ELEVATION OF SERUM TRANSCOBALAMIN II IN PATIENTS WITH SCRUB TYPHUS

Cheeraratana Cheeramakara[1], Wanyarat Thanomsak[1], Kriyaporn Songmeang[1], Apichart Nontprasert[2], Charnnarong Sanghirun[1], Nopachai Suthisai[1] and Suvit Areekul[1]

[1]Department of Tropical Radioisotopes, Faculty of Tropical Medicine, Mahidol University; [2]Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Abstract. Serum transcobalamin II levels were measured in scrub typhus patients. Eighteen out of fifty-two patients admitted to Maharat Nakhon Ratchasima Hospital were diagnosed with scrub typhus infection. The serum unsaturated vitamin B_{12} binding protein (UBBC) and total vitamin B_{12} binding protein (TBBC) levels in these patients were significantly higher than in normal subjects ($p<0.001$). The mean serum transcobalamin II level in the typhus patients was also significantly higher than in the normal subjects ($p=0.004$). There was a significant correlation between serum TCII levels and typhus IgM or IgG titers ($p<0.05$), but not to total IgM levels. These findings indicate that patients with scrub typhus had stimulation of the recticuloendothelial system as a result of a considerable increase in transcobalamin II levels.

INTRODUCTION

Scrub typhus is transmitted to man by the bite of trombiculid mites infected with *Orientia tsutsugamushi*. It is endemic in numerous countries in the Asia-Pacific Region, including Thailand. The symptoms of this disease typically include prolonged fever, headache, maculopapular rash, eschar (pathognomic lesion), lymphadenopathy, and central nervous system abnormalities (Silpapojakul, 1997; Richards *et al*, 1997).

Transcobalamin II (TCII) is a plasma protein which plays an important role in the transportation of vitamin B_{12} into tissues. Increased serum TCII levels have been reported in patients with stimulation of the recticuloendothelial system and inflammatory diseases, such as acute leukemia, lymphoma, systemic lupus erythematosis, dermatomyositis, rheumatoid arthritis, multiple myeloma and lysosomal storage defects, such as Gaucher's disease (Gilbert and Weinreb, 1976; Carmel and Hollander, 1978; Laser *et al*, 1985). Patients with proliferative mononuclear phagocytic systems, such as malignant histiocytosis, also had elevated serum TCII (Fehr and Vecchi, 1985). It has been suggested that monocytes and macrophages may be the sites of TCII synthesis (Laser *et al*, 1985; Arnalich *et al*, 1990). As reactive macrophage hyperplasia occurs frequently in patients with typhus, it is of interest to study TCII levels in patients with scrub typhus.

Correspondence: Cheeraratana Cheeramakara, Department of Tropical Radioisotopes, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand.
Tel. 66 (0) 2643-5585, Fax: 66 (0) 2643-5585
E-mail: tmccr@mahidol.ac.th

MATERIALS AND METHODS

Fifty-two patients with clinical signs and symptoms of scrub typhus, such as eschar, fever and headache, were investigated. Patients were admitted to Maharat Nakhon Ratchasima Hospital, Nakhon Ratchasima Province, Thailand. Clinical history, age and other pertinent information were recorded. The following tests were performed by staff of the hospital laboratory : multiple blood film examinations for malaria parasites, bacterial cultures with blood, urine and stool samples, serologic tests for melioidosis, bacterial agglutination tests for typhoid fever, and an indirect immunoperoxidase test (IIP). The diagnosis of scrub typhus was confirmed by a demonstration of rising IgG (≥1:1,600) and IgM (≥1:400) titers against the rickettsial antigen.

Indirect immunoperoxidase test (IIP)

The IIP test for specific immunoglobulin

against the scrub typhus antigen was modified from Suto (1980) and Yamamoto and Minamishima (1982). Serum and conjugated antibody were diluted using phosphate buffered saline (pH 7.3) and incubated for 30 minutes at 37ºC. Peroxidase conjugated antihuman IgG, IgM, or whole IgM was used.

Determination of transcobalamin levels

Transcobalamin determination was performed by using a modified method described by Selhub *et al* (1976). Three transcobalamins, TCI, TCII and TCIII, were measured by filtration through stack charged cellulose (DE-81) disks. A reaction mixture containing serum was incubated with excess ^{57}Co-B_{12} of high specific activity, diluted with 0.1 M sodium borate buffer (pH 8.5), and passed through the filter stack by applying a vacuum. Under these conditions, TCII was selectively and quantitatively adsorbed onto the cellulose-nitrate filter while both the TCI and TCIII were adsorbed onto the DE-81 filters. Then, TCIII was selectively eluted from the filters by applying a 0.05 M monopotassium phosphate solution (pH 4.6).

Unsaturated vitamin B_{12} binding protein (UBBC)

UBBC was present as the sum of TCI, TCII and TCIII in pg of ^{57}Co-B_{12} per ml of serum.

Determination of vitamin B_{12}

Vitamin B_{12} level was measured using a modified method of radioisotope dilution and coated charcoal technique (Lau *et al*, 1965; Kidroni and Grossowicz, 1969). This technique used chicken serum as a vitamin B_{12} binder and PVP-coated charcoal to separate the bound vitamin B_{12} from the free vitamin B_{12}.

Total vitamin B_{12} binding protein (TBBC)

TBBC was calculated from the sum of vitamin B_{12} and UBBC.

Statistical analysis

The relationships between the serum transcobalamin II and the other biochemical parameters were determined by the Pearsons correlation method. The differences between the means of the serum vitamin B_{12} and vitamin B_{12} binding protein levels in patients and controls were compared by the Student's *t*-test for independent samples. A $p<0.05$ was considered statistically significant.

RESULTS

Fifty-two patients with suspected scrub typhus were investigated for scrub typhus. Only 18 patients were confirmed to be infected with scrub typhus by the Indirect Immunoperoxidase (IIP) test. These patients with scrub typhus were investigated further in this study.

The mean values for serum vitamin B_{12}, UBBC, and TBBC in the patients (1,936±946.19 pg/ml, 4,167±1,463.27 pg/ml, and 6,140± 2,587.67 pg/ml, respectively) were significantly higher than the 60 control subjects (575±157.32 pg/ml, 1,450± 364.08 pg/ml, and 1974±359.86 pg/ml, respectively) ($p<0.001$). The mean value of serum transcobalamin II in the patients (2,761±1,069.84 pg/ml) was also significantly higher than the controls (1,083±296.22 pg/ml) ($p=0.004$). Fourteen out of eighteen patients (78%) had an elevated serum TCII level, over 2,000 pg/ml. The demographic details of these 18 patients with scrub typhus are shown in Table 1.

There was a direct association between serum TC II levels and IgM/IgG titres/ total IgM in this study (Fig 1). There was no correlation between serum TCII levels and total IgM levels ($p=0.376$),or liver and renal function tests ($p>0.117$), as shown in Table 2. A correlation was found between IgM titers and total IgM levels in these infected patients ($y=138.118 + 0.0602 x$, $r=0.767$, $p<0.001$ (Fig 2).

DISCUSSION

In this study, patients with scrub typhus had elevated serum TCII levels which returned to normal after treatment. These findings confirm previous reports of elevated serum TCII levels in patients with both cerebral malaria and scrub typhus (Areekul *et al*, 1995a,b). The exact mechanism of the elevated TCII levels is not known. It could be either an increased synthesis or a decreased clearance of TCII from the blood stream.

Elevated serum TCII levels have been reported previously in patients with renal failure (Carmel *et al*, 2001), and malaria with renal failure (Areekul *et al*, 1993). TCII levels have been reported to be associated with BUN and creatinine levels (Areekul *et al*, 1993, 1995a). None of

Table 1
Demographic details of 18 patients with scrub typhus.

No.	Sex	Age (years)	IIP[a] IgM[b]	IIP[a] IgG[c]	TCII (pg/ml)	Liver function SGOT (U/ ml)	Liver function SGPT (U/ ml)	Liver function AP (U/ l)	Renal function BUN (mg/dl)	Renal function Creatinine (mg/dl)	IgM (mg/dl)
1	F	36	1:3,200	1:3,200	2,471	25	11	30	9	1.2	388
2	M	23	1:6,400	1:12,800	3,527	232	154	214	16	0.9	402
3	F	29	1:6,400	1:6,400	2,137	60	91	67	9	1.2	634
4	M	28	1:12,800	1:12,800	3,722	49	65	45	12	1.6	733
5	M	16	1:6,400	1:6,400	1,054	44	48	160	5	0.9	300
6	M	19	1:6,400	1:6,400	2,414	90	96	196	12	1.1	430
6	M	30	1:12,800	1:12,800	4,188	24	26	40	11	0.9	425
8	F	26	1:6,400	1:6,400	3,748	91	85	111	15	1	324
9	M	34	1:800	1:3,200	1,900	47	17	45	10	1.2	203
10	M	38	1:12,800	1:12,800	4,256	41	42	38	17	0.5	1,371
11	M	59	1:800	1:800	2,868	96	70	70	13	0.8	288
12	F	51	1:3,200	1:6,400	1,586	81	87	154	11	0.9	347
13	M	43	1:12,800	1:6,400	1,250	119	71	95	9	1.2	1,303
14	M	33	1:6,400	1:12,800	2,146	127	72	124	9	1.5	745
15	F	60	1:800	1:1,600	2,556	147	151	69	17	1	185
16	F	40	1:3,200	1:6,400	2,087	39	31	15	12	1.3	367
17	M	41	1:6,400	1:6,400	3,096	124	107	145	32	2.3	447
18	M	38	1:12,800	1:12,800	4,708	30	17	75	11	1.1	871

IIP[a]=Indirect immunoperoxidase; IgM[b]=The IgM antibody titer was positive if > 1:400; IgG[c]=The IgG antibody titer was positive if > 1:1,600

Table 2
A correlation between serum transcobalamin II levels and IgM, IgG titers, total IgM, liver function and renal function tests in patients with scrub typhus.

TCII versus	Regression formula		r	p-value
IgM titer	Y=1,054.373+2.048	x	0.499	0.035
IgG titer	Y=1,624.2993+2.1797	x	0.5701	0.0135
Total IgM	Y=344.841+0.0715	x	0.2219	0.376
SGOT	Y=93.073-0.0042	x	-0.0836	0.741
SGPT	Y=76.885-0.0028	x	-0.073	0.774
AP	Y=125.879-0.0115	x	-0.207	0.409
BUN	Y=7.1199+0.0021	x	0.383	0.117
Creatinine	Y=1.243-0.00004	x	-0.099	0.695

the patients with scrub typhus infection in this study had any signs or symtomps of renal insufficiency, azotemia, or abnormal BUN or creatinine levels except case No. 17. The increased serum TCII levels could not be due to increased TCII-B_{12} uptake by proximal tubular cells or reduced degradation of TCII by lysosomal enzymes as described in an earlier report (Areekul *et al*, 1993).

The finding of increased TCII levels in patients with multiple myeloma and lymphoproliferative disorders suggests that macrophages, plasma cells and B-lymphocytes may be a cellular source for TCII synthesis (Carmel, 1985). TCII levels in Gaucher's disease also indicates the reticuloendothelial system may play a role in serum TCII metabolism (Gilbert and Weinreb, 1976). A marked rise in serum TCII levels in patients with rheumatoid arthritis also indicates that the mononuclear phagocytic system was stimulated and synthesized this protein (Arnalich *et al*, 1990). Rickettsiae preferentially infect endothelial cells of small blood vessels and macrophages in perivascular inflammatory infiltrate. A perivascular inflammatory response developed at infection sites with polymorphonuclear cells, monocytes, macrophages, lymphocytes and occasionally plasma cells. Rickettsiae infect causes damage to endothelial cells and causes vasculitis and perivasculitis of small veins, arteries and capillar-

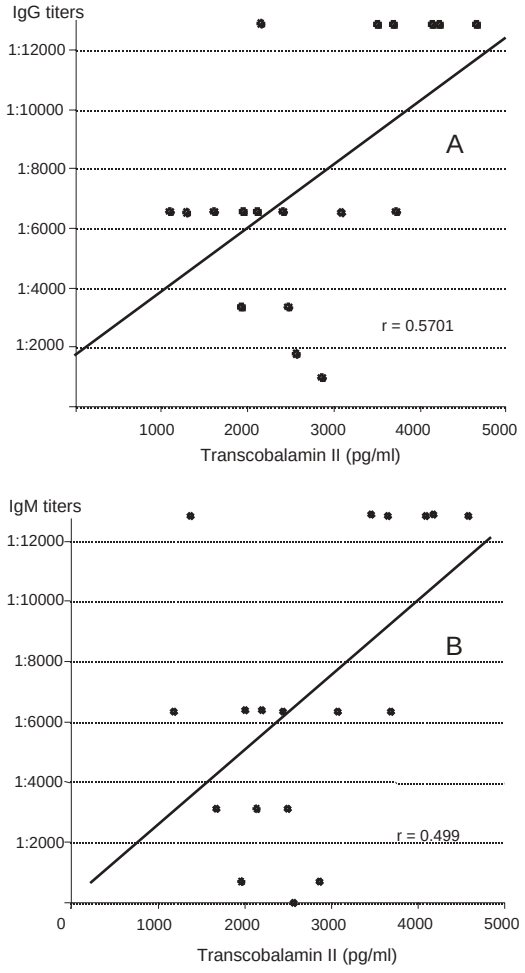

Fig 1–Correlation between transcobalamin II levels in 18 scrub typhus patients and IgG titers (A) and IgM titers (B).

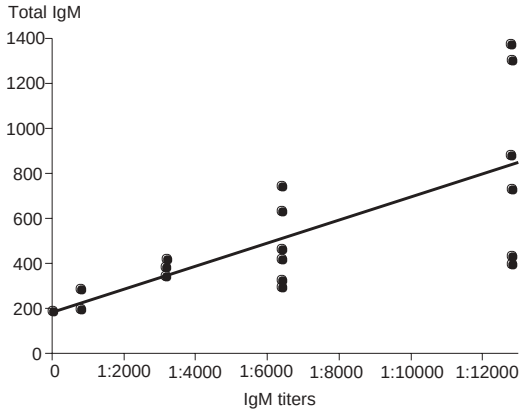

Fig 2–Correlation between IgM titers and total IgM in 18 scrub typhus patients.

ies of the brain, lung, liver, kidney and other organs.

It is possible that this stimulated mononuclear phagocytic system may be responsible for the increased synthesis and released of TCII into the circulation. It has been shown *in vitro* that mouse peritoneal macrophages, and human monocytes and macrophages secrete considerable amounts of TCII (Rachmilewitz *et al*, 1978; Rabinowitz *et al,* 1982).TCII levels in peripheral blood monocytes isolated from patients suffering from acute inflammatory diseases of the bowel, such as shigellosis, and chronic inflammatory diseases, such as Crohn' s and ulcerative colitis, are three to four times higher than in normal subjects (Rachmilewitz *et al*, 1980).

In primary scrub typhus infections, IgM levels rose quickly, while IgG levels followed more slowly. With reinfection, IgM level increases were delayed but reached the same heights as with primary infection (Bourgeois *et al*, 1982). Our study shows a direct relationship between serum TCII levels and IgM or IgG titers. These findings indicated that IgM and IgG titers are synthesized in parallel with TCII. More than 50% of patients with multiple myeloma and high serum TCII levels had elevated gamma globulin levels, and those with Gaucher's disease had diffuse hypergammaglobulinemia. It has been suggested that high TCII levels in these 2 diseases may be related to the immunoglobulin abnormalities (Carmel and Hollander, 1978; Pratt *et al*, 1966). Increased total IgM and IgG levels have also been reported in patients with typhoid, malaria, amebiasis and trypanosomiasis (Tobie *et al,* 1966; Houba and Allison, 1966; Lehman *et al*, 1972; Braga *et al*, 2002).

Serum TCII levels in patients with malaria but without renal insufficiency, or in amaebic liver abscess, have been found to be normal (Areekul *et al*, 1995c). The findings of no relationship between serum TCII levels and total IgM levels in our study indicate elevated TCII levels are not due to increased IgM levels.

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