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SHORT REPORT

In-house ELISA for the diagnosis of childhood tuberculosis

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There are no conventional methods for diagnosis of childhood tuberculosis. Children can not cough out sputa and thus sputum for smear and culture methods are not useful for them. Tuberculin skin tests may give false positive results in children who were immunized with BCG. Chest X-ray may give mimic results with other lung disorders. A rapid and reliable test for diagnosis of childhood tuberculosis would have great applicability as a clinical tool. Therefore, use of whole cell soluble *Mycobacterium tuberculosis* antigen ELISA (in-house test) as a serodiagnostic test for childhood tuberculosis was studied.

This study was carried out from May 2002 to April 2003. It was a cross-sectional, laboratory and hospital-based study. The coating antigen was prepared in the Tuberculosis Culture Laboratory and Immunology Research Division, Department of Medical Research (Lower Myanmar). It was prepared from *Mycobacterium tuberculosis* cultured on 3% Ogawa media. Whole cell soluble *My. tuberculosis* antigen was prepared using the method of Nicholl's (1975)[1]. Phenolized non-viable tubercle bacilli were collected, treated with N sodium hydroxide and heated at 80°C for 30 minutes. After washing and centrifuging, antigen soluble in double distilled water was used as the coating antigen in the ELISA test.

This study was done on 50 children admitted to the North Okkalapa General Hospital and Thingangyun Sanpya Hospital. The consents were asked from their parents or guardians. After getting informed consent chest X-rays and tuberculin skin tests were carried out on all cases. The parents and guardians of all subjects were interviewed on the BCG vaccination status of the subject. Two milliliters of blood were taken and whole cell soluble *Mycobacterium tuberculosis* antigen ELISA (in-house ELISA) was done on their sera.

Sera samples were assayed to detect the IgM antibodies, whole cell soluble *My. Tb.* antigen was used as the coating antigen, horse-radish peroxidase conjugated goat anti human IgM (Dako, Denmark) was used as the conjugate and orthophenylene diamine (OPD) (Wako, Osaka, Japan) was used as the substrate. Mean optical density (OD) values were obtained from the samples tested in duplicate. Sera samples from normal apparently healthy children who had visited the Vaccine and Diagnostic Clinic of Department of Medical Research (Lower Myanmar) were used as negative controls. The cut-off value of controls was OD-0.264.

There was no significant association between ELISA and BCG status (X^2=0.08, p=0.786). The sensitivity and specificity of

the in-house ELISA test was 72% and 80% respectively for IgM in childhood tuberculosis. Our findings point to a reasonable specificity (80%) and sensitivity (72%) of the test when IgM antibody titers are considered for the diagnosis of childhood tuberculosis.

REFERENCE

1. Nicholls, A. C. A serodiagnostic test for tuberculosis. *Journal of Clinical Pathology* 1975; 28: 850-853.