Thesis Title IgM-Capture Biotin-Streptavidin Enzyme-linked Immunosorbent Assay for Detection of Antibody to Dengue Viruses

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ABSTRACT

Dengue hemorrhagic fever has become one of the major public health problems in Thailand. The important factor in the management and control of dengue fever (DF) and dengue haemorrhagic fever (DHF) is the availability of a laboratory diagnostic test to confirm dengue infection. This study attempted to detect immunoglobulin M (IgM) dengue antibody by using a biotin-streptavidin system based on IgM-Capture ELISA to improve the sensitivity and specificity of the test. Two hundred and fifty serum samples, 102 dengue patients, 100 non dengue patients who were admitted in Ratchaburi hospital with febrile syndrome and 48 healthy students, were collected from the children with age under 15 years between March and September, 1991. All serum samples were tested by HI method against dengue and JE viruses, 2 cases (1.96%) showed a primary dengue infection whereas 100 cases (98.04%) had a secondary response. Antibody levels (GMT$\pm$SD)

to JE antigen were the highest followed by DEN-4, DEN-3, DEN-2, and DEN-1. In acute sera, those were 851±9, 573±9, 437±7, 416±6 and 363±8, respectively. In convalescent sera, those were 11,482±4, 8,128±4, 8,128±4, 8,128±4, and 6,457±4, respectively. To develop IgM-BS-ELISA, high titer of antibody to flavivirus from DHF cases was purified by using ion exchange chromatography (DEAE-cellulose) and labelled with biotin. If serum sample has IgM, it will bind to anti-human IgM coated in microtiter plate. After the tetravalent dengue antigens, anti-flavivirus IgG labelled with biotin, streptavidin-peroxidase conjugate and substrate were added. The color was developed and the reaction was stopped by using sulfuric acid. The absorbance was read by spectrophotometer at the wavelength of 490 nm. By using HI test as gold standard, the sensitivity and specificity of IgM-BS-ELISA in acute sera were 83.33% and 95.27%, respectively. The predictive values of positive and negative results were 92.39% and 89.24%, respectively. The efficiency of test was 90.4%. In convalescent sera, the sensitivity and specificity of IgM-BS-ELISA were 100% and 92.56%, respectively. The predictive values of positive and negative results were 90.26% and 100%, respectively. The efficiency of test was 95.6%. The agreement rate of HI and IgM-BS-ELISA was good. K (kappa) value was 0.79 for acute sera and 0.91 for convalescent sera. Correlation between IgM-BS-ELISA and HI was quite good. The correlation coefficients (r) were 0.7603 for acute sera and 0.8489 for convalescent sera. The results indicate that the IgM-BS-ELISA has high sensitivity and specificity. It is simple to perform, rapid and inexpensive (5-6 bath/test). The total test time is about 9 hrs. Therefore the IgM-BS-ELISA can be applied efficiently in diagnosis of dengue infection by using single serum and implemented for a large scale study.