Serum Ornithine Carbamoyl Transferase as a Surrogate Marker in Malaria

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Abstract

Ornithine carbamoyl transferase (OCT) activity and other liver function tests were studied in a total of 50 patients of clinical malaria and 15 controls. They were grouped as group I (positive for malarial parasite on peripheral blood smear, n=18), group II (negative for malarial parasite on peripheral blood smear (PBS) but responded to antimalarials, n=17) and group III (peripheral blood smear negative and did not respond to antimalarial therapy, n=15). The mean OCT levels were significantly raised in group I (6.79 ± 1.84 IU/L, p value = 0.006) and group II (5.0 ± 1.13 IU/L, p value = 0.014) as compared to controls (2.5 ± 1.13 IU/L) and returned to normal after treatment. In contrast, group III had normal levels except in a case of kala azar and septicemia where OCT levels were high and increased further on treatment. Taking PBS positivity as a gold standard of diagnostic criteria, OCT had a sensitivity of 83% and specificity of 86% with a high positive predictive value of 88% as compared to ALT which had a lower sensitivity of 55% and specificity of 80%. The clinical response rate in PBS negative cases of fever having high OCT level was 83% as compared to 35% in cases with normal OCT level, making OCT a good surrogate marker of malaria. OCT levels could also be of prognostic significance as 2 cases of cerebral malaria had high OCT levels of 11.1 U/L and 10.7 IU/L, respectively.

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Key Words : Malaria; Ornithine carbamoyl transferase

Introduction

OCT (EC 2.1, 3.3) is a key enzyme in urea cycle, catalysing the reaction of ornithine with carbamoyl phosphate to form citrulline. It is a cell sap enzyme [1] present in mitochondria. OCT is almost totally liver specific [2] and a minor degree of hepatocyte damage could create readily detectable elevation in blood levels. The OCT level in serum is of considerable value in diagnosis of liver disease, it being raised to 100 times the upper limit of normal in infective hepatitis [3]. Raised activities are also reported in non icteric cholecystitis and malignant diseases affecting liver [3]. Einheber et al [4] used measurements of OCT as an index of liver damage in acute *P. berghei* infections in mice produced by intra peritoneal infection of parasitised erythrocytes and found plasma levels of OCT elevated to five times above those of control mice on fifth and sixth day of infection, indicating liver cell damage.

Pre-erythrocytic schizogony with hepatic parenchymal damage is part and parcel of natural history of malaria. Signs of liver damage and derangement of liver function tests of varying degrees of severity have been reported in all four naturally acquired human malaria infections and artificially induced malaria in experimental animals. Increased levels of serum bilirubin are seen in *P. vivax* malaria [5] and *P. falciparum* malaria [6,7]. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) have been reported to be more than three times normal without any clinical or serological evidence to suggest viral hepatitis

[8,9]. Increases in aminotransferases, although generally regarded as reflecting liver cell changes in acute malaria could be contributed, to varying extent, by enzymes released from lysed erythrocytes and other tissues [10].

Since OCT is more specific to liver damage, study of serum OCT levels in cases of malaria was undertaken to study the degree of hepatic damage in malaria and to see if OCT estimation could be used as a surrogate and/or prognostic marker in malaria.

Material and Methods

The study was carried out on 50 patients of suspected clinical malaria who reported to a large hospital, and 15 healthy controls. Clinical malaria was suspected in patients with short history of high grade fever, chills with or without hepatosplenomegaly, after ruling out other conditions like urinary tract infections, upper respiratory tract infections and acute cholecystitis. History of intake of hepatotoxic drugs, jaundice and malaria was noted. The patients were evaluated by PBS for malarial parasite and were considered smear positive if examination revealed any stage of malarial parasite - ring form, trophozoite, schizont or gametocyte. All the patients of suspected clinical malaria were treated with a standard course of antimalarials. Blood samples were collected before treatment and on the third day after commencement of treatment and in a few cases also on the fifth day.

The patients were grouped into three groups -

Group I : Peripheral blood smear positive for malaria

Group II : Peripheral blood smear negative for malarial parasite but responded to antimalarial therapy

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Group III : Peripheral blood smear negative for malarial parasite and did not respond to antimalarial therapy.

Tests done on samples before and after treatment included - serum bilirubin (total, conjugated, unconjugated - method of Mallory and Evelyn), serum total proteins (modified Biuret method), serum albumin (BCG dye binding method), blood urea, serum creatinine, and serum enzymes.

Enzyme studies included -

1. Estimation of ALT, AST and alkaline phosphatase (ALP) which were carried out by a kinetic method on Erbachem - 5 Clinical Chemistry analyser

2. Serum adenosine deaminase (ADA) - method of Guisti [11].

3. OCT - The method used for estimation of OCT was as described by Somani [12]. The citrulline formed from ornithine and carbamoyl phosphate catalysed by OCT, is reacted with diacetyl monoxime at low acidic conditions [13]. Standard curve was plotted at various concentrations of citrulline for colour yield. There was a linear relationship between citrulline concentration and absorbance at 464nm (Fig 1). The method was found to be very sensitive for estimation of citrulline as it gave a molar extinction coefficient of $3.062 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$, with an absorbance of 0.19 IU/L⁻¹ making the method sensitive to a level of $\pm 0.05 \text{ IU/L}^{-1}$ of OCT.

The procedure consisted of incubating 100 μl serum with 250 μl of ornithine (10 mM) and 250 μl of carbamoyl phosphate (8mM) at 37°C for 30 minutes in the presence of 5 μl of urease and 500 μl of tricine buffer at pH 8.2. Proteins were precipitated with 600 μl of trichloroacetic acid (20%). Supernatant (500 μl) was reacted with 1.5 ml of colouring reagent prepared fresh by mixing diacetyl monoxime (8g/litre in 5% acetic acid), antipyrine (9 g/litre in water) and H₂SO₄ (mixture of 400 ml analytical grade H₂SO₄ and 600 ml of water) in the ratio of 75:15:10 and incubated at 100°C for 25 minutes, rapidly cooled over ice and read at 464 nm on Beckman DU25 spectrophotometer. The precaution was taken of not touching the inside of the tubes to avoid contamination with urea.

Results

There were 18 patients in group I (15 positive for *P. vivax* and 3 positive for *P. falciparum*), 17 patients in group II (negative for malarial parasite by PBS but responded to antimalarials) and 15 patients in group III (smear negative and did not respond to antimalarials). Group III patients were followed up and cause of fever was established wherever possible. 15 normal healthy controls were also analysed.

The mean serum bilirubin, ALT and AST were found raised in group I and II. The mean levels were significantly higher when compared to controls ($p < 0.01$ in both group I & II). There was a significant decrease in serum bilirubin, ALT and AST levels within three days after commencement of treatment in Group I. The enzymes ALT and AST however, returned to normal only on 5th day. The controls had a mean OCT level of 2.5 IU/L. The upper limit of normal (ULN) was taken as 5 IU/L (mean + 2SD).

As seen from Figure 2 and Table 1, the mean OCT levels in group I and II were raised and these were statistically significant when compared to controls (p value = 0.006 and 0.014 respectively). Amongst the slide positive malaria cases, all the 3 cases of falciparum malaria had raised OCT levels, the maximum being 11.1 IU/L which decreased to 4.8 IU/L on third day after treatment. Amongst the *P. vivax* malaria, 12 cases out of 15 had raised OCT levels. There was a significant drop in the level of OCT, in both group I & II, to normal levels on the third day after treatment (p value 0.004 and 0.020, respectively).

Amongst non responders (Group III) OCT was raised in 2 cases - one of these on follow up was diagnosed as a case

Fig. 2: Scatter diagram showing distribution of OCT values in

Table 1

Levels of serum bilirubin, ALT, AST and OCT before and third day after treatment

	Before treatment mean (SD)				After treatment (3 rd day) mean (SD)			
	Bilirubin (mg/dl)	ALT IU/L	AST IU/L	OCT IU/L	Bilirubin mg/dl	ALT IU/L	AST IU/L	OCT IU/L
Group I (18)	0.92 (0.33)	59.77 (25.6)	65.38 (36.19)	6.79 (1.84)	0.69 (0.15)	51.11 (18.14)	52.33 (19.25)	4.05 (0.74)
Group II (17)	0.70 (0.17)	48.29 (7.19)	41.70 (9.16)	5.01 (1.13)	0.53 (0.09)	37.17 (10.1)	35.29 (9.92)	4.29 (0.91)
Group III (15)	0.62 (0.12)	40.40 (10.5)	39.66 (8.38)	3.96 (0.99)	0.55 (0.11)	39.00 (8.5)	40.66 (8.9)	3.89 (0.93)
Controls (15)	0.46 (0.16)	26.10 (11.9)	28.10 (7.5)	2.5 (1.30)

Fig. 3 : Scatter diagram of OCT vs AST in Group I

Fig. 4 : Scatter diagram of OCT vs ALT in Group I

normal on third day, showed increasing levels in these two cases. Correlation of OCT with AST was better than ALT (r value 0.8 and 0.77 respectively), though this difference was not statistically significant (Fig 3 & 4).

OCT as a surrogate marker of malaria, taking PBS positivity as gold standard of diagnostic criteria, had a sensitivity of 83% and specificity of 86% with a high positive predictive value of 88% when other causes of fever and hepatic disease had been excluded on clinical examination, while ALT had a lower sensitivity (55%) and specificity (80%)(Table 2). The levels of serum ALP, ADA, proteins, urea and creatinine in patient groups were not significantly different from control.

Table 2

Analysis of OCT and ALT levels in Group I and III

Enzyme level	Slide positive for MP Group I	Slide negative for MP Group III
OCT level		
Normal n=16	3	13
Raised n=17	15	2
ALT level		
Normal n=20	8	12
Increased n=13	10	3

Clinical response rate in all the cases of fever with negative smear for malaria in this study (Group II and Group III combined) was analyzed. It was found that 10 out of 12 cases (83%) with raised OCT level became afebrile after treatment, where as only 7 out of 20 cases (35%) with normal OCT levels responded to treatment. Response rate with reference to ALT in this smear negative group was 56% in patients with normal ALT levels (15 out of 27) and 40% in patients with raised ALT levels (2 out of 5) (Table 3).

Discussion

As pre-erythrocytic schizogony with hepatic parenchymal damage is part and parcel of natural history of malaria, derangements in liver function do occur and have been reported by many workers in the past [5-9]. This is especially so in falciparum malaria

Table 3
Analysis of cases of fever with negative smears for malaria (n=32)

Enzyme level	Responded to treatment	Not responded to treatment	Percent clinical response
OCT level			
Normal n=20	7	13	35
Raised n=12	10	2	83
ALT level			
Normal n=27	15	12	56
Raised n=5	2	3	40

enzyme with almost exclusive distribution in liver is a better index of hepatic insult. We found that 3 cases of falciparum malaria and 12 out of 15 cases of vivax infection had significantly raised levels of OCT as compared to controls. Further OCT levels decreased to less than upper limit of normal on treatment with antimalarials, earlier than enzymes like ALT and AST in groups I and II. Amongst the non responders (Group-III), the levels of OCT were normal except in 2 cases where there was rise in the enzyme levels - one was a case of kala azar and the other a case of terminal septicaemia.

Taking slide positivity as a gold standard of diagnostic criteria of malaria, OCT had a higher sensitivity of 83%, specificity of 86% and positive predictive value of 88% as compared to ALT. The high positive predictive value was further supported on analysing all the cases of fever which were slide negative as a whole, where 83% of patients with high OCT levels responded to treatment, proving the utility of OCT as a surrogate marker in malaria.

In this study only 3 cases of falciparum malaria were present, of which 2 had cerebral complications. Both these cases had high OCT levels >2 times ULN (11.1 IU/l and 10.8 IU/l) which returned to normal on treatment, suggesting more studies to be done in areas

with high incidence of falciparum malaria to pass a definite judgement on the prognostic utility of OCT.

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Tarun - I am a well known collector of antiques.

Seema - I guess so, I saw your wife the other day.

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Seema - I have changed my mind.

Tarun - I see, does this one work better?

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