SERUM LEVELS OF THROMBOMODULIN, INTERCELLULAR ADHESION MOLECULE-1, VASCULAR CELL ADHESION MOLECULE-1, AND E-SELECTIN IN THE ACUTE PHASE OF PLASMODIUM VIVAX MALARIA

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Abstract. Elevated plasma or serum levels of thrombomodulin (TM), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin have been reported in several diseases. However, plasma or serum levels of TM, ICAM-1, VCAM-1, and E-selectin have not been investigated in the acute phase of Plasmodium vivax malaria. Serum TM, ICAM-1, VCAM-1, E-selectin, and creatinine levels were determined in six Japanese patients in the acute phase of vivax malaria and in seven healthy Japanese controls. Parasitemias of the peripheral blood were < 0.1% in five patients and 0.8% in one patient. The patients’ mean ± SD serum levels of TM, ICAM-1, VCAM-1, and E-selectin were 5.7 ± 1.3 Fujirebio units/ml, 709 ± 397 ng/ml, 2,112 ± 782 ng/ml, and 99 ± 28 ng/ml, respectively, and all were significantly greater than those in the controls (TM; P < 0.005, ICAM-1; P < 0.025, VCAM-1; P < 0.005, E-selectin; P < 0.025). However, no significant difference was identified between patients and controls for serum creatinine values. The serum levels of TM and VCAM-1 were not related to parasitemia. The elevation of serum TM levels suggests that endothelial cell damage occurs in the acute phase of vivax malaria.

Plasmodium vivax malaria is a distinctive, acute, systemic, febrile disease caused by infection with P. vivax. Although vivax malaria is generally not fatal, this parasitic disease is a major clinical and public health problem in developing countries, particularly in tropical and subtropical areas, and it is now becoming an important problem as an infectious disease imported into developed countries from developing areas.

Thrombomodulin (TM), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin are mainly present on endothelial cell surfaces, but they are also found in circulating plasma or serum, and their serum levels have recently been reported to be elevated in the acute phase of falciparum malaria. However, plasma and serum levels of TM, ICAM-1, VCAM-1, and E-selectin have not been investigated in patients with vivax malaria. In the present study, serum levels of TM, ICAM-1, VCAM-1, and E-selectin were measured in patients in the acute phase of vivax malaria prior to treatment.

SUBJECTS AND METHODS

Patients and controls. Serum samples of six Japanese patients in the acute phase of vivax malaria (four men and two women, mean ± SD age = 35.3 ± 11.5 years, age range = 26–57) were tested for levels of TM, ICAM-1, VCAM-1, E-selectin, and creatinine prior to treatment. The period from onset of illness to the time the samples were taken was 3–6 days (mean = 4.5 days). Parasitemia was also investigated when the samples were taken. All patients had contracted the disease outside Japan and were diagnosed by confirming the presence of P. vivax in the blood. They had no evidence of malignant diseases, collagen diseases, vascular diseases, or infectious diseases other than vivax malaria. None of the patients had received effective antimalarial treatment before admission and they had no prior empiric self-administration of antimalarials before diagnosis. All patients received a standard regimen consisting of chloroquine and primaquine directly after the blood drawing for determination of levels of TM, ICAM-1, VCAM-1, E-selectin, and creatinine, and good clinical and parasitologic therapeutic effects were obtained.

Serum samples were also taken from seven healthy Japanese subjects (five men and two women, mean ± SD age = 33.4 ± 12.2 years, age range = 26–56) with no history of malaria. These volunteers served as controls. The serum samples were stored at −35°C until assayed.

This study was approved by the Clinical Research Committee of the Tokyo Metropolitan Bokutoh General Hospital. Informed consent for participation in the study was obtained from all patients and controls.

Assays. Serum TM was measured with commercially available one-step sandwich enzyme immunoassay kits (Fujirebio, Tokyo, Japan), and serum ICAM-1, VCAM-1, and E-selectin were measured with commercially available one-step sandwich ELISA kits (R & D Systems Europe, Abingdon, United Kingdom). The assays were performed according to the manufacturer’s instructions with the reagents provided. The serum creatinine levels were also measured with a creatinase enzyme method (Iatron, Tokyo, Japan).

Statistical analysis. Statistical analysis was performed using the Student’s t-test to assess the differences between values of the patients and controls. A level of P < 0.05 was considered statistically significant.

RESULTS

Parasitemias. Parasitemia in the peripheral blood when the serum samples were taken were < 0.1% in five patients and 0.8% in one patient.

Serum TM levels. The serum TM levels (mean ± SD) in the patients with vivax malaria (5.7 ± 1.3 Fujirebio units [FU]/ml) were significantly higher than those in the controls (3.2 ± 0.7 FU/ml) (P < 0.005). The results are shown in Figure 1. The TM levels of the patient with a parasitemia of 0.8% and the five patients with parasitemias < 0.1% were 6.7 FU/ml and 3.9–6.9 FU/ml, respectively.

Serum ICAM-1 levels. The serum ICAM-1 levels (mean
were 140 ng/ml and 52±108 ng/ml, respectively.

The ICAM-1 levels of the patient with a parasitemia of 0.8% and the five patients with parasitemias < 0.1% were 1,450 ng/ml and 408–773 ng/ml, respectively.

Serum ICAM-1 levels. The serum ICAM-1 levels (mean ± SD) in the patients with vivax malaria (2,112 ± 782 ng/ml) were significantly higher than those in the controls (522 ± 56 ng/ml) (P < 0.005). The results are shown in Figure 1. The ICAM-1 level of the patient with a parasitemia of 0.8% and the five patients with parasitemias < 0.1% were 2,750 ng/ml and 1,380–3,200 ng/ml, respectively.

Serum E-selectin levels. The serum E-selectin levels (mean ± SD) in the patients with vivax malaria (99 ± 28 ng/ml) were significantly higher than those in the controls (56 ± 25 ng/ml) (P < 0.025). The results are shown in Figure 1. The E-selectin levels of the patient with a parasitemia of 0.8% and the five patients with parasitemias < 0.1% were 140 ng/ml and 52–108 ng/ml, respectively.

Serum creatinine levels. Serum creatinine levels (mean ± SD) in the patients with vivax malaria and controls were 0.83 ± 0.10 mg/dL and 0.70 ± 0.14 mg/dL, respectively, with no significant difference identified between them. The creatinine level in the patient with a parasitemia of 0.8% was 0.7 mg/dL.

### DISCUSSION

The serum or plasma levels of TM, ICAM-1, VCAM-1, and E-selectin have been reported to be increased in a variety of infectious diseases,\textsuperscript{1,5} malignant diseases,\textsuperscript{5} collagen diseases,\textsuperscript{5,6} and vascular diseases.\textsuperscript{5,7-9} However, none of the patients in the present study were known to have any disease other than malaria known to cause increased levels of TM and adhesion molecules. Although only small numbers of patients and controls were used, the present study revealed an elevation of the serum levels of TM, ICAM-1, VCAM-1, and E-selectin in the acute phase of vivax malaria.

Thrombomodulin, a vascular endothelial cell receptor for thrombin, is widely distributed on the surface of the endothelia of arteries, veins, capillaries, and lymphatics in all human organs and tissues except for the brain.\textsuperscript{10,11} It is believed to be filtered through the kidney since it is found both in the plasma and the urine of healthy subjects,\textsuperscript{12} and also since patients with chronic renal failure have increased plasma TM values.\textsuperscript{7} There were no significant differences between the patients and controls in serum creatinine levels in the present study, suggesting that the elevated serum TM, ICAM-1, VCAM-1, and E-selectin levels in the patients investigated could not be attributed to delayed clearance from the kidney. An in vitro experiment has shown that TM was not secreted by endothelial cells on stimulation but rather was released after cellular damage of endothelial cells.\textsuperscript{13} Moreover, the importance of the determination of soluble TM levels for the estimation of endothelial cell injury has been established in vitro.\textsuperscript{13,14} Thus, in the patients in the present study, the elevated serum TM levels are thought to be attributable to endothelial cell damage induced by the interaction between the infected and uninfected red blood cells and endothelial cells. It is well known that both infected and uninfected red blood cells are sequestered in falciparum malaria patients, with higher rates of sequestration noted in serious cases. Red blood cells parasitized with \textit{P. falciparum} adhere \textit{in vitro} to ICAM-1,\textsuperscript{15} VCAM-1,\textsuperscript{16} and E-selectin.\textsuperscript{16} The chondroitin sulfate chain of TM is a receptor for \textit{P. falciparum}-infected erythrocytes.\textsuperscript{17} Binding of red blood cells parasitized with \textit{P. falciparum} to TM, ICAM-1, VCAM-1, and E-selectin on the endothelial cell surface may be one of the causes of sequestration. Mild sequestration may also occur in the blood vessels of patients with vivax malaria, and this mode of sequestration may have resulted in injury to the endothelial cells in the patients in the present study. However, no histopathologic investigation to confirm this hypothesis was performed in this study. Thus, elevation of the serum levels of TM suggests that endothelial cell damage occurs in the acute phase of vivax malaria.

Intercellular adhesion molecule-1 is widely expressed at a low level on a variety of cells, including endothelial cells, epithelial cells, fibroblasts, leukocytes, hepatocytes, basal keratinocytes, and some types of tumor cells.\textsuperscript{5,16-19} Vascular cell adhesion molecule-1 is present on endothelial cells, epithelial cells, tissue macrophages, and dendritic cells.\textsuperscript{5,20} E-selectin is found on cytokine-activated endothelial cells.\textsuperscript{21}

![Figure 1. Serum thrombomodulin (TM), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin levels in patients in the acute phase of \textit{Plasmodium vivax} malaria and in healthy controls. Vertical bars indicate the mean ± SD.](image-url)
There are two possible explanations for the elevated serum levels of ICAM-1, VCAM-1, and E-selectin identified in the patients investigated. First, since ICAM-1, VCAM-1, and E-selectin have been demonstrated in vitro in supernatants of cytokine-stimulated endothelial cells,2,23 and that the soluble levels of ICAM-1 and E-selectin have been reported to be significantly correlated with the levels of soluble 55-kD tumor necrosis factor receptor and interleukin-2 receptor in falciparum malaria,1,3 it is suggested that the elevation was caused by release from cytokine-stimulated endothelial and/or other cells. Second, elevated levels of TM, ICAM-1, VCAM-1, and E-selectin can be attributed to damage of endothelial cells. Further studies on the mechanism of elevation of serum levels of TM, ICAM-1, VCAM-1, and E-selectin in malaria patients are needed.

Although the present study showed vivax malaria, in addition to falciparum malaria, leads to an elevation of serum levels of TM, ICAM-1, VCAM-1, and E-selectin, it is unclear which of these two malaria species leads to greater elevation of these levels. To resolve this problem, serum levels of these substances in patients with vivax malaria and falciparum malaria should be measured and compared under similar conditions using similar assay kits.

It has been reported that soluble E-selectin was related to parasitemia in patients with falciparum malaria.1 Although the patient in this study with a parasitemia of 0.8% showed the highest levels of ICAM-1 and E-selectin, the serum levels of TM and VCAM-1 did not show an evident association with the level of parasitemia. While these findings suggest that TM and VCAM-1 are not reliable indicators of parasitemia in patients with vivax malaria, the limited number of patients in the present study prevents any definitive conclusion as to whether serum levels of ICAM-1 and E-selectin are related to parasitemia in patients in the acute phase of vivax malaria.

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