STUDY ON SERUM TRANSCOBALAMIN II IN PATIENTS WITH MURINE TYPHUS

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Abstract. We measured the serum transcobalamin II in murine typhus- infected patients (n = 16) admitted to the Hospital for Tropical Diseases in 1996-1997, compared with healthy controls (n = 60). The results showed that the transcobalamin II (TCII) and total serum unsaturated vitaminB_{12} binding capacity (UBBC) in patients with murine typhus (2,126.5 pg/ml, range 1,262-4,568 and 3,771.5 pg/ml, range 1,576-6,763 pg/ml) were statistically significantly higher than normal subjects (987.5 pg/ml, range 678-2,000 pg/ml and 1,402 pg/ml, range 932-2,470 ml) ($p<0.001$). Serum TCII levels in patients (63%) were elevated during the febrile period and returned to normal post-treatment. These findings suggest that patients with murine typhus had stimulation of reticulo-endothelial system, spleen, mesenteric lymph nodes, liver and skin and then released TCII into the blood circulation. The elevation in TCII may be used for confirming a diagnosis of murine typhus.

INTRODUCTION

Murine typhus, caused by *Rickettsia typhi* (*R.mooseri*), occurs in many areas of the world. There is increasing evidence from many countries that murine typhus constitutes a large proportion of acute febrile diseases, especially in areas with a large rodent population. Disease symptoms vary from unrecognized, through mild, severe and fatal. Severity of disease has been associated with old age, hepatic and renal dysfunction, central nervous system abnormalities, and pulmonary compromise if diagnosis was delayed.

Several reports suggest that murine typhus still causes health problems in Thailand. A sero-survey in rural northern Thailand detected antibodies in 7-11% of subjects examined (Sankasuwan *et al*, 1969). Clinical murine typhus has been documented in refugees in northern Thailand at an estimated annual incidence of 2.2% in one camp and 0.5 % in another (Brown *et al*, 1988). In southern Thailand, murine typhus was also found in 7 % of febrile-illness patients in Songkhla Province (Silpapojakul *et al*, 1987).

Elevation of serum TCII levels were reported in patients who had stimulation of recticulo endothelial system and inflammatory diseases *eg* acute leukemia and lymphoma, systemic lupus erythematosus, dermatomyositis, rheumatoid arthritis, multiple myeloma as well as lysosomal storage defect such as Gaurhcer's disease (Gilbert and Weinreb 1976; Carmel and Hollander, 1978; Laser *et al*, 1985) and scrub typhus (Cheeramakara *et al*, 2005).

We investigated serum transcobalamin II levels as a determinant of murine typhus in patients compared with control.

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MATERIALS AND METHODS

Patients

Thirty-six patients with suspected murine typhus admitted to the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Thailand in 1996-1997 were screened for murine typhus infection. Diagnosis of murine typhus was based on clinically compatible history and confirmed by Weil-Felix agglutination test with positive OX-19 result (a 4-fold rise in titer, or ≥1:640). Blood samples were taken on day 1 of admission and the following weeks for detection of murine typhus antibodies. Sixteen patients were confirmed as murine typhus and recruited into this study. The healthy controls were also included (n=60).

Determination of Weil-Felix OX-19 test

The assay was performed by microtiter technique using Proteus OX-K , OX-2 and OX-19 antigen (Wellcome Diagnostic, Dartford. UK) and the microtiter plate method, as describe by Gaultney *et al* (1971).

Determination of transcobalamin level

The assay was fractionated and quantitatively measured, as described by Selhub *et al* (1976).

Unsaturated vitamin B_{12} binding capacity (UBBC)

UBBC was expressed as amount in picogram of ^{57}Co-B_{12} bound per ml of serum. The value of UBBC was calculated from the sum of TCI, TCII and TCIII.

Statistical analysis

The relationships between serum transcobalamin II and the other biochemical parameters were determined by the Pearson's correlation method. The differences between the means of serum transcobalamin II and vitamin B_{12} binding capacity levels in patients and controls were compared by Mann-Whitney *U* test for independent samples, at $\alpha = 0.05$.

RESULTS

The demographics of 16 patients with murine typhus are shown in Table 1. There was no significant difference in hematological and biochemical tests between patients and controls. Ten of 16 patients (62%) had elevated TCII during a febrile illness and returned to normal after treatment. The mean values of serum transcobalamin II in the patients (2,126 pg/ml, range 1,262 to 4,568 pg/ml) was significantly higher than those of 60 normal subjects (987 pg/ml, range 678 to 2,000 pg/ml) ($p < 0.001$) (Fig 1a). The mean value of UBBC levels in patients (3,771 pg/ml, range 1,576 to 6,763 pg/ml) was also significantly higher than those of controls (1,402 pg/ml, range 932 to 2,470 pg/ml) ($p<0.001$)(Fig 1b). There were no correlations between TCII and Hb, Ht, DB, TB, BUN and creatinine in patients with murine typhus ($p>0.05$).

DISCUSSION

The diagnosis of murine typhus relies mainly on serological methods, of which micro-immunofluorescence is the standard reference test. It has been reported that Weil-Felix test, a simple and inexpensive method, was quite specific for murine typhus, in spite of the non-rickettsial nature of the antigen used. The specificity at the frequency quoted cut-off titers of 1:160 or 1:320 were and 98%, respectively (Silpapojakul *et al*, 1995). However, the corresponding sensitivity with acute sera at these titers was only moderate, probably due to the relatively late appearance of the reactive antibodies, which appear at the end of the first week or during the second week of illness (Stuart and Pullen, 1945). As paired sera were obtained from patients with negative or low OX-19 titer on admission, diagnosis of murine typhus is definite in all of these 16 patients.

Table 1
Demographic details of 16 patients with murine typhus.

No	Sex	Age (yrs)	Hb g/dl	Hct %	WBC x10^9/l	Weil-Felix			TCII (pg/ml)	Total UBBC	Liver function			Renal function	
						OX2	OX19	OXK			SGOT (U/ml)	SGPT (U/ml)	AP (U/ l)	BUN (mg/dl)	Creat (mg/dl)
1	M	42	6.1	18	3.6	1:80	1:640	1:40	2,668[a]	4,899	127	97	40	19	0.9
2	F	30	9.5	30	7.5	1:160	1:160	1:80	3,499[a]	3,975	149	108	51	8	2.6
3	M	58	11.8	36	7	neg	1:640	1:80	2,057[a]	3,774	678	561	75	3	0.8
4	F	51	13.2	41	8	1:320	1:640	1:80	1,599	2,437	76	140	42	12	0.8
5	M	27	13.8	41	7.9	1:80	1:32	neg	1,382	2,431	15	12	24	10	0.7
6	M	37	13.8	41	7.9	1:20	1:640	1:20	3,305[a]	5,721	74	56	33	11	0.8
7	M	24	13.5	43	10	1:80	1:640	1:40	1,262	3,833	50	110	32	14	0.9
8	M	35	14.0	42	13.2	neg	1:640	1:40	2,196[a]	4,019	107	170	77	10	1.2
9	F	31	14.2	42	5.5	1:40	1:160	neg	1,544	2,226	74	56	33	11	0.8
10	M	40	14.3	41	12.2	1:40	1:640	1:20	4,568[a]	6,763	52	90	41	10	0.9
11	M	52	13.7	40	6.8	neg	1:640	1:20	3,958[a]	2,437	146	109	131	20	1.1
12	F	50	13.5	43	10	1:80	1:>640	1:80	3,004[a]	4,022	76	140	42	12	0.8
13	M	48	15.4	46	11.7	1:20	1:>640	1:80	3,000[a]	3,769	187	154	75	14	0.9
14	F	55	12.4	37	7.9	1:320	1:640	1:40	2,943[a]	3,661	282	307	132	10	0.9
15	M	33	14.1	43	5.5	1:320	1:>640	1:80	1,338	1,576	108	165	23	9	0.9
16	F	30	12.9	42	8	1:40	1:>640	1:20	1,707	3,450	64	113	24	10	1.0

[a]Patients with high TCII

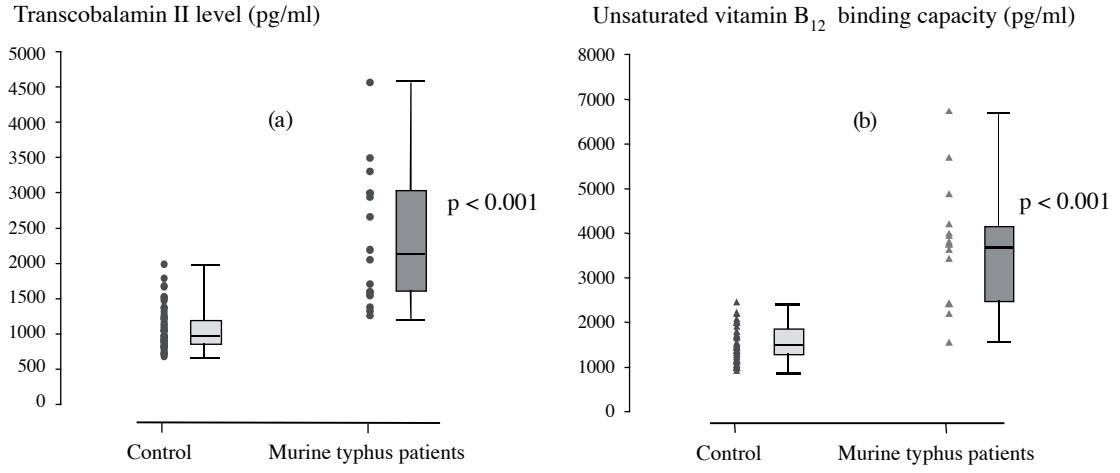

Fig 1– Comparison of serum transcobalamin II level (a) and unsaturated vitaminB_{12} binding capacity level (b) in patients with murine typhus compared with healthy controls. The horizontal line represents median. The box represents 25-75th percecntiles. The bar represents range.

Result in the present study show that 10 of 16 patients with murine typhus had elevated serum TCII levels, which returned to normal post-treatment. The mechanism causing increasesd serum TCII levels are not exactly known, but it could be due to either increased production and release or decreased catabolism and clearance of this protein.

Vasculitis of the small blood vessels consists of focal areas of rickettsial infection of endothelial cell, accompanied by perivascular accumulation of monocytes, macrophages and lymphocytes, indicating that rickettsiae can infect and grow in human endothelial cells, macrophages monocytes, lymphocytes and fibroblast, causing activation and proliferation of these cells (Wisseman and Waddell, 1983). Several mammalial cells, such as liver cells, skin fibroblasts, macrophages, monocytes, plasma cells and B lymphocytes have been reported to synthesize and secrete TCII. The increased serum TCII levels in murine typhus infection are therefore highly due to the increased synthesis of this protein by the proliferative cell from the reticulo-endothelial system, such as the spleen, mesenteric lymph nodes, liver and skin. This is evident from a finding of mild to moderate hepatic injury in murine typhus, which probably results from widespread infection of hepatic sinusoidal lining cells and endothelial vessels in the portal region with injury to adjacent hepatocytes and biliary structures (Dumler *et al*, 1991). As no oliguria, azotemia or hypotension were observed in the present patients, it is very unlikely that the reduced uptake and decreased TCII catabolism by the tubular cells are responsible for the elevated serum TCII as reported in falciparum-infected patients with renal insufficiency (Areekul *et al*, 1993).

As antibodies detected by Weil-Felix OX-19 test appear in acute sera of patients with murine typhus relatively late, *ie*, at the end of the first week or during the second week of illness (Stuart and Pullen, 1945), it is necessary to repeat the Weil-Felix test on convalescent serum. Determination of serum TCII may be helpful in confirming a diagnosis of murine typhus, especially during the first week of illness.

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