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Short Communication

Serum Levels of sICAM-1 and sE-Selectin in Patients with Dengue Virus Infection

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SUMMARY: The purpose of this study was to measure the serum level of sICAM-1 and sE-selectin as markers for endothelial damage in patients with dengue fever (DF) and dengue hemorrhagic fever (DHF). Twenty-nine patients with serologically-proven dengue virus infection (age 10.6 ± 2.4 years, 55% male, DF = 13 and DHF = 16) were enrolled. Serum samples were collected from 25 healthy children (age 10.6 ± 2.3 years, 40% male) as the control group. A follow-up was done at a mean interval of 15.9 ± 1.6 days. The level of sICAM-1 at the toxic stage was significantly elevated compared to its level at the follow-up (494.1 ± 107.4 versus 358.2 ± 67.6 ng/ml, $P = 0.001$), but no difference was found between patients with DF and patients with DHF (444.1 ± 158.0 versus 465.1 ± 154.6 ng/ml, $P = 0.74$). The sICAM-1 level at the follow-up was similar to that of the control group (396.9 ± 113.0 ng/ml, $P = 0.56$). The level of sE-selectin at the toxic stage was not different from its level at the follow-up (75.9 ± 33.0 versus 75.5 ± 31.7 ng/ml, $P = 0.96$), and no difference was found between the DF group and the DHF group (64.1 ± 25.7 versus 78.8 ± 39.9 ng/ml, $P = 0.30$). These levels were not elevated compared to the sE-selectin level that was determined in 8 patients in the control group (94.7 ± 20.5 ng/ml, $P = 0.12$). In conclusion, there is evidence of endothelial activation by an increased sICAM-1 level in patients with dengue virus infection. However, the degree of endothelial activation alone may be similar for patients with DF and patients with DHF, and this fact by itself cannot explain the difference between the two clinical syndromes of dengue virus infection. The sE-selectin level was not elevated for patients included in this study.

Dengue virus infection is one of the most important emerging infectious diseases in tropical countries (1; available online at http://www.who.int/csr/resources/publications/dengue/Denguepublication/en/). The two clinical syndromes caused by dengue virus infection are undifferentiated fever (dengue fever [DF]) and a clinical entity characterized by vascular leakage, thrombocytopenia, and coagulopathy (dengue hemorrhagic fever [DHF]). Endothelial cell activation and damage has been thought to be the cause for the vascular leakage in DHF (2). The purposes of this study were, first, to search for evidence of endothelial activation in patients with dengue virus infection by measuring the serum level of sICAM-1 and sE-selectin during various stages of the illness and, secondly, to find out if there was any difference in the degree of endothelial activation between patients with DHF and patients with DF.

Blood samples at the febrile, toxic, and convalescent stages of dengue virus infection and at the follow-up (15.9 ± 1.6 days after defervescence) were collected from 29 patients (age 10.6 ± 2.4 years, male/female = 16/13, DF = 13 and DHF = 16). To provide a control group, serum samples were collected from 25 healthy children (age 10.6 ± 2.3 years, 40% male) who came to the clinic as part of the follow-up for hepatitis B virus vaccination (given at birth). Sera were separated and stored at −70°C until analysis. All patients were serologically confirmed to have dengue virus infection by the enzyme-linked immunosorbent assay (ELISA) method. Diagnosis and grading of DHF were done according to criteria published by the World Health Organization (WHO) (1). The toxic stage was defined as the day of defervescence or the presence of hemoconcentration and/or shock. The convalescent stage was defined as 24 - 48 h after the toxic stage and a time when the patient was recovering. Follow-up was done at least 1 week after the toxic stage. The study was approved by the Ethic Committee of the Faculty of Medicine, Chulalongkorn University, Thailand. Written informed consent was obtained from each subject and/or appropriate guardian prior to enrollment.

A total of 80 serum samples from DF/DHF patients were analyzed for sICAM-1 and sE-selectin. The demographic and clinical data of all patients are summarized in Table 1. Serum levels of sICAM-1 and sE-selectin were determined by the commercial ELISA kit (R & D system, Minneapolis, Minn., USA). The levels of sICAM-1 and sE-selectin in all 29

Table 1. Demographic and clinical data of the 29 patients

	DF ($n = 13$)	DHF ($n = 16$, 5 DSS)	P
Age (year)	9.8 ± 2.3	11.4 ± 2.4	0.07
Sex (male/female)	8/5	8/8	0.71
Highest Hct	41.8 ± 3.4	45.5 ± 3.8	0.01
Lowest platelet count (per mm^3)	75,800 ± 16,000	28,300 ± 25,300	<0.01
Duration of fever (days)	3.4 ± 0.7	3.8 ± 0.9	0.29

DF, dengue fever; DHF, dengue hemorrhagic fever; DSS, dengue shock syndrome; Hct, hematocrit.

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patients at different stages are shown in Figure 1. The level of sICAM-1 at the toxic stage was significantly elevated compared to its level at the follow-up (494.1 ± 107.4 versus 358.2 ± 67.6 ng/ml, $P = 0.001$ by paired t test), but no difference was found between its levels in patients with DF and in patients with DHF (444.1 ± 158.0 versus 465.1 ± 154.6 ng/ml, $P = 0.74$ by unpaired t test). The distribution and level of sICAM-1 at the follow-up were similar for the patients in the study and for those in the control group (Figure 1, last column of the left figure; $P = 0.56$ by unpaired t test). The level of sE-selectin at the toxic stage was not different from its level at the follow-up (75.9 ± 33.0 versus 75.5 ± 31.7 ng/ml, $P = 0.96$ by paired t test), and no difference in these levels was found between patients with DF and patients with DHF (64.1 ± 25.7 versus 78.8 ± 39.9 ng/ml, $P = 0.30$ by unpaired t test). Furthermore, these levels were not elevated compared to the levels of sE-selectin that were determined in 8 patients in the control group (Figure 1, last column of the right figure; $P = 0.12$ by unpaired t test). Comparisons of the levels of both adhesion molecules between patients at the toxic stage with DF and with DHF are shown in Figure 2.

From these results, a few conclusions can be made. First, this study demonstrates that evidence of endothelial activation in patients with dengue virus infection can be seen by an elevation of sICAM-1 level even though the degree of endothelial activation does not seem to differentiate patients with DHF from those with DF. Our data suggests that the degree of endothelial activation and damage alone cannot explain the existence of vascular leakage in patients with DHF and that other explanatory factors need to be sought through further study.

Secondly, we found that only sICAM-1 and not sE-selectin was elevated during the toxic stage of dengue virus infection. We did not study sVCAM-1, sP-selectin, or sL-selectin since previous studies had reported the level of these adhesion molecules in patients with dengue virus infection (3,4). These findings and the data from the sVCAM-1 studies (3,4) were in agreement with the in vitro findings regarding the upregulation of gene expression for sICAM-1 and sVCAM-1 in endothelial cell culture treated with culture fluid from monocyte inoculated with dengue virus-antibody complex (5). In this study (5), sE-selectin expression was more transient, a finding which concurs with the finding in our current study that there was no significant elevation of sE-selectin. This is in contrast to studies of adhesion molecules in patients with systemic inflammatory response syndrome and/or sepsis in which the sE-selectin level often showed a marked elevation (6,7). A unique pattern of endothelial cell activation and damage may be characteristic of DF and DHF. Combining the data from this and previous studies (3,4), only sVCAM-1 had been shown to be higher in the serum of patients with DHF compared to patients with DF while sICAM-1, sE-selectin, sP-selectin, and sL-selectin levels were not higher. The significance of these findings is unknown at this time. Further studies are needed to elucidate the cause(s) and importance of endothelial activation and damage in the pathogenesis of the illnesses caused by dengue virus infection.

The level of sICAM-1 at the toxic stage was significantly higher than its level at the follow-up in dengue-infected patients ($P = 0.001$ by paired t test), but its level for these patients failed to demonstrate a significant elevation when compared to the healthy control group ($P = 0.12$ by unpaired t test). This discrepancy here to show a significant difference was probably due to the higher statistical power of paired data

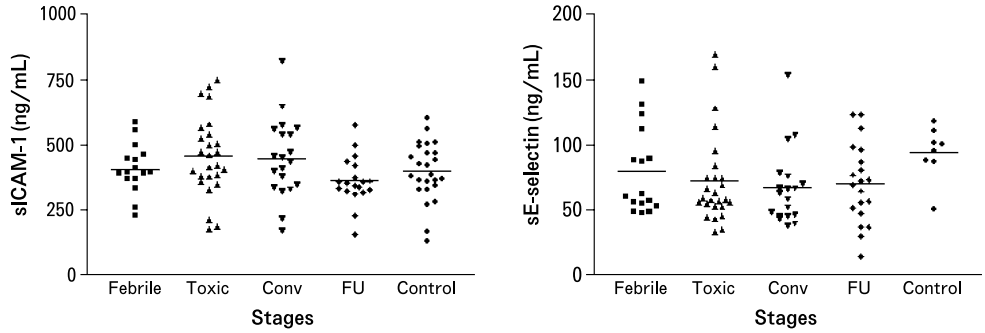

Fig. 1. Serum levels of sICAM-1 (left) and sE-selectin (right) during different stages of dengue virus infection. The horizontal lines represent the mean of sICAM-1 or sE-selectin level at each particular stage. Conv, convalescent stage; FU, at follow-up. Febrile serum was obtained at the mean interval of 1.2 ± 0.4 days before defervescence, convalescent serum at 1.6 ± 0.6 days after defervescence, and follow-up serum at 15.9 ± 1.6 days after defervescence.

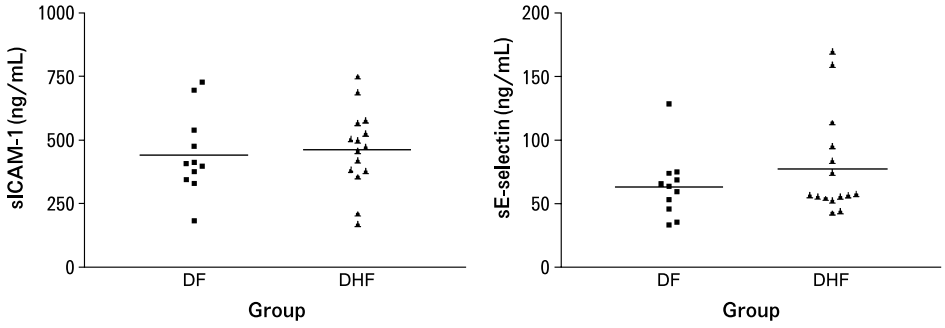

Fig. 2. Serum levels of sICAM-1 (left) and sE-selectin (right) during toxic stage (or at defervescence) in patients with dengue fever (DF) and dengue hemorrhagic fever (DHF). The horizontal lines represent the mean of sICAM-1 or sE-selectin level in each group.

compared to unpaired data. Because the level of significance was high for the difference found between the sICAM-1 level during the toxic stage and the follow-up, and because the sICAM-1 level at the follow-up for the dengue patients closely matched the level for the control group (Figure 1), we have concluded that sICAM-1 was elevated during the toxic stage of the dengue virus infection.

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