The Myanmar Health Sciences Research Journal, Vol. 17, No. 2, 2005

SHORT REPORT

Assessment of whole cell soluble *Mycobacterium tuberculosis* antigen ELISA (in-house ELISA) test in serodiagnosis of pulmonary tuberculosis

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A rapid, reliable and inexpensive serological test for diagnosis of tuberculosis would have great applicability as a clinical, epidemiological and public health tool. Therefore, use of whole cell soluble *Mycobacterium tuberculosis* antigen ELISA (in-house test) as a serodiagnostic test for pulmonary tuberculosis was studied.

This study was carried out from February 2002 to January 2003. It was a cross-sectional, laboratory and institutional-based study. Coating antigen was prepared in 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 *M. tuberculosis* antigen was prepared using the method of Nicholl's (1975). 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 coating antigen in ELISA test.

Sputa and 2 ml of blood samples were obtained from 40 smear positive and 54 smear negative pulmonary tuberculosis cases who attended the Union Tuberculosis Institute, Aung San, during July to September 2002. One hundred and thirty six blood samples were also collected from normal apparently healthy blood donors from National Blood Center (as a control group). Informed consents were taken from all participants. Sputa were decontaminated and processed into culture at TB laboratory, DMR (LM). Cultures were made onto Ogawa slant under 37°C incubation for 8 weeks.

In-house ELISA test was carried out as follows: Fifty µl of 10 µg/ml concen-trated soluble antigens were coated onto duplicate wells, 96-well microtitre plate (Fastec Microplate U. Fujirebio Inc.) and placed at 4˚C overnight. Plate was then washed three times with phosphate buffer saline containing 0.05% of Tween 20 (PBST) and 100µl of blocking agent was added to each well and incubated at 37˚C for 30 minutes. It was sucked out and 100µl of diluted sera (1:40 dilution) was added to each well and incubated at 37˚C for 1 hour and washed with PBST for 3 times. Then 50µl of goat anti-human IgG conjugated horse-radish peroxidase, diluted in 1:1500 with dilution buffer was added to each well and incubated at 37˚C for 1 hour. It was washed with PBST for 3 times again. After that, 100µl of substrate solution (orthophenylene-diamine) was added to each well and incubated at 37˚C for 15 minutes. Reaction was stopped using concentrated sulphuric acid and readings were taken on an ELISA reader at 492nm. Result was considered positive if OD value was higher than 0.264 (previously calculated cut-off point of control mean + 2 SD).

For smear positive pulmonary tuberculosis group, sputum for culture and in-house

ELISA test were positive in 40/40 and 39/40 samples respectively and therefore sensitivity was 100% and 97.5% respectively. For smear negative pulmonary tuberculosis group, sputum for culture and in-house ELISA test were positive in 29/54 and 38/54 samples respectively and therefore sensitivity was 53.71% and 70.37% respectively. For control group, 114/136 samples were negative in in-house ELISA test and thus specificity of this test is 83.82%.

Therefore, sensitivity of in-house ELISA test on pulmonary tuberculosis in our community was 97.5% and 70.37% for smear positive and negative pulmonary tuberculosis respectively and specificity was 83.82%. Therefore, this study indicates that in-house test is beneficial for screening of pulmonary tuberculosis (both AFB smear positive and negative cases).

REFERENCE

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