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Original article

D-dimer as an indicator of dengue severity

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Background: Dengue infection is a problem of global concern. The clinical spectrum of the disease varies from an acute febrile course accompanied by mild hemorrhagic manifestations with uneventful recovery to refractory shock and massive bleeding with high mortality. Several mechanisms may be involved in the pathogenesis of bleeding, namely: vasculopathy, thrombocytopenia, coagulopathy and dissiminated intravascular coagulopathy (DIC).
Objective: To determine the relationship between D-dimer (DD) levels and clinical outcome in dengue patients.
Method: Children with suspected dengue infection admitted to King Chulalongkorn Memorial Hospital were enrolled. D-dimer (DD) was sequentially measured during the course of illness using whole blood and a rapid semiquantitative system (SimpliRed). Diagnosis of dengue infection was confirmed by serology and WHO criteria were used for classifying dengue severity.
Results: 41 dengue patients, 22 girls and 19 boys were recruited in the study. The mean age was 9.68 years. There were 12 (29.3 %) cases of dengue fever (DF) and 29 (70.7 %) cases of dengue hemorrhagic fever (DHF). DD was more significantly present in the DHF group (87 %) than in the DF group (13%) ($P<0.03$). The sensitivity and specificity of DD in predicting severe dengue infection (DHF) were 90% and 67 %, respectively. Sequential analysis of DD showed higher levels at all stages of dengue infection. It correlated with the disease severity.
Conclusion: Semiquantitative DD assay measurements in children suffering from dengue infection significantly correlated with dengue severity.

***Keywords*:** D-dimer, dengue.

Dengue infection is a global concern as it is an expanding public health problem in the tropical and subtropical world. Reports suggest 2.5 billion people are at risk for dengue with up to 100 million dengue virus infections each year and more than 25,000 reported deaths annually.The clinical spectrum of disease varies from an acute febrile course to refractory shock and massive bleeding with high mortality. The pathogenesis of hemorrhage in dengue virus infection is not fully understood. Mechanisms of bleeding in dengue infection are vasculopathy, thrombocytopenia, coagulopathy and dissiminated intravascular cogulopathy (DIC). The coagulation-fibrinolysis system appears to be abnormal during infection manifesting as decreased fibrinogen levels, increase levels of fibrinogen degradation products (FDP), prolonged partial thromboplastin time, low levels of coagulation factors VIII and XII, plasminogen, prothrombin, and α-2-antiplasmin [1, 2].

The presence of the D-dimer (DD) indicated activation of the coagulation system resulting from the destruction of cross-linked fibrin and reflects clot formation and lysis [3, 4]. Thus the D-dimer assay, a specific marker for cross-linked fibrin, is often used as a marker for DIC [5-8].

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We hypothesized that DD status would correlate with dengue severity. Therefore, we performed a prospective descriptive study to determine the relationship between DD and clinical outcome in dengue patients.

Materials and methods

Dengue patients

This study was performed with the approval of the Ethics Committee of King Chulalongkorn Memorial Hospital and with consent of the participating parents. The study group consisted of 41 children (0-15 years of age) with suspected dengue infection admitted to King Chulalongkorn Memorial Hospital during October 2004 to October 2005.

Sample collection

DD was sequentially measured during the course of illness using whole blood and a rapid semiquantitation system (SimpliRed; AGEN Biomedical Limited; Brisbane, Australia). The SimpliRed D-dimer Test was a rapid quantitative in-vitro test for the detection of cross-linked fibrin degradation products in whole blood. A fingerstick sample was suitable for testing.

In the presence of DD, this assay leads to RBC agglutination. For each sample, at least 10 µl of blood was placed in a test well and mixed with reagent for 2 minutes. If there was no agglutination, we confirmed the absence of DD by performing a negative control. If agglutination indicating the presence of DD was noted, its intensity was graded as either weak (1+) or strong (2+) as outlined by the manufacturer. The stronger intensity of reaction corresponded to higher levels of DD. The lower limit of detection was 120 ng/ml. This level is in accordance with the upper limit of normal for DD.

Diagnosis of dengue infection

The diagnosis was confirmed using an enzyme-linked immunosorbent assay (ELISA) for IgM and IgG antibodies to dengue virus performed at the Armed Forces Research Institute of Medical Sciences (AFRIMS). Serum anti dengue IgM above 40 units or 2-fold increase of serum anti IgG with absolute level above 100 units comfirmed the diagnosis.WHO criteria were used for classifying dengueseverity.

Statistical analysis

Continuous data were reported as means. Chi-square test was performed to compare categorical variables unless the expected size of any one cell is small. In those instances we used the Fisher's Exact Test. Analyses were performed using SPSS for Windows. All reported P values are two-tailed, and value <0.05 were considered statistically significant.

Results

Forty-one patients with serological confirmed dengue infection were studied. The age of patients ranged between 3-15 years with a mean age of 9.68 years. Twenty-two patients (54 %) were girls and 19 patients (46 %) were boys. There were 12 (29.3 %) cases of dengue fever (DF) and 29 (70.7 %) cases of dengue hemorrhagic fever (DHF). Most cases of DHF were DHF grade I and II.

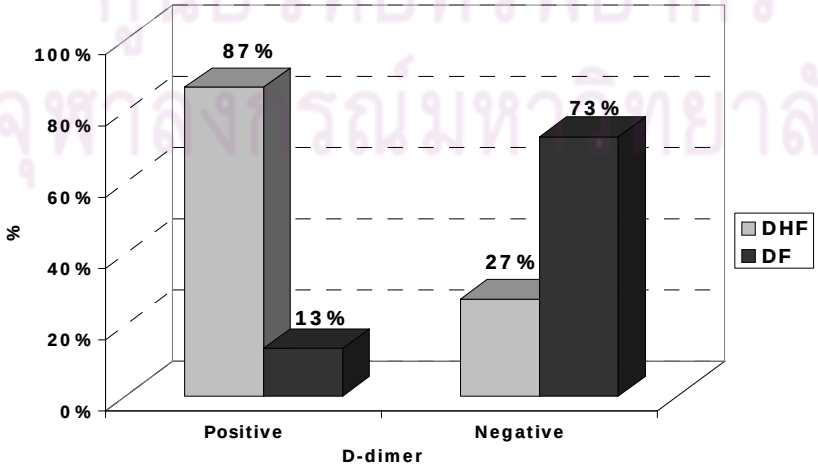

Fig. 1 D-dimer results in DHF in comparison to DF (P-value <0.001).

Figure 1 shows DD results in DHF in comparison to DF. DD was found to be positive in 26 (87 %) DHF and 4 (13 %) DF patients. This showed that DD was more significantly present in the DHF group (87 %) than DF group (13 %) (P-value <0.01).

The sensitivity and specificity of DD in predicting DHF were 90 % and 67 %, respectively. The positive and negative predictive values of DD were 87 % and 72 %, respectively.

Correlation of DD levels with dengue severity in all stages of the disease is presented in **Figs. 2-4**. Weak (1+) and strong (2+) intensity of DD was mostly found in DHF grade I and II. Dengue severity was found to be positively correlated with DD levels in all stages of disease namely febrile, toxic and convalescent stages (P-value <0.05).

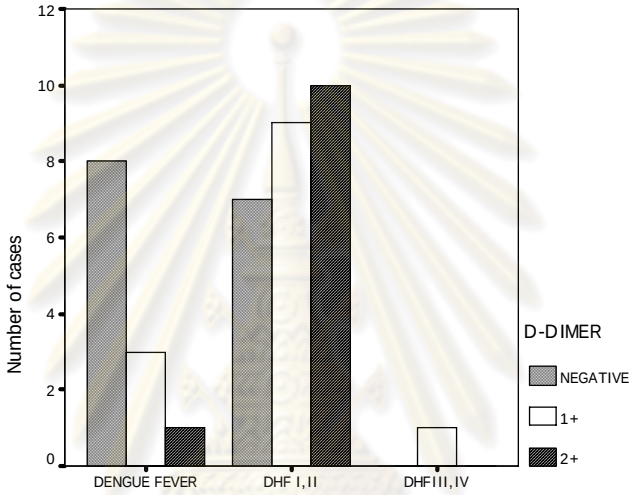

Fig. 2 D-dimer results in DF in comparison to DHF grade I, II and DHF grade III, IV (febrile stage) (P-value: 0.023).

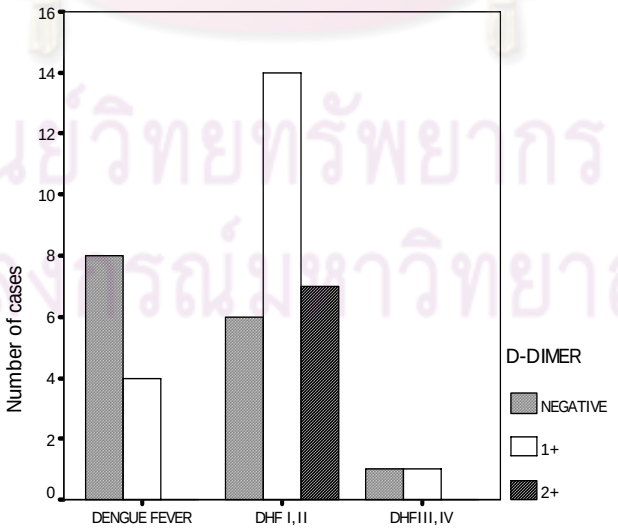

Fig. 3 D-dimer results in DF in comparison to DHF grade I, II and DHF grade III, IV (toxic stage) (P-value: 0.038).

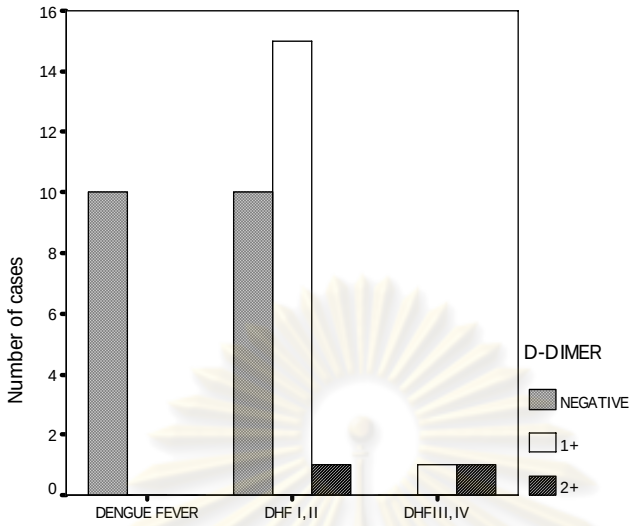

Fig. 4 D-dimer results in DF in comparison to DHF grade I, II and DHF grade III, IV (covalescent stage) (P-value < 0.01).

Discussion

The pathogenesis of hemorrhage in dengue virus infection is not fully understood, several studies showed abnormal hemostasis including DIC in DHF [9, 10]. The presence of the D-dimer (DD) indicated activation of the coagulation system resulting from the destruction of cross-linked fibrin and reflects clot formation and lysis [3, 4]; thus D-dimer assay, a specific marker for cross-linked fibrin, is often used as a marker for DIC [5-8].

Our study showed significantly higher DD levels in DHF patients compared with DF patients with the sensitivity of DD in predicting DHF of 90 %. DD was also found to be positively correlated with dengue severity in all stages of disease namely febrile, toxic and convalescent (P-value <0.05). Detection of DD in the febrile stage of dengue infection may be beneficial for predicting the clinical course of the disease. It may help clinicians in predicting dengue severity before the patients progress into toxic stage so that close monitoring and proper management can be arranged. The semiquantitative DD assay measurements (SimpliRed D-dimer test), is simple and non-invasive and can be easily performed at the bedside.

Detection of DD at the febrile stage, suggests that DIC and activation of fibrinolytic system occur early in patients with dengue virus infection in the before onset of severe hemorrhagic manifestations. This may provide the basis for considering future studies for early intervention.

Our results should be interpreted with caution in view of the small number of DHF patients grade III, IV and the total number of patients in the study. We believed that a larger study would have shown an association of DD and dengue severity.

In summary, semiquantitative DD assay measurements in children suffering from dengue infection significantly correlate with dengue severity in all stages of disease.

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