SERUM TRANSCOBALAMIN II LEVELS IN PATIENTS WITH MALARIA INFECTION

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Abstract. Serum transcobalamin II (TCII) levels were determined in 56 patients with *P. falciparum* malaria infection. They were divided into 3 groups: severe (malarial parasite > 5% or patients with cerebral malaria or renal insufficiency), moderate (1-5% infection without complictions) and mild (1% infection). Elevated serum TCII values were found only in patients with severe malaria infection. These values correlated directly with parasitemia, direct bilirubin, total bilirubin, serum glutamic oxalacetic transminase, serum glutamic pyruvate transminase, blood urea nitrogen and creatinine, but were not correlated with alkaline phosphatase. As 17 patients with azotemia had elevated serum TCII levels while other 3 patients with normal BUN and creatinine concentrations had serum TCII levels within the normal limits. These findings indicated that malarial patients with renal insufficiency had increased serum TCII. A possible mechanism is the reduced TCII-B_{12} that filtered through the glomeruli due to the reduced renal blood flow with the decreased its uptake by proximal tubular cells resulting in the decreased degradation of TCII by the tubular lysosomal enzymes. Determination of serum TCII level may be used as an indicator of renal function in malarial patients with renal insufficiency.

INTRODUCTION

Transcobalamin II (TCII) is a vitamin B_{12} binding protein that functions as a transport protein and carries vitamin B_{12} into cells. It facilitates the uptake of vitamin B_{12} by a variety of cells both *in vitro* and *in vivo*. High serum TCII levels have been reported in many clinical disorders such as Gaucher's disease, multiple myeloma, systemic lupus erythrematosus, rheumatoid arthritis, dermatomyositis, chronic monocytic leukemia and immunosuppressed recipients or renal allografts (Rachimilewitz *et al,* 1978; Gilbert and Weinreb, 1976; Carmel and Hollander 1978; Frater-Schroder *et al,* 1980; Carmel and Coltman 1971). Recently, it has been reported that some patients with typhoid fever also had elevated serum TCII (Areekul *et al,* 1995b). It was suggested that the increased serum TCII in these conditions are due to the increased production by the stimulated reticuloendothelial system. As monocytosis with reactive macrophage hyperplasia are usually encountered in malarial patients. The objective of the present study was to determine serum TCII in patients with *Plasmodium falciparum* infection.

MATERIALS AND METHODS

The study was carried out on 56 patients of both sexes suffering from *P. falciparum* admitted to the Bangkok Hospital for Tropical Diseases. They were classified into 3 groups according to their peripheral blood parasitemia : mild (< 1%), moderate (1% - 5%) and severe (> 5%). Patients were also included in the severe malaria group if they had blood urea nitrogen > 20 mg/dl or serum creatinine > 3 mg/dl or other clinical manifestations of severity such as coma or renal insufficiency.

Blood samples were taken on day one and then at two-day intervals. Urine was collected and volumes recorded daily. Vitamin B_{12} and its binding proteins were determined in blood and urine samples. Vitamin B_{12} level was determined by the radioisotope dilution and coated charcoal technique (Lau *et al,* 1965). Transcobalamins (TC) were fractionated and quantitatively measured by the method of Selhub *et al* (1976). Blood counts and routine biochemistry were determined. Quantitation of malarial parasites was done by counting the parasites per 1,000 red blood cells. Hemoglobin concentration was determined by the cyanmethemoglobin method and hematocrit by centrifuging the blood at 10,000*g* for 5 minutes.

RESULTS

Altogether 56 patients were studied, 20 (36%), 16 (28%) and 20 (36%) patients were classified as having mild, moderate and severe malaria, respectively. The hematological and biochemical data of these patients are shown in Table 1.

Results of serum TCII levels in these 3 groups of patients are illustrated in Fig 1. It is evident from this figure that serum TCII levels were elevated in only a group of severe malaria patients. Seventeen out of 20 patients in this group had serum TCII level over 2,000 pg/ml which is the upper limit of normal subject in this laboratory. The clinical details and the individual serum TCII value of these severe malaria patients are shown in Table 2. Out of 20 patients, 16 (80%), 17 (85%) and 11 (55%) patients had cerebral malaria, renal insufficiency and jaundice, respectively. There were direct relationships between serum TCII levels and parasitemia, blood urea nitrogen (BUN), creatinine, direct and total bilirubin, serum glutamic oxalacetic acid (SGOT) and serum glutamic pyruvate transminase (SGPT) as shown in Table 3. However, no correlation between serum TCII and alkaline phosphatase was observed in these patients ($p > 0.05$). The correlation between BUN and serum TCII levels is illustrated in Fig 2.

Table 1

Mean values ± SD of hematological and biochemical data of patients on admission.

	Patient group		
	Mild (n = 20)	Moderate (n = 16)	Severe (n = 20)
Hb (g/dl)	11.1 ± 2.0	11.1 ± 1.8	9.5 ± 2.7
Ht (%)	34 ± 6	35 ± 6	30 ± 7
Parasite count × 10^5/μl	0.06 ± 0.01	0.48 ± 0.12	3.94 ± 2.64
AP (U/dl)	26 ± 8	36 ± 17	41 ± 15
SGOT (U/ml)	33 ± 16	35 ± 22	131 ± 117
SGPT (U/ml)	25 ± 18	31 ± 20	53 ± 42
DB (mg/dl)	0.32 ± 0.27	0.90 ± 0.30	4.36 ± 5.15
TB (mg/dl)	1.29 ± 0.56	1.80 ± 1.00	8.20 ± 8.74
BUN (mg/dl)	14 ± 4	18 ± 2	68 ± 42
Creatinine (mg/dl)	1.12 ± 0.14	1.20 ± 0.20	2.67 ± 2.20
Serum TCII (pg/ml)	1,092 ± 402	1,028 ± 247	3,255 ± 1,283

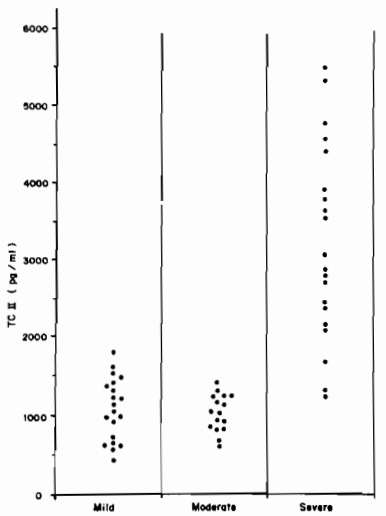

Fig 1–Serum TCII levels in 3 groups of patients with mild, moderate and severe infections.

DISCUSSION

Results in the present study show that elevated serum TCII levels are found only in patients with severe malaria infection and show a direct relationship with parasitemia. However, five out of 20 patients (25%) had parasitemia less than 5%, but their serum TCII levels were elevated. On the other hand, three patients had normal TCII levels with high parasitemia in 2 patients (No. 4 and 15) and a moderated

Table 2

Serum TCII levels and complications in severe malaria patients.

Case No.	Cerebral malaria	Renal insufficiency	Jaundice	Parasite (10^5/μl)	BUN (mg/dl)	Creatinine (mg/dl)	TCII (pg/ml)
1	+	+	-	4.51	49	1.4	2754
2	+	+	+	8.26	93	3.5	4667
3	+	+	-	0.50	34	1.7	2193
4	+	-	-	2.41	19	1.0	1256
5	+	+	-	1.39	36	1.3	2910
6	-	+	-	2.53	65	2.1	2169
7	-	+	+	1.59	36	1.2	3635
8	+	+	+	3.34	67	1.3	2800
9	+	+	+	4.54	41	1.3	4469
10	+	-	-	1.44	13	0.7	1714
11	+	+	-	7.59	35	1.3	2486
12	+	+	+	8.97	65	1.8	3802
13	+	+	+	1.44	61	2.4	2451
14	+	+	-	3.24	102	2.2	3136
15	-	-	-	2.19	15	0.8	1277
16	+	+	+	3.80	117	7.7	3938
17	+	+	+	2.58	86	3.5	5383
18	+	+	+	3.32	118	5.7	4802
19	-	+	+	8.27	139	4.3	5574
20	+	+	+	6.83	159	8.1	3684
Total	16	17	11	-	-	-	-

Table 3

The relationships between serum TCII levels and other parameters in 20 severe malaria patients.

	Regression equation	r	T	P
Parasitemia Vs TCII	$y = 2{,}308.107 + 240.511 \times$	0.494	2.410	0.0269
DB *vs* TCII	$y = 2{,}581.770 + 154.393 \times$	0.620	3.354	0.0035
TB *vs* TCII	$y = 2{,}567.886 + 83.840 \times$	0.571	2.951	0.0085
AP *vs* TCII	$y = 1{,}752.515 + 36.213 \times$	0.428	2.010	0.0596
SGOT *vs* TCII	$y = 2{,}336.491 + 7.006 \times$	0.638	3.519	0.0024
SGPT *vs* TCII	$y = 2{,}522.491 + 13.873 \times$	0.451	2.145	0.0458
BUN *vs* TCII	$y = 1{,}833.094 + 21.065 \times$	0.695	4.096	0.0006
Creatinine *vs* TCII	$y = 2{,}405.428 + 318.788 \times$	0.546	2.765	0.0127

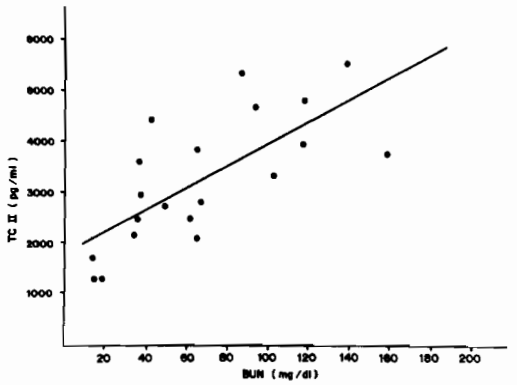

Fig 2–The relationship between serum TCII and BUN concentrations in 20 patients with severe malaria infection. The regression equation is
$y = 1833.094 + 21.065 \times (r = 0.695; p = 0.0006)$

parasitemia in one patient (3.9% infection in No. 10). Therefore, the number of malarial parasites may not be responsible for the elevated serum TCII levels in these patients.

Results in the present study showed a direct relationship between serum TCII levels and liver function tests such as DB, TB, SGOT and SGPT. These findings indicate that increased serum TCII levels can be detected in malarial patients with hepatic impairment.

It has been shown in a previous report that patients with acute liver involvement such as viral hepatitis had reduced all TC values due to the rise in serum vitamin B_{12} level which was a temporary flooding of the blood stream with vitamin B_{12} released from the damaged liver cells (Areekul *et al,* 1977). Serum TCI and TCII values were found to decrease 25% and 50%, respectively in these patients. Since all the endogenous vitamin B_{12} was carried on the TCI, the raised serum vitamin B_{12} may saturate TCI and therefore markedly reduced the unsaturated TCI. Reduced TCII in these patients may result from defective synthesis by the diseased liver. The possibility that damaged liver cells in malarial patients synthesis and release more TCII into the circulation is therefore very unlikely.

Many previous reports have suggested a relationship between serum TCII levels and reticuloendothelial cell activity. A marked rise in serum TCII levels reported in patients with autoimmune disorders such as SLE, rheumatoid arthritis and dermatomyositis has been interpreted as a response to the stimulation and proliferation of cells of the mononuclear phagocytic system (Arnalich *et al,* 1990; Gilbert and Weinreb, 1976). A general rise of TCII levels frequently reported in multiple myeloma and lymphoproliferative disorders also suggested that macrophages, plasma cells and B lymphocytes may be considered possible cellular sources for TCII synthesis (Carmel, 1985). This was supported by a finding that TCII is synthesized in part by mouse peritoneal macrophages as well as by human monocytes, and macrophages produced and secreted considerable amounts of TCII into the medium (Rabinowitz *et al,* 1982; Rachmilewitz *et al,* 1978).

Malarial parasites have been known to evoke a striking hyperplasia of reticuloendothelial macrophages especially those of the liver, spleen and bone marrow. This was manifested by the progressive transformation of sinusoidal lining cells to actively phagocytizing macrophages. These cells will enlarge, proliferative and characteristically contain malarial pigment or hemozoin in addition to the phagocytised parasites and erythrocytes as a result of an overall accelerated phagocytic activity of the reticuloendothelial system (Sherman *et al,* 1965; Sheagren *et al,* 1976; Areekul *et al,* 1973). However, there are no data or evidence to indicate that this stimulated and proliferative mononuclear phagocytic system in malarial infection increases the production and secretion of TCII into the circulation.

Results in the present study showed the correlations between serum TCII levels and BUN or creatinine concentration. Three out of 20 patients who had normal BUN and creatinine concentrations had serum TCII levels within the normal limits (No. 4, 10 and 15), while the other 17 patients with azotemia had elevated serum TCII levels. These findings indicate that elevated serum TCII levels occur only in malarial patients with renal insufficiency. This is in accordance with reports of increased serum TCII levels in malarial patients with pre-renal azotemia and cerebral malarial patients with renal insufficiency (Areekul *et al,* 1993, 1995a).

The mechanisms causing increased serum TCII levels in malarial patients with azotemia are not exactly known. One possibility is the renal ischemia resulting from hypovolemia, blood hyperviscosity and occlusion of capillaries by infected red cells,

catecholamine effects, intravascular hemolysis and intravascular coagulation and cellular injury from released free oxyen radicals (Sinniah *et al,* 1988). A decreased glomerular filtration rate from the reduced renal blood flow can reduce the amount of TCII-B_{12} that filtered through the glomeruli resulting in the decreased its uptake by proximal tubular cells and the degradation of TCII by the lysosomal enzymes. The decreased TCII catabolism by the tubular cells will therefore prolong intravascular TCII survival resulting in elevated serum TCII levles. Findings of decreased clearance and urinary excretion of vitamin B_{12} and TCII in 4 malarial patients with azotemia support this explanation (Areekul *et al,* 1993).

This study shows that elevated serum TCII levels are found in patients with severe malaria infection or patients with complications such as cerebral malaria or renal insufficiency. It suggests that determination of serum TCII may be useful for determining the renal function in malarial patients with renal insufficiency.

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