species.

MATERIALS AND METHODS

Normal control

This experimental study was conducted at the department of Pharmaceutical Chemistry, Faculty of pharmacy, Gomal University over a period of one and half years, on normal controls and malaria patients infected by Plasmodium Falciparum and Plasmodium Vivax. Blood samples were collected from patients visited Gomal Medical College teaching Hospital D.I. Khan with the consent of patients and doctors.

The age of normal control and patients ranged from 20-50 years. Mean 34.4 years. There were 25 male and 25 female in each group of Normal controls (N), patients infected by Plasmodium Falciparum (F) and Plasmodium Vivax (V).

Malaria diagnosis

Malaria was diagnosed with the blood smear staining method of Warhusted and William 1996. The stages of malaria were confirmed by microscopic examination. It was also confirmed that the patients were not taking any antimalarial drugs or any food supplement.

Blood samples

Blood samples were collected into centrifuge tubes and left to stand at room temperature fro 10 minutes and centrifuged at 3000 rpm for 15 minutes. The separated serum samples were stored at -20°C for determination.

Antioxidant activity

The concentration of antioxidants Vitamin C, Vitamin E and GHS was estimated by FRAP assay according to the procedure described by Pulido *et al*. (2005).

All chemicals and reagents of analytical grade purchased from Sigma, Aldrich and Fluka.

STATISTICAL ANALYSIS

The values are expressed as mean ±SEM. The statistical analysis of data was performed using student t-test, value $P<0.01$ were considered significant as compared to control.

RESULT

Normal control

25 Male normal individual were selected are ranged between 20-50 years with mean age was 34.4 years. Mean ± SEM serum antioxidant values (µ mol/l) for Vitamin C, Vitamin E and GSH were obtained 465.95 ± 8.0, 503.99 ± 8.8 and 2520.19 ± 44.1 respectively.

25 normal female subjects were selected are ranged between 20-50 years the mean age was 34.1 years, mean ±SEM antioxidant values for Vitamin C, Vitamin E and GSH were estimated as 439.70 ± 8.1, 484.95 ± 8.9 and 2425.03 ± 44.5 respectively. Values are shown in table.

Malaria Falciparum

25 Male infected by Plasmodium Falciparum patients were selected age ranged 20-50 years the mean age is 34.2 years. Serum antioxidant values (µ mol/l) obtained by FRAP method in malaria disease caused by plasmodium Falciparum as Vitamin C, Vitamin E and GSH were observed 319.31±15.2, 352.18±16.8 and 1761.08±84.2 respectively. Values are shown in table.

25 female infected by plasmodium Falciparum patients

Table: Mean ±SEM FRAP values (µ mol/l) Vitamin C, Vitamin E and GSH levels in Normal controls Malaria caused by Plasmodium Falciparum and Plasmodium Vivax.

Sex and Age	Normal Controls	Malaria (Falciparm)	Malaria (Vivax)
Vitamin C (µ mol/l)			
Male 20-50 years	465.95±8.0	319.31±15.2***	332.21±12.3***
Female 20-50 years	439.70±8.1	277.33±13.4***	284.55±11.7***
Vitamin E (µ mol/l)			
	Mean Age		
Males 20-50 years	503.99±8.8	352.18±16.8***	366.41±13.6**
Females 20-50 years	484.95±8.9	305.87±14.8***	313.83±12.7**
Glutathione			
	Mean Age		
Males 20-50 years	2520.19±44.1	1761.08±84.2***	1832.22±68.1***
Females 20-50 years	2425.03±44.8	1529.54±74.4***	1569.32±64**

Level of Significance *** = very highly significant, **= highly significant, *= Significant
Differences were considered significant at $P<0.01$, as compared to control.

were selected age ranged 20-50 years, the mean age was 34.5 years. Mean ±SEM antioxidant values for Vitamin C, Vitamin E and GSH were observed 277.33±13.4, 305.87±14.8 and 1529.54±74.4 respectively. Values are shown in table.

Malaria Vivax

25 Male patients infected by Plasmodium Vivax were selected between 20-50 years age, the mean age was 36 years. Mean ± SEM Serum antioxidant value (µ mol/l) for Vitamin C, Vitamin E and glutathione were calculated as 332.21 ± 12.3, 366.41 ± 13.6 and 1832.22 ± 68.1 respectively.

25 Female patients infected by Plasmodium Vivax were selected between 20-50 years age, the mean age was 34

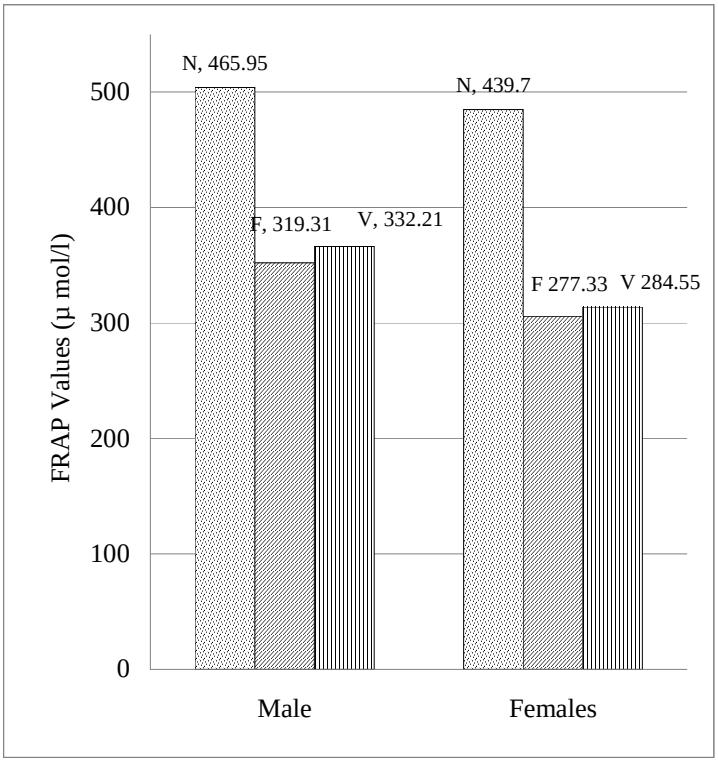

Fig. 1: The Comparative levels of Vitamin C FRAP values in male and female of Normal Controls (N), Malaria caused by Plasmodium Falciparum (F) and Plasmodium Vivax (V) patients.

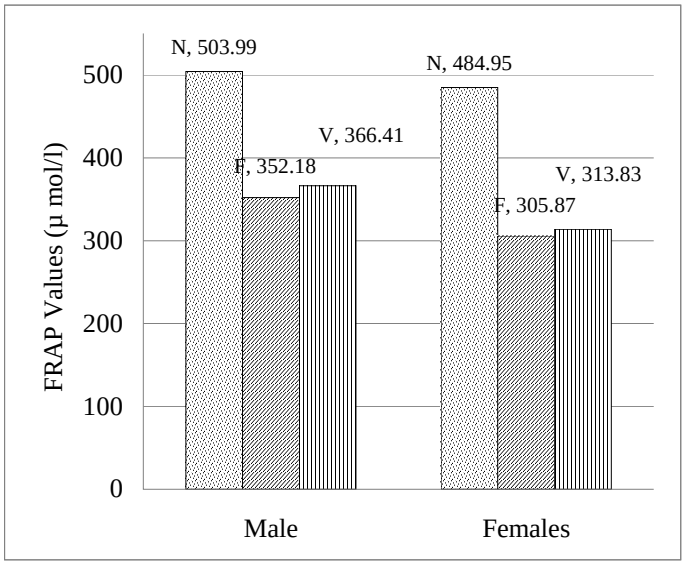

Fig. 2: Comparative levels of Vitamin E FRAP values in male and female (20-50 years) in Normal controls (N), Malaria caused by Plasmodium Falciparum (F) and Malaria caused by Plasmodium Vivax (V) patients.

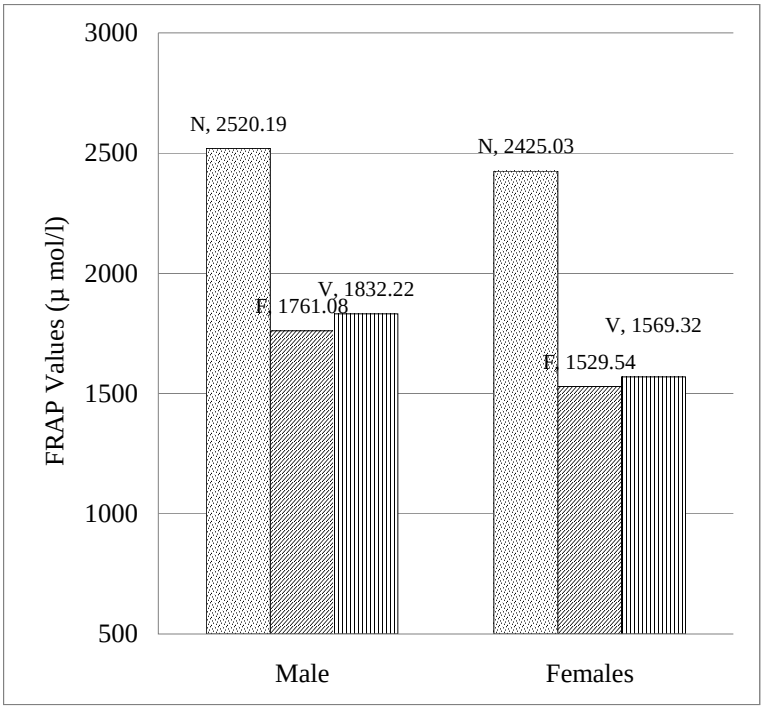

Fig. 3: Comparative levels of GSH FRAP values in male and female (20-50 year age) in Normal controls (N), patients infected by Plasmodium Falciparum (F) and Plasmodium Vivax (V).

years. Mean ±SEM serum antioxidant values obtained by FRAP method for Vitamin C, Vitamin E and GSH were obtained 284.55 ± 11.7, 313.83 ± 12.9 and 1569.32 ± 64 respectively are given in table.

DISCUSSION

The result of antioxidant status of Malaria caused by Plasmodium Falciparum and Plasmodium Vivax was found in age and sex matched groups, the vitamin C content in male and female are shown in table and fig. 1 were very highly significant lowered as compared with normal control group with same age limit. The Vitamin C value of antioxidant capacity of female group was also highly significant decreased. The result shows that in Malaria caused by Plasmodium Falciparum and Plasmodium Vivax the oxidative stress is due to the over load in reactive oxygen species which create an increase in the oxygen free radicals and failure of normal defense mechanism that decreases the antioxidant blood serum levels (Hunt and Stocker, 1990). The vitamin C is an aqueous phase as antioxidant present in high concentration. In tissue the concentration of Vitamin C decrease significantly because it is consumed in the generation and activation of Vitamin E.

The result of Vitamin E values (µ mol/l) male measured by FRAP method in patients of which where affected by Plasmodium Falciparum and Plasmodium Vivax. The Mean ± SEM values are summarixed in table and shown in fig. 2. The Vitamin E values in both Malaria disease in patients were found decreased very highly significant as compared with normal controls. The Vitamin E is a lipid phase antioxidant that partition into cell membranes and converts superoxide anion, Hydroxyl and other chemical radicals to less reactive forms. It acts by donating hydrogen ion to the radicals, a stable Vitamin E radical is formed which may also be able to quench the effect of reactive oxygen species. The antioxidant such as superoxide radicals, Hydrogen peroxide, Hydroxyl radicals and lipid peroxides play an important role in human disease. Pathogenesis of an infecting agent cause an over production of free radical species and failure of normal defense mechanism that decreases antioxidant level which leads to decreases elimination of reactive substances.(Thurnhann *et al.*, 1988)

Glutathione (GSH) is a very important for secondary defense against reactive oxygen species. The values of antioxidant glutathione were compared in decease with normal controls are summarized in table and shown in fig. 3. The serum antioxidant glutathione level in malaria caused by Plasmodium Falciparum patients were shown a very highly significant decrease when compared with malaria caused by Plasmodium Vivax. The reduction of glutathione in diseased state was due to quench free radicals by donating electrons from their oxidizable functional group. The oxidative stress can also occur due to decreased levels of glutathione in certain tissues. (Maxwell, 1995)

In conclusion the antioxidant levels of secondary antioxidant levels of Vitamin C, Vitamin E and glutathione decreased in malaria disease caused by Plasmodium Falciparum and Plasmodium Vivax. The antioxidant values decreased significantly in malaria caused by Plasmodium Vivax as compared with Plasmodium Falciparum in both genders. In treatment of malaria disease with antimalarial drugs some antioxidants of secondary defense system must be given to patients as preventive measure which will help in early recovery by increasing the antioxidant defense system in the body.

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