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Antioxidant Status of Bilirubin and Uric Acid in Patients Diagnosed with *Plasmodium falciparum* Malaria in Douala

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Abstract: Oxidative stress and changes in antioxidant status have been implicated in the pathogenesis of malaria. To assess the antioxidant level of bilirubin and uric acid associated with falciparum malaria infection, 60 untreated patients (30 men and 30 women) in Douala, Cameroon were screened for the study. Sixty five healthy individuals (29 men and 36 women) were used as controls. Total and conjugated bilirubin were calculated using Jendrassik-Grof method while uric acid was determined using Barham-Trinder method. It was observed that total and conjugated bilirubins were significantly ($p < 0.001$) higher in malaria patients (10.722 ± 4.043 and 3.627 ± 1.571 mg L⁻¹, respectively) when compared to control (6.830 ± 2.436 and 1.777 ± 0.729 mg L⁻¹) and these bilirubin levels increased significantly with parasite count ($p < 0.050$). There was also significant increased ($p = 0.021$) of uric acid in malaria patients (56.262 ± 13.963 mg L⁻¹) compared to controls (49.838 ± 15.419 mg L⁻¹). No significant differences based on sex were observed on uric acid, parasite count, total and conjugated bilirubins in malaria patients. Positive correlations were obtained between parasite count and total bilirubin ($r = 0.320$, $p < 0.050$), conjugated bilirubin ($r = 0.477$, $p < 0.001$), uric acid ($r = 0.060$, $p > 0.050$) and between total and conjugated bilirubin ($r = 0.729$, $p < 0.001$). From this study, it has been hypothesized that the augmentation of plasma level of bilirubin and uric acid could provide more protection against oxidative stress induced by malaria.

Key words: Antioxidant, bilirubin, uric acid, oxidative stress, malaria

INTRODUCTION

Malaria is known as the world's most important tropical parasitic infectious disease to human (Donovan *et al.*, 2007). It generates 300 to 500 millions of case and an average of 2 million deaths every year (Kochar *et al.*, 2003). It is caused by protozoan parasite of the genus *Plasmodium* and transmitted from one human to another by the bite of female infected anopheles mosquitoes (Pete, 1999). In recent times, there has been considerable interest in oxidative stress caused by Reactive Oxygen Species (ROS) and its involvement in the disease process (Brownlee *et al.*, 2007; Ochs-Balcom *et al.*, 2006; Dudka, 2006). ROS are natural by-products of the normal metabolism of oxygen playing an important role in cell signalling (Hancock *et al.*, 2001). They are formed permanently in the Human cells at different levels (Guzman *et al.*, 2001). Malaria infection has shown to generate abnormally large quantities of ROS (Farombi *et al.*, 2003; Siddiqi and Alhomida, 1999)

as well as changes in antioxidant defence system (Prasannachandra *et al.*, 2006). Antioxidants are scavengers of ROS and include bilirubin and uric acid. Urate is a final oxidation product of purine catabolism. Bilirubin is a breakdown product of hemoglobin. It circulates in the bloodstream in two forms: free bilirubin and conjugated with polar compounds like glucuronic acid (Chang *et al.*, 2007). These molecules have been considerably studied as waste metabolites (Alexander and Gutman, 2005; Hammerman *et al.*, 1998) but, recent studies demonstrated their efficiency against ROS (Temme *et al.*, 2001; Kehrer, 2000; Ivanova and Ivanova, 2000). It looks important to use the benefit effects of these molecules, which at a small scale, protect human cells against expansion and gravity of some diseases.

There are few published reports on biochemical parameters changes in plasmodium infection especially regarding oxidative stress and antioxidant status. The present study was therefore undertaken to estimate the levels of bilirubins and uric acid in malarial patients.

MATERIALS AND METHODS

The studied population recruited in January 2003 consisted of 60 untreated patients with *Plasmodium falciparum* malaria (30 men and 30 women) between the age of 15 to 74 years at Douala's Laquintinie hospital, Cameroon and 65 controls (29 men and 36 women) between 16 and 79 year old. Only patients with general signs of malaria like muscle pain, headache, chills, fever and fatigue were used. They were asked if they had any other illness than malaria, but no laboratory or clinical diagnosis were done to confirm their answers. So all malaria patients who did not report any other disease or ailment were included in the study. Children under 15, pregnant women and breastfeeding mothers were excluded. Blood sample were removed from the studied population with their informed consent. For malaria investigation, a finger prick blood sample was taken to prepare thick and thin blood film by the routine giemsa-stained that is used to determine the presence or absence of malaria parasites. Approximately 4 mL of venous blood samples were collected from confirmed malaria patients and control individuals. Plasma was obtained after centrifugation of blood at 3000 rpm for 10 min at room temperature and used to estimate the concentration of uric acid, total and conjugated bilirubin. Total and conjugated bilirubin analyses were carried out using Diazo reaction method with diazotized sulfamyl acid (Jendrassik and Grof, 1938). Uric acid concentration was determined according to Barham and Trinder (1972) method. Statistical analysis was performed using SPSS 10.0 for windows. Values are expressed as mean±standard deviation. Student Newman Keuls test was used to assess significance of difference and correlations between Parasite count, uric acid, total and conjugated bilirubins were determined using Pearson correlation.

RESULTS

Table 1 shows the mean±SD of biochemical parameters studied in malaria patients and controls. Student t-test indicates that plasma concentrations of total and conjugated bilirubin increased significantly ($p<0.001$) in malaria patients compared to controls. Uric acid status is significantly higher in malaria patients than control group ($p = 0.021$). Comparison based on sex of patients revealed that there were not statistical differences of parasite counts, uric acid and bilirubins levels between male and female. Table 2 and 3 indicate correlations between the different parameters. Parasite counts correlated positively with total bilirubin ($r = 0.320$,

Table 1: Comparison of plasma level of uric acid, total and conjugated bilirubin (mean±SD) in patients and controls

Groups	Total bilirubin	Conjugated bilirubin (mg L ⁻¹)	Uric acid
Malarial patients (N = 60)	10.722±4.043 ^A 3.1-19.5 ^B	3.627±1.571 0.3-8.1	56.262±13.963 29.7-92.1
Controls (N = 65)	6.830±2.436 3.2-11.3	1.777±0.729 0.1-3.4	49.838±15.419 18.2-88.3
t-value	-6.966	-8.153	-2.388
p-value	<0.001	<0.001	<0.050

^AMean±SD, ^BRange

Table 2: Correlations between parameters in malarial patients

Parameters	Total bilirubin	Conjugated bilirubin	Uric acid
Parasite count	0.320 ^A <0.050 ^{B*}	0.477 <0.001***	0.060 0.847 ^{NS}
Total bilirubin		0.729 <0.001***	-0.058 0.662 ^{NS}
Conjugated bilirubin			0.132 0.315 ^{NS}

^APearson's correlation coefficient, ^BProbability value, *Significant at 0.05 probability level, **Significant at 0.01 probability level, ***Significant at 0.001 probability level, NS = Not Significant

Table 3: Correlation between parameters in controls

Parameters	Conjugated bilirubin	Uric acid
Total bilirubin	0.369 ^A <0.010 ^{B***}	0.050 0.695 ^{NS}
Conjugated bilirubin		-0.018 0.890 ^{NS}

^APearson's correlation coefficient, ^BProbability value, *Significant at 0.05 probability level, **Significant at 0.01 probability level, ***Significant at 0.001 probability level, NS = Not Significant

$p<0.050$) and conjugated bilirubin ($r = 0.477$, $p<0.001$). Parasite count was weakly positively correlated with uric acid ($r = 0.060$, $p>0.050$). In malaria patients, strong and positive correlation was also found between total and conjugated bilirubin ($r = 0.729$, $p<0.001$).

DISCUSSION

In controls, plasma levels of total and conjugated bilirubin are 6.830 ± 2.436 and 1.777 ± 0.729 mg L⁻¹ respectively. They are within the references values established which are 3 to 10 mg L⁻¹ for the total and 1 to 3 mg L⁻¹ for the conjugated bilirubin (Alexander *et al.*, 2004). This study shows that bilirubins levels are increased significantly in patients infected with malaria parasites compared to controls (Table 1). According to WHO, bilirubin level remain in the range of 7 to 10 mg L⁻¹ in *Plasmodium falciparum* patients (WHO, 2000). We obtained in this study high total bilirubin level (10.772 mg L⁻¹). It is believed that hyperbilirubinemia in malaria patients occurs as a result of intravascular haemolysis of red blood cells and degradation of hemoglobin, a major nutrient source used by the malaria parasite (Goldberg *et al.*, 1990). Haemolysis alone is

unlikely to produce conjugated hyperbilirubinemia. Many studies revealed hepatic impairment associated with malaria infection (Kochar *et al.*, 2003; Levesque *et al.*, 1999). Hepatic dysfunction may be due to alteration in vascular flow through the organ as parasitised erythrocytes adhere to endothelial cells blocking sinusoids and leading to the decrease in excretion of conjugated bilirubin through bile canaliculus. This is likely to explain the high plasma level of conjugated bilirubin obtained in malaria patients. Parasite index are positively and significantly correlated with plasma levels of total and conjugated bilirubin. These results corroborate with those of Selvam and Mathews (1992) and Ravichandiran *et al.* (1996) who obtained significant correlations between malaria infection and plasma bilirubins levels with Indian patients.

Plasma uric acid increased significantly ($p = 0.021$) in malaria patients compared to controls (Table 1). Increase rate of purine catabolism and production of urate is a general phenomenon during malaria infection because of haemolysis and oxidation of nucleic acids by reactive oxygen species produced (Siddiqi and Alhomida, 1999). The non-significant increase of plasma uric acid with parasite count is not in concomitance with the report of Das *et al.* (1993) who obtained significant increase of uric acid level with plasmodium falciparum infection. The low parasitic index in our study is likely to explain this difference because Das *et al.* (1993) were having 42 patients with severe malaria infection.

From this study, we can conclude that there is an increase in plasma level of bilirubins and uric acid in malaria patients, which positively augment the ability of the host to fight against reactive oxygen species produced during infection.

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