

# AMINOTRANSFERASE ACTIVITIES INCREASE IN MALARIAL INFECTIONS

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(Received 21 February, 2006; Revision Accepted 5 April, 2006)

## ABSTRACT

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum and red blood cell lysate of two hundred malaria patients comprising of one hundred and sixty adults (aged 18-45 years) and forty children (aged 1-13 years) were assayed. Eighty age and sex-matched apparently healthy individuals were used as control. The AST activities in both serum ( $16.7 \pm 15.7\mu\text{L}$ ) and RBC lysate ( $79.0 \pm 57.3\mu\text{L}$ ) in malaria subjects were significantly higher ( $P < 0.05$ ) than the corresponding levels in non malaria positive subjects. The ALT activity in the serum of malaria positive subjects ( $7.9 \pm 6.6\mu\text{L}$ ) was significantly higher than its activity in non malaria subjects, but its activity in RBC lysate ( $6.8 \pm 4.9\mu\text{L}$ ) was not ( $P > 0.05$ ). There was no significant correlation between AST and ALT activities in both serum and RBC lysate, and parasite densities ( $P > 0.05$ ). Also there was no significant correlation between the ratio of serum and RBC lysate enzymes activities and parasite densities ( $P > 0.05$ ). A very marked increase was observed in RBC lysate AST activity ( $79.0 \pm 57.3\mu\text{L}$ ) of malaria positive subjects compared to non malaria subjects ( $38.0 \pm 25.8\mu\text{L}$ ). The marked increase in the AST RBC lysate activity may be due to parasite enzyme. These parameters were not significantly associated with the level of parasitaemia probably due to fluctuation in peripheral plasma load with hepatic erythrocytic cycle.

**KEYWORDS:** Aminotransferases activities, malarial infection, increase.

## INTRODUCTION

Malaria, a parasitic disease with a very high morbidity and mortality is re-emerging as World Number One killer infection. Once nearly eradicated, the disease now affects more than 300 million and kills more than 3 million every year (Kikkilaya, 2005). In malarial infections, liver is usually the first organ to be attacked by the sporozoites particularly in falciparum malaria and impairment of hepatic function is common in severe malaria (Mishra *et al.*, 2003).

In liver phase of malaria development, the proliferation and maturation of the parasites with their subsequent release via hepatocyte rupture is associated with hepatic abnormalities (Willairatana *et al.*, 1996). Histologic examination of parasitized liver showed dilated hepatic sinusoids with hypertrophied kuffer cells and parasitized red cells in the sinusoid. (Pongoponrath *et al.*, 1998). Elevated levels of aminotransferases have been reported in severe malaria hepatitis with a higher level of alkaline aminotransferase (ALT) (Willairatana *et al.*, 1996). But in malaria infection, higher level of serum aspartate aminotransferase (AST) is obtained because of the involvement of red blood cells and intracellular AST is released into the blood upon the rupture of parasitized red cells (Kamath *et al.*, 1996, Theal *et al.*, 1996). Hyperbilirunaemia is also a common feature (Murlty *et al.*, 1998).

Liver involvement with severe malaria may manifest as jaundice, hepatomegaly with elevated liver enzymes (Rajesh *et al.* 1996). In malaria hepatitis or hepatopathy, these enzymes are mildly raised but may be normal (Kamath *et al.*, 1996). Alanine

aminotranferase elevation of up to 5 times may be seen in malaria parasitaemia but never reaches the level observed in viral hepatitis (WHO, 2000 and Wilairatama *et al.*, 1996). In severe and complicated cases of malaria involving ischemia with circulatory shock, enzyme level might be so high to be confused with viral hepatitis infection (Mishra *et al.*, 2003). This study attempts to find out if malaria parasite AST and ALT activities contribute to the activity observed in serum of malaria positive subject.

## Subjects and Methods

One hundred and twenty subjects attending the Children emergency unit of the University of Calabar Teaching Hospital and General hospital, all in Calabar Municipality, Cross River State of Nigeria were examined while the remaining 80 apparently healthy children of the University of Calabar community were used as control subjects.

A written permission was sought and obtained from the Ethical committee in the two hospitals and also a verbal informed consent was sought and obtained from each patient guardian before inclusion into the study.

**Blood Collection:** Seven milliliters (7ml) of venous blood was collected from each subject and 3 and 4 ml of it were dispensed into EDTA and plain tubes respectively. The blood in the plain tube was allowed to stand and retract for 30 minutes. The tubes were centrifuged at 3 rpm for 5 minutes before the plasma/serum was separated from them and stored at  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . The RBC from the EDTA bottle was washed 3 times with physiological saline before the cells were

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lysed with equal volume of distilled water. Each lysate was then adjusted to 10g/dl concentration for the analysis.

**Assay of Samples:** Assay of aspartate transferase (AST) and alanine tranferase (ALT) was done using a test kit supplied by Randox laboratories (United Kingdom) catalogue numbers AS 101 and AL100 which is based on the method of Reitman and Frankel (1957).

**Malaria Detection:** For malaria parasite detection, Thick and thin blood films were made on each blood sample (Laveran, 1980). Both films were placed on a flat surface or bench to air dry. There after the thin films were fixed in absolute methanol for 5 seconds and allowed to dry. Both the thick and thin films were then stained in freshly prepared 2% Giemsa stain for 30 minutes (Payne, 1988). At the end of the staining, the films were removed from the stain, rinsed in buffered water of PH 7.2 and stood vertically to dry. Both the stained thick and thin films were examined microscopically using the X 100 objective lens with oil immersion. The thin blood films were used for speciation of Plasmodium while thick films were used for detection and quantification of malaria parasitaemia.

**Statistics/Calculation:** Calculation of malaria parasite density was made by counting the parasite per white blood cells in thick blood films and parasite density determined by multiplying the figure by 8000 which is an average white blood cell count per microlitre ( $\mu$ l) of blood (Shute, 1986).

SPSS and Microsoft excel programmes respectively were used for the data analysis

## RESULTS

Table 1 compares aspartrate aminotransferase (AST) and alanine aminotransferase (ALT) activities in malaria and non-malaria subjects. The result of AST and ALT in both serum and RBC were significantly higher in malaria patients than in controls. Significant difference existed between the serum and RBC lysate AST levels in malaria patients compared with the controls ( $P < 0.05$ ). Also, there was a significant difference between the serum ALT activities of malaria patients and the controls but no such difference was observed between the RBC lysate ALT of malaria patients and the controls ( $P > 0.05$ ).

The mean value of AST activity in RBC lysate, ( $79.0 \pm 15.7$ ) of malaria patients was markedly higher than the corresponding activity in controls ( $38.0 \pm 25.8 \mu$ L;  $P < 0.05$ )

TABLE I: COMPARISM OF AST, ALT ACTIVITIES IN MALARIA AND NON-MALARIA SUBJECTS

Parameters	Controls n = 40	Malaria Subjects n =60	Calculated t	P. Value
Serum AST( $\mu$ /L)	7.5 $\pm$ 2.5	16.7 $\pm$ 15.7	3.77	P < 0.05
RBC AST ( $\mu$ /L)	38.0 $\pm$ 25.8	79.0 $\pm$ 57.3	4.23	P < 0.05
Serum ALT ( $\mu$ /L)	4.8 $\pm$ 2.8	7.9 $\pm$ 6.6	2.60	P < 0.05
RBC ALT ( $\mu$ /L)	5.3 $\pm$ 5.1	6.8 $\pm$ 4.9	1.57	P > 0.05

AST = Asparate aminotransferase

ALT = Alanine aminotransferase

RBC = Red blood cell

t = Student's t-test

P = Probability

Table II shows the correlation analysis between AST and ALT activities in serum and RBC lysate and parasite density. There was no significant correlation between the activities of AST and ALT in serum and RBC lysate; and parasite densities ( $P > 0.05$ ). The correlation analysis between serum/RBC ratio of AST and ALT; and parasite density is shown in Table III. No significant correlation was observed between the ratios of serum and RBC level of AST and ALT, ( $P > 0.05$ ) and parasite densities (Table III).

## DISCUSSION

This work measured the activities of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in serum and red blood cell (RBC) haemolysate of malaria infected patients. *P. falciparum* was found to be

the only specie of malaria parasite detected in the subjects examined.

The involvement of liver and red blood cells in the Plasmodium life cycle may contribute to the increment in the activities of aminotransferases in the serum of malaria infected patients. ALT which is liver specific is mainly localized in the cytosol, while AST is found in both cytosol and mitochondria. Any inflammatory reaction on hepatocytes results in leakage of these enzymes into blood. But because malaria infection does not necessarily involve inflammatory reaction only a mild elevation was observed. The higher activity of AST observed in the serum is not necessarily as a result of hepatocellular damage but mainly due to hemolysis of parasitized red blood cells.

It was observed that significantly the activities of AST and ALT in malaria patients ( $P < 0.05$ ) were twice

TABLE II: CORRELATION ANALYSIS BETWEEN AST AND ALT ACTIVITIES IN SERUM AND RBC LYSATE; AND PARASITE DENSITY

Parameters	Enzyme Activity ( $\mu\text{L}$ ) n = 60	Parasite Density ( $\mu\text{l}$ ) n = 60	Calculated r	Calculated t	P. Value
Serum AST	16.7 $\pm$ 15.7	113.4 $\pm$ 106.3	0.20	1.70	P > 0.05
RBC AST	79.0 $\pm$ 57.3	113.4 $\pm$ 106.3	0.16	1.33	P > 0.05
Serum ALT	7.9 $\pm$ 6.6	113.4 $\pm$ 106.3	0.02	0.15	P > 0.05
RBC ALT	6.8 $\pm$ 4.9	113.4 $\pm$ 106.3	0.13	1.06	P > 0.05

AST = Aspartate aminotransferase

ALT = Alanine aminotransferase

RBC = Red blood cell

p = Probability

t = Student's t-test

r = Correlation

TABLE III: CORRELATION ANALYSIS BETWEEN SERUM / RBC RATIO OF AST AND ALT; AND PARASITE DENSITY

Parameters	Enzyme Density ratio ( $\mu\text{L}$ )	Parasite Density $\mu\text{l}$	Calculated r	Calculated t	P. Value
Serum / RBC ratio of AST	0.27 $\pm$ 0.25	113.4 $\pm$ 106.3	0.00	0.00	P > 0.05
Serum / RBC ratio of ALT	0.91 $\pm$ 0.70	113.4 $\pm$ 106.3	0.16	1.17	P > 0.05

AST = Aspartate aminotransferase

ALT = Alanine aminotransferase

RBC = Red blood cell

r = Correlation

t = Student's t-test

p = Probability

the level of control (Table 1), but the activity of AST in serum and RBC haemolysate of malaria subjects was twice and eleven times (11x) that of serum and RBC haemolysate ALT respectively. This supports the work done by Mishra *et al.*, (2003) confirming that the higher level of AST encountered results from massive red cell destruction.

There was a significant difference in the serum and RBC AST as well as serum ALT of malaria patients ( $P < 0.05$ ) compared with the control, but no significant difference was found in RBC ALT of malaria patients compared with the control. This supports the earlier finding by Willaraitana *et al.*, (1996) that in malaria hepatopathy, there is an increase in serum aminotransferases and higher level of AST found was as

a result of intravascular hemolysis of parasitized red cells. (Mishra *et al.*, 2003).

The activity of the erythrocytic AST was more marked than the ALT activity suggesting that AST would be a more sensitive marker of malaria than ALT. the higher activity of AST may be a reflection of its dual location in the cytoplasm as well as in the mitochondria compared to ALT which is present only in the cytosol. The marked increase in the activity of AST in malaria positive sample may raise the activity of these enzymes in patients being investigated for other ailments.

Comparison of the AST and ALT activities in the haemolysate of malaria positive and malaria negative control haemolysate showed significantly higher activities ( $P < 0.05$ ) in the malaria positive lysate. Since

the haemoglobin concentrations were adjusted to the same level and the obvious difference between the two (malaria and non-malaria) is the presence of malaria parasite in the haemolysate then it may be speculated that the excess AST and ALT activity was contributed by the parasite themselves. This view is supported by the known fact that malaria parasite do synthesize their own enzyme to augment host enzyme system (Read, 1972; Shahabuddin *et al.*, 1994). These enzymes would provide additional enzymes activity to the activity that is usually present in the plasma when red cells rupture in the erythrocytic phase of malaria schizogony. This is similar to the overflow into plasma when the hepatocytes rupture during the exoerythrocytic phase. Correlation analysis done on the activities of these enzymes and parasite density showed that there was no significant correlation ( $P>0.05$ ) between the serum and RBC AST and ALT and parasite density ( $r = 2.8$ ). No significant correlation also existed between the ratios of serum and RBC AST and ALT and parasite density in malaria subjects ( $r = 3.8$ ,  $P>0.05$ ). However, a marked increase in AST activity was observed in the red cell lysate of malaria subjects compared to controls. This study has shown there is increased activity of AST and ALT in malaria infection but the very high level of AST in RBC lysate may be linked to parasite enzyme since malaria parasite have their own enzyme system. (Shahabuddin, 1994; Read, 1972).

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