SERODIAGNOSIS OF TUBERCULOSIS USING TWO ELISA SYSTEMS

Swati Banerjee, Sonika Gupta, Niraj Shende, Satish Kumar and Bhaskar C. Harinath

Jamnala Bajaj Tropical Disease Research Centre and Department of Biochemistry, Mahatma Gandhi Institute of Medical Sciences, SEVAGRAM, WARDHA-442 102, MAHARASHTRA, INDIA

Running title: Serodiagnosis of tuberculosis

ABSTRACT

Serodiagnosis by ELISA has been widely explored over the years, in the diagnosis of tuberculosis. Two ELISA systems were evaluated for detection of mycobacterial antibodies in pulmonary and extra pulmonary tuberculosis. The two test assays explored were ERBA LISA (TB IgG) test (Anda Biologicals) which uses A60 antigen complex found in the cytosol of typical and atypical mycobacteria, and SEVA TB (IgG) ELISA, which uses a 31 kDa, glycoprotein antigen purified from *M. tb* H₃₇Ra culture filtrate. Sera from 98 proven tuberculosis [pulmonary TB (48), tuberculous lymphadenopathy (30), tuberculous meningitis (15) & genito-urinary TB (5)] were studied along with 32 healthy controls. The overall positivity obtained using ERBA LISA (TB IgG) test and SEVA TB (IgG) ELISA test was 72.9% and 91.6% in pulmonary tuberculosis, 43.3% and 76.6% in tuberculous lymphadenopathy respectively. The sensitivity of ERBA LISA test in tuberculous meningitis and genito-urinary TB was significantly low (26.6% & 40% respectively) compared to sensitivity obtained using SEVA TB ELISA (86.6% & 60% respectively) with overall specificity of 60% and 87.5%. Thus SEVA TB IgG ELISA test was found to be more sensitive than ERBA LISA in detecting IgG antibodies in tuberculous sera, in particular in extra pulmonary tuberculosis cases.

KEY WORDS

ELISA, ES-31, serodiagnosis, tuberculosis

INTRODUCTION

Tuberculosis is a leading cause of morbidity and mortality in the world, particularly in India and developing countries and has been declared a global emergency. Conventional techniques such as acid-fast smear and culture studies are either not sufficiently efficient and specific or require an extended turnaround time from the laboratory. Moreover extra pulmonary tuberculosis is difficult to diagnose as in many cases clinical, radiological and laboratory findings are non-specific, and acid-fast smear and culture of AFB are rarely positive. Therefore a number of alternative diagnostic tests

that use molecular, chromatographic and immunological methods have been explored. While molecular methods overcome the insensitivity and time consumption, they depend on obtaining specimen from the site of infection, are expensive and require expertise. It is often difficult to obtain specimen in childhood tuberculosis and in some cases of extra pulmonary tuberculosis.

Immunological methods measure specific cellular or humoral responses of the host to detect presence of infection or disease. They do not require a specimen from the site of infection. Numerous serological tests that use various antigens such as secretory, heat shock proteins, lipopolysaccharide and peptides have been developed(1). These tests use various modifications of enzyme-linked immunosorbent assay (ELISA) to detect different antibody classes.

Despite extensive work on mycobacterial antigens over the last 100 years, so far only a few serodiagnostic kits are available, based on detection of antibodies to tuberculous specific antigens, for

Author for Correspondence:

Dr. B. C. Harinath
Director Professor
JB Tropical Disease Research Centre
Mahatma Gandhi Institute of Medical Sciences
Sevagram-442 102 (Wardha) M. S. (India)
Tele Fax: (07152) 84038
E-mail: jbtldr@nagpur.dot.net.in
bc_harinath@yahoo.com

wide clinical use. Many workers have explored the use of A60 antigen in detection of tuberculous antibody from pulmonary and extrapulmonary TB sera with varying success (32.1%-88.5%) (2-9).

In the present study, we have evaluated two serological assay systems for detection of IgG antibodies from tuberculous sera, namely a commercially available ERBA LISA (TB IgG) test kit (from Anda Biologicals, Strausberg, France) which uses A60 antigen complex and an indigenously developed SEVA TB (IgG) ELISA test which uses a 31kDa secretory antigen protein, which was reported to be more promising than other secretory antigen purified from *M. tb* H₃₇Ra Culture filtrate(11).

MATERIALS AND METHODS

Study population

The study population comprises of 4 groups. Group I- AFB smear positive, culture positive pulmonary tuberculosis patients (35), Group II- AFB smear negative, culture negative but ATT responded pulmonary tuberculosis patients (13), Group III- Extra pulmonary tuberculosis patients which included cytologically and histopathologically confirmed tuberculous lymphadenopathy (30), clinically diagnosed & ATT responded tuberculous meningitis (15) and genito-urinary tuberculosis cases (5). Group IV- Healthy individuals of this locality (32) served as healthy controls. Blood samples were collected, sera were separated, and stored at -20°C with 0.1% sodium azide.

ELISA:

ELISA using both the kits was performed on blood (serum) obtained from all study cases by both the assay systems.

a) ERBA LISA (TB IgG) Test:

The test was performed to detect IgG antibodies to A60 antigen using commercially available kits (Anda Biologicals, Strausberg, France) according to manufacturer's instructions. Serum dilution of 1:100 was used in the assay. Positive and negative reference sera were included in run along with test sera. For determining IgG units, the curve was constructed by plotting the OD values of different reference sera. Thereafter, concentration of IgG antibodies in test serum in terms of units/ml were determined by extrapolating the OD value of serum

against the reference curve (10).

b) SEVA TB (IgG) ELISA Test:

Stick indirect penicillinase ELISA was performed as described by Nair et al(11), to detect IgG antibodies in human sera. Five µl of optimally diluted antigen [ES-31 (0.2µg/ml), a glycoprotein with metalloprotease activity having N-terminal sequence NTGQS (Asp-Thr-Gly-Glu-Ser) purified by fast protein liquid chromatography from culture filtrate of *M.tb* H₃₇Ra as described by Nair et al(11) previously] applied to cellulose acetate membrane squares fixed to plastic strip, optimally diluted human sera (1:600) and anti human IgG penicillinase conjugate (1:1000) were used in this assay. The sera showing complete decolourisation of blue colour substrate at least 5 min. earlier than the negative control denoted a positive reaction.

RESULTS

Since the sensitivity and specificity of any serological test is dependent on the appropriate cut-off values chosen in the test, we initially determined the most suitable cut-off values for IgG antibodies in ERBA LISA assay system as suggested by manufacturers to differentiate the tuberculous cases from controls in this region. Three different values i.e. 100 (chosen arbitrarily), 125 and 225 units/ml (according to manufactures instructions) were considered. However, cut-off of 125 units/ml was chosen as this produced a moderate ratio of sensitivity (52.6%) and specificity (60%) (Table 1) in this population.

Serological analysis (disease wise break-up) using both assays is depicted in Table 2. Of the 35 smear positive, culture positive pulmonary tuberculosis cases, 68.5% (24/35) were found positive using Anda TB (IgG) test, whereas using SEVA TB ELISA test significantly higher positivity (91.4%) was obtained. However in smear negative, culture negative pulmonary TB group quite better positivity (84.6) was obtained compared to smear positive cases using ERBA LISA test, but still slightly lesser than that obtained using SEVA TB ELISA test (92.3%).

In extra pulmonary tuberculosis three groups were included i.e. tuberculous lymphadenopathy (TBLN), tuberculous meningitis (TBM) and genito urinary tuberculosis (GUTB). In all these three groups significantly low positivity using ERBA LISA test was obtained i.e. 43.3% in TBLN, 26.6% in TBM &

40% in GUTB, compared to the positivity obtained using SEVA TB ELISA i.e. 76.6%, 86.6%, & 60% in TBLN, TBM & GUTB respectively.

Of 32 healthy controls 19(59.4%) had a negative ERBA LISA TB IgG test and 28 had a negative SEVA TB IgG test, thus giving a specificity of 59.4% and 87.5% respectively.

Using SEVA TB ELISA quite better positive & negative predictive values were obtained (95.4% & 65.1% respectively) compared to that obtained by using ERBA LISA Test (80.5% & 30.1% respectively) (Table3).

DISCUSSION

The diagnosis of tuberculosis continues to be surrounded by uncertainty in spite of the availability of a variety of tests. Of today's available tests such as conventional tests and various molecular biology tests, serology is an attractive possibility in diagnosis and patient's management. Despite extensive work on various antigens no single reliable test is available for clinical use.

This study was performed to evaluate indigenously developed SEVA TB (IgG) ELISA assay and ERBA LISA (TB IgG) kit from Anda Biologicals, strausberg, France, one of the few commercially available & widely used diagnostic kit. Many workers have reported use of antigen A60 for diagnosis of active pulmonary TB cases with varying sensitivity in the range of 48.0% - 88.5%(2-5). Our findings are in correlation with these authors. The sensitivity of the assay was improved in smear negative cases, where in fact in absence of smear positivity, detection of maximum number of cases is essential, but still it was slightly lesser than that of SEVA TB ELISA which detected more number of cases from smear negative (92.3%) as well as smear positive cases (91.4%). However number of negative cases analysed was comparatively lesser than smear positive cases, hence more number of samples are needed to confirm the findings. The antigen used in SEVA TB ELISA assay is a 31kDa glycoprotein culture filtrate antigen(11). Many workers have reported use of culture filtrate antigens in the diagnosis of tuberculosis with varying sensitivity (48-94%) and specificity (87-100%)(12-16).

The diagnosis of extra pulmonary tuberculosis is difficult, delayed in many cases and many a time done on circumstantial evidences alone. Moreover, because of the difficulty in obtaining specimens for microbiological study, serodiagnosis seems to be an ideally suited diagnostic modality for extra

pulmonary tuberculosis. Antibodies against A60 antigen have been detected by many workers in the range of 32.1%-84% in extra pulmonary tuberculosis sera (6-9). In our study using A60 antigen, IgG antibodies could be detected in overall 38% of sera from extra pulmonary cases (Table 3). One of the authors has also reported the sensitivity in EPTB in such low range (32.1%). This low sensitivity in our study, may be observed due to variations in antibody response in different regions or differences in antigen expression in different conditions. Using SEVA TB ELISA assay, however the antibodies against 31kDa antigen could be detected in significantly more number of sera (78%) indicating 31kDa could be a better marker for (detecting) diagnosing extra pulmonary as well as pulmonary TB cases. Further it has shown less cross reactivity with the control sera (12.5%) compared to A60 antigen (40.6%) (Table 2). The observed cross reactivity may possibly due to the exposure to environment mycobacteria or latent infection.

Serodiagnosis by ELISA may have greatest utility in developing countries because it is relatively inexpensive and the higher prevalence of tuberculosis in those areas will increase its positive predictive value, however these values vary markedly with the prevalence of the disease in a given community. Thus higher positive predictive & negative predictive value of SEVA TB ELISA make it a useful tool in diagnosis by confirming the clinical suspicion. Thus this test could potentially be used for smear negative tuberculosis and those subgroups of patients with tuberculosis from whom specimens are difficult to obtain i.e. extra pulmonary tuberculosis and childhood tuberculosis to aid in clinical decision making.

Thus evaluation of these two assays showed that SEVA TB (IgG) ELISA had advantages over the commercially available ERBA LISA (TB IgG) test kit, such as cost effectiveness, requiring no special equipment (being visual reaction) and having higher sensitivity, specificity, positive & negative predictive value and thus can be of better clinical utility.

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Table 1

Comparison of the sensitivity and specificity of ERBA LISA test, at three cut-off values.

IgG units/ml	Overall Sensitivity (%) (PTB + EPTB)	Overall Specificity (%)
100	67.3	46.8
125	52.6	60
225	31.6	75

Table 2

Comparative analysis of tuberculosis sera by ERBA LISA (TB IgG) kit and SEVA TB (IgG) assay.

Group (sera)	Total No. Screened	ERBA LISA Test + ve No. (%) *	SEVA TB ELISA + ve No. (%) **
Pulmonary TB (S+, C+)	35	24 (68.5)	32 (91.4)
Pulmonary TB (S-, C-)	13	11 (84.6)	12 (92.3)
TB lymphadenitis	30	13 (43.3)	23 (76.6)
TB meningitis	15	4 (26.6)	13 (86.6)
Genito-urinary TB	5	2 (40.0)	3 (60.0)
Healthy Controls	32	13 (40.6)	4 (12.5)

* Threshold for positive reaction 125 U/ml.

**Threshold for positive reaction 1:600 dilution.

Table 3
Comparative analysis of both assays using different parameters.

Parameters	ERBA LISA Test (%)	SEVA TB ELISA Test (%)
Overall sensitivity (PTB)	72.9	91.6
Overall sensitivity (EPTB)	38	78
Overall specificity	60	87.5
PPV	80.5	95.4
NPV	30.1	65.1

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