Thesis Title Detection of Dengue Virus Antigens in Sera and Peripheral Blood Leukocytes by Biotin-Streptavidin Enzyme-Linked Immunosorbent Assay

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ABSTRACT

Dengue virus infection remains the major public health problem. Dengue hemorrhagic fever is the most severe manifestation of an acute dengue virus infection which can develop dengue shock syndrome and causes significant number of deaths in children. The laboratory diagnostic tests for confirmation of dengue infection will be useful for treatment, control and surveillance in order to reduce morbidity and mortality rates of disease.

This study attempts to detect dengue antigens in sera compared with peripheral blood leukocytes (PBLs) by using a biotin-streptavidin system in ELISA to improve the sensitivity and specificity of the test. One hundred and seventy eight blood samples (acute sera and PBLs) were collected from 106 dengue and 72 non-dengue patients aged

under 15 years at Khonkaen Hospital between July and November, 1994. Age of dengue infected patients were in the range of 5-9 year (54.7%), 10-14 year (30.2%) and 0-4 year (15.1%). The male and female ratio was about 1:1. They were classified by clinical manifestations and laboratory findings as dengue fever (DF ; 19 cases, 17.9%) and dengue hemorrhagic fever (DHF ; 87 cases, 82.1%). Thirty six (34.0%) cases were dengue shock syndrome (DSS) with no death. The clinical features including high fever (>38.0 ^{0}c), anorexia, drawsiness, hepatomegaly, flush face, headache and abdominal pain were observed mainly in DF cases. Moreover, the unusual manifestations such as hepatomegaly (73.7%), petechiae (26.3%), epistaxis (26.3%), hemoconcentration (21.1%) and thrombocytopenia (15.8%) were reported. The clinical features of DHF cases included high fever, anorexia, drawsiness, abdominal pain, vomitting, lymphadenopathy and flush face. Tourniquet test positive (81.6%),hepatomegaly (77.0%),hematemesis/melena (48.3%), petechiae (34.5%), epistaxis (31.0%), gum bleeding (13.8%) and ecchymosis (9.2%) were registered with 46.0% of thrombocytopenia and 60.9% of hemoconcentration.

Among 106 DF/DHF cases confirmed by hemagglutination inhibition (HI) test, almost of them (98.1%) were secondary infection. Only 2 cases (1.9%) were primary dengue infection. Detection of dengue viral antigens by BS-ELISA showed 18.9% of sensitivity and 97.2% of specificity in acute sera. The predictive values of positive and negative results were 90.9% and 44.9%, respectively. The efficiency of test was 50.6%. In acute PBLs, the sensitivity and specificity were 53.8% and 95.8%, respectively. The predictive values of positive

and negative results were 95.0% and 58.5%, respectively. The efficiency of test was 70.8%. The detection of dengue antigens in 106 dengue cases by BS-ELISA were compared between sera and PBLs. Using PBLs, the detection was greater than sera 6.3 times with significant difference ($p<0.001$). When both sera and PBLs were determined in combination by BS-ELISA, the sensitivity and specificity increased (60.4% and 93.1%, respectively).

Considering the severity of disease, the presence of dengue antigens in acute sera determined by BS-ELISA did not depend on DF or the grade of DHF except DHF 4 (DF= 21.1%, DHF1= 12.5%, DHF2= 21.1%, DHF3= 18.2%, DHF4= 66.7%). For acute PBLs, the positive cases of dengue antigens detection increased when the infection was severe (DF= 36.8%, DHF1= 59.4%, DHF2= 52.6%, DHF3= 54.5% and DHF4= 100%). The serum samples collected between day 2 and day 7 after the onset of the disease positive by BS-ELISA increased gradually (6.7%, 15.0%, 15.6%, 30.8%, 20.0% and 33.3%, respectively). Using PBLs, the dengue antigens were detected with higher percentage (46.7%, 50.0%, 68.8%, 42.3%, 60.6% and 33.3%, respectively) than sera and the peak was on day 4.

The results indicate that with some improvements, the detection of dengue antigens in acute PBLs by BS-ELISA can be applied efficiently in diagnosis of dengue infection. In addition, if both serum samples and PBLs are collected and determined by BS-ELISA, the positive detection will increase.