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Comparative analysis of ES-31 and ES-41 antigens in the detection of tubercular IgG antibodies and study on combine sensitivity of antibody-antigen

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ABSTRACT

Immunoscreening was carried out by ELISA for the samples from the patients admitted in Kastruba Hospital, Sevagram. Blood samples of patients of pulmonary and extrapulmonary tuberculosis were examined for presence of tuberculosis specific antibody, antigen and combine sensitivity was also tested. Antigen preparation namely *M.tb*$H_{37}R_a$ ES-31 and 41 were used for the detection of tubercular IgG antibody whereas affinity purified anti ES-31 antibody was used for tubercular circulating free and immune complexed (CIC) antigen. ES-31 showed more sensitivity in pulmonary, lymphnode, CNS, genito uninary TB and ES-41 showed more in abdominal, osteoarticular TB, percentage positivity in combined Ab/ Ag/ CIC-Ag sensitivity was found more.

Key words: ES-31, ES- 41, Pulmonary, Extra-pulmonary, Tuberculosis.

INTRODUCTION

Generally the diagnosis of TB is made on the basis of clinical, radiological and bacteriological examinations. For the later case smear and culture examination for the presence of AFB in clinical samples is done. The limitation of smear examination for AFB is that it may give false negative results and require a high degree of bacillary load of at least 50,000 bacilli/ ml and subject to inherent errors like contamination[1]. Another demerit is that it is positive in open cases only[2], thus it is not helpful in extra pulmonary form of TB, particularly in TBM/ childhood TB where sputum is not available. The limitations of bacterial culture are that the results are available only after 4-6 weeks and though reliable for pulmonary TB but not so for extra pulmonary form[3]. X-ray is not suitable for field studies in developing countries like India and also the reports are non specific. Despite an enormous amount of research we still do not have simple, sensitive and specific test for the diagnosis of TB in active form[4].

The diagnosis of extra pulmonary TB (EPTB) is difficult, delayed and frequently made on circumstantial evidence alone and delay in diagnosis might have threatening consequences.

Attempts have been made to improve the sensitivity and the speed of detection of TB bacilli of components thereof by other methods such as radiometric determination of bacterial growth (BACTEC system), gas chromatography/ mass spectrometry and DNA hybridization. All of these have meet with problems of either sensitivity or prohibitive cost. Thus, when the current criteria for diagnosis such as clinical and radiological signs and bacteriological and biochemical methods that aid in

diagnosis are unreliable, there is a search for alternative test which can stand with its merits in vigorous clinical trials. Serodiagnostic tests have attracted considerable attention of the investigators.

The history of serodiagnosis starts with Robert Koch's unsuccessful attempt to diagnose the disease by direct aggulutination of TB bacilli. Subsequently several workers tried several ways of immunoassays like agglutination of RBC, complement fixation, preparation in fluid and gels and radio immunoassays, but hardly any one is accepted for routine clinical use.

ELISA is having the added advantage as it is sensitive, specific, reproducible and useful in field studies to detect mycobacterial antigens[5-9] or antibodies[10,11].

The choice of antigen is the major determinant for the success of any serological test. A wide variety of antigens have been applied ranging from crude antigens such as culture filtrate antigen, whole cell sonicate antigen, mycobacterium saline[5] extract antigen to purified antigens such as antigen, glycolipid antigen, Lipo arabinomannan etc. Grange suggested that the study of antibody response to those antigens that are actively secreted by living mycobacteria rather than cytoplasmic antigens liberated by sonication or other mechanical means are more fruitful for TB diagnosis[12]. The most pronounced specific antibody, which correlated with active disease was IgG[13,14].

In our laboratory studies using different antigen preparations (whole protein to purified proteins) have been employed for exploring their potential in detecting tubercular IgG antibodies in tuberculosis[15,16]. On exploring use of ES-31 and ES- 41 antigens of *M. tb*H_{37}Ra in stick penicillinase ELISA for the detection of seroreactivity in pulmonary TB and EPTB, ES-31 showed good potential in detecting IgG antibody in TB Lymphnode, Tubercular meningitis and pulmonary TB[17] while ES-41 in abdominal TB and Bone and Joint TB. Hence, the present study was undertaken to evaluate ES-31 and ES-41 antigen in immunodiagnosis of pulmonary and extra pulmonary tuberculosis.

EXPERIMENTAL

The study subjects comprised of patients with tuberculosis, non- tuberculous disease control and healthy controls, who attended Kastruba Hospital, Sevagram during 1999-2001. Tuberculosis groups were showing group for pulmonary tuberculosis (having progressive tuberculosis proven bacteriologically), lymphnode tuberculosis (confirmed by histopathologically), Central nervous system (CNS) tuberculosis (confirmed by CSF studies or response to anti- tubercular therapy), Genitourinary tuberculosis (confirmed by histopathologically, radiology , CT and clinical study), Abdominal tuberculosis (confirmed by biopsy, radiology, ascitic fluid examination and clinical study) and Osteoarticular tuberculosis (confirmed by clinico- radiological measures).

Sera were collected before starting of antitubercular and antibiotic treatment. Approximately 3 cc of blood was collected in sterile vials. Sera was separated and stored at -20°C after addition of 0.1% sodium azide as preservative. Pure laboratory strain of *Mycobacterium tuberculosis* H_{37}Ra from Tuberculosis Research Centre, Chennai was used in this study. Lowentstein – Jensen (L-J) medium was used for seed culture and modified synthetic Sautan medium was used as subculture medium. *Mycobacterium tuberculosis* ES antigen was isolated and labelled as *M.tb* ES antigen and stored in aliquots at – 20°C with sodium azide (0.1%) until use. ES-31 & 41 antigen was isolated by affinity chromatography. Polyclonal antibodies to ES- 31 and 41 antigen were raised in goat and specific antibodies against ES –31 and 41 antigen isolated from immune sera using ES-31 and 41 antigen coupled CN Br- activated sepharose B column[18].

Stick indirect penicillinase ELISA for tuberculous IgG antibody detection was carried out. Stick sandwich penicillinase ELISA was carried out for detection of circulating TB antigen and CIC-antigen in sera samples with some modification. The concentration of ES-31 & 41 antigen was 1ng/ stick while that of affinity purified goat anti ES-31 and 41 antibody was 1 mg per stick. Optimum sera dilution in case of antibody detection was 1:600, while in case of antigen detection was 1:300. In the

detection of CIC antigen sera samples were pre-treated with glycine HCl buffer (0.1 M pH 2.8) followed by heating at 65°C for 15 minutes[19]. Combined sensitivity were also tested. The results obtained are depicted in Table 1-2.

RESULTS AND DISCUSSION

In a preliminary study, a total of 52 sera samples belonging to different tubercular groups viz. pulmonary TB, abdominal TB, lymphnode TB, CNS TB, osteoarticular TB, genitourinary TB along with disease controls and healthy controls were analysed for the detection of tubercular IgG antibody using ES-31 and ES- 41 antigens. Serological analysis of tuberculosis cases on ATT by penicillinase ELISA showed presence of antibody in 11 cases out of 22 cases; out of 11 cases which were negative for antibody, 10 each were positive for antigen and CIC antigen with a combined positively of 95%.

Table 1: Comparative analysis of *M. tb* ES-31 and ES-41 antigen in the detection of tubercular IgG antibodies by indirect ELISA pulmonary and extra pulmonary TB

Groups Tuberculosis	No screened	No. showing positive reaction with* ES-31	ES-41
1. Pulmonary TB	5	4(80)	2(40)
2. Extra-pulmonary TB			
a) Abdominal TB	5	1(20)	4(80)
b) LN TB	5	4(80)	2(40)
c) CNS TB	5	4(80)	1(20)
d) Osteoarticular TB	5	1(20)	4(80)
e) Genitourinary TB	5	3(60)	1(20)
3. Disease control	12	4(33)	5(42)
Bronchial asthma	2	0	1
Chronic Bronchitis	2	1	1
Irritable bowel syndrome	2	1	0
Non specific Lymphadenopathy	3	1	2
Pyogenic meningitis	2	1	0
Rheumatoid arthritis	1	0	1
4. Healthy control	10	3(30)	2(20)

Figure in parenthesis denotes percentage positivity
*Sera showing positive reaction at 1: 600dilution.

The study has shown that *M. tb* ES –31 antigen is more sensitive in detecting tubercular IgG antibody in pulmonary TB cases (80%), in some systemic groups of extra pulmonary TB like, Lymphnode TB (80%), Central Nervous System (CNS) TB (80%) and genitourinary TB(60%) compared to *M. tb* ES –41 antigen which showed less sensitivity (40%, 40%, 20% respectively), whereas ES-41 antigen is more sensitive in some groups of extra pulmonary TB, like, abdominal TB(80%) and osteoarticular TB(80%) compared to ES-31 antigen which showed less sensitivity in these two forms of extra pulmonary TB (20% each). Percentage positivity in combined antibody / antigen / CIC – antigen sensitivity was found more as depicted in Table 2.

Table 2: Combine sensitivity of antibody, antigen or CIC –Ag screened with ES –31, ES-41 antigens and affinity purified anti ES – 31 antibody

Groups	No screened	No. showing positive reaction for	
		Antibody#	Combined (Ab /Ag /CIC- Ag)
Pulmonary TB	33	29(89)	32(97)
Lymphnode TB	10	07(70)	09(90)
CNS TB	12	08(67)	10(83)
Genitourinary TB	07	05(71)	6(86)
Abdominal TB*	21	15(71)	19(90)
Osteoarticular TB*	07	05(71)	06(86)
TB cases on ATT	22	11(50)	21(95)

Figures in parenthesis indicate percentage positivity
* Screened with ES –41 antigen
# Sera showing positive reaction at 1: 600dilution

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