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ASSAY OF TUBERCULAR ANTIBODY, CIRCULATING FREE AND IMMUNE COMPLEXED ANTIGEN IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS

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ABSTRACT

Analysis of tubercular antibody, circulating free and immune complexed antigen (CIC-Ag) was done in confirmed pulmonary tuberculosis sera by ELISA, using ES-31 antigen and affinity purified anti ES-31 antibody. Twenty three of 25 (92%) tuberculosis sera were positive for IgG antibody to ES-31 antigen. Using anti ES-31 antibody, free tubercular antigen could be detected in 20 of 25 (80%) cases whereas circulating immune complexed antigen (CIC-Ag) in 18 of 25 (72%) cases by sandwich ELISA. Of the two sera showing absence of antibody, one showed presence of free and CIC-Ag whereas the other showed the presence of free antigen. Thus antigen assay may be used as an adjunct tool for confirmation of pulmonary tuberculosis.

KEY WORDS: Antibody, Antigen, ELISA, ES-31 Antigen, Immunodiagnosis, Pulmonary tuberculosis.

INTRODUCTION

In spite of extensive work on various mycobacterial antigens, over the last 100 years, no rapid and reliable test is available for the diagnosis of tuberculosis. Today's conventional methods of diagnosis like smear and culture are cumbersome, time consuming and less sensitive. Therefore, development of simple, rapid and reliable test is a need of the day. The use of serology i.e. detection of antibodies or antigen or immune complexed antigen using polyclonal or monoclonal antibody reagents as a diagnostic adjunct for detection of tuberculosis (TB) is widespread.

Many workers have explored the use of culture filtrate antigens of *Mycobacterium tuberculosis* (*M.tb*) in ELISA with variable results (1, 2). The diagnostic potential of *M.tb* $H_{37}Ra$ culture filtrate antigens in detection of tuberculous IgG antibodies has been already reported from our laboratory (3, 4). The objective of the present study was to assess the diagnostic potential of antibody, circulating free antigen and immune complexed antigen (CIC-Ag) assays in sera samples of pulmonary tuberculosis (PTB) cases.

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MATERIALS AND METHODS

Patients

Blood samples were collected from 25 bacteriologically confirmed (smear+, culture+) pulmonary tuberculosis (PTB) cases attending Kasturba Hospital, Sevagram, prior to antituberculosis therapy (ATT). Blood samples collected from 18 cases of other diseases including non-tubercular pulmonary diseases and leprosy cases and 20 from healthy individuals of this locality served as negative controls. Sera were separated and stored at –20°C with 0.01% sodium azide.

Isolation of *M.tb* $H_{37}Ra$ excretory-secretory antigen ESAS-7F (ES-31)

M.tb ESAS-7F antigen was isolated on cation

exchanger, Resource 'S', 1 ml column by fast protein liquid chromatography (FPLC) (Pharmacia Biotech: Sweden) as described by Nair *et al* (5). Since, ESAS-7F was further demonstrated as 31 kDa protein molecule on SDS-PAGE, it will be further termed as ES-31.

Isolation of affinity purified goat anti ES-31 antibodies

Polyclonal antibodies to *M.tb* ESAS-7 antigen were raised in goat and specific antibodies against ES-31 antigen were isolated from immune sera using ES-31 antigen coupled cynogen bromide (CNBr)-activated sepharose-4B column as described earlier (4).

ELISA

Stick indirect penicillinase ELISA for tuberculous IgG antibody detection was carried out as described by Nair *et. al.* (4). Stick sandwich-penicillinase ELISA was carried out for detection of circulating free antigen and circulating immune complexed antigen (CIC-Ag) in sera samples as described by Nair *et al* (4). The concentration of affinity purified goat anti ES-31 antibody was 1µg per stick and serum dilution used was 1:300. While detecting CIC-Ag, serum sample was pretreated with glycine-HCl buffer (0.1M, pH 2.8) followed by heating at 65°C for 15 mins, as described by Prasad *et al* (6).

RESULTS

M.tb ES-31 antigen was employed in indirect stick penicillinase ELISA for detection of tuberculous IgG antibody in different groups of sera samples. The results are summarized in Table 1. At 600 cut off for positive reaction, 92% (23/ 25) of pulmonary TB sera showed positive reaction whereas 5.2% (2/ 38) disease and healthy controls (one each) showed cross reaction with tuberculous antigen.

When affinity purified goat anti ES-31 antibodies were employed in sandwich penicillinase ELISA 80% (20/ 25) and 72% (18/ 25) of sera showed positivity for free tubercular antigen and CIC-Ag respectively, whereas only 5.2% (2/ 38) and 2.6% (1/ 38) control sera showed positive reaction for free and CIC-Ag respectively.

Table 1. Comparative analysis of antibody, circulating free & immune complexed antigen positivity in pulmonary tuberculosis.

Group (sera)	Total no. screened	No. (%) showing +ve reaction for		
		Ab*	Ag**	CIC-Ag**
Pulmonary Tb (S+C+)	25	23 (92)	20 (80)	18 (72)
Disease Controls	18	1 (5.5)	1 (5.5)	1 (5.5)
COAD	6	1	-	-
Leprosy	4	-	-	-
Chronic bronchitis	4	-	1	1
Pneumonia	4	-	-	-
Healthy Controls	20	1 (5)	1 (5)	-

**Sera showing positive reaction at 1:600 dilution*
***Sera showing positive reaction at 1:300 dilution*

Out of two negative for tuberculous IgG antibody, one case showed positive reaction for free antigen, while other showed positive reaction for both circulating free and CIC-Ag.

DISCUSSION

Despite importance of serodiagnosis of tuberculosis which has been well emphasized and reported by many workers (2-4,6), studies on the correlation of antibody and free and CIC-Ag parameters are quite scanty. Therefore in this study, tuberculous IgG antibody and free and CIC-Ag have been detected using purified ES-31 antigen and affinity purified anti ES-31 antibody to possibly pick up the maximum true positive TB cases.

Detection of tuberculous antibodies and antigen has been reported by many workers with a wide range of sensitivity (48-94%) and specificity (87-100%) (7-11), whereas in this study a sensitivity of 92% and comparable specificity of 94.7% were achieved indicating the importance of humoral immune response to *M.tb* ES-31 in diagnosis of tuberculosis. The concomitant presence of *M.tb* ES-31 free antigen in 80% and CIC-Ag in 72% of patients samples was detected. Simultaneously in these assay systems, TB antigen and affinity purified antibody have shown

very little false positivity. Thus the present study reported an overall better specificity of 95.5% compared to that reported by Lodam *et al* (3) and Sood *et al* (12).

Although the assays carried out to detect antibody, antigen, and CIC-Ag were found to have well comparable sensitivities and specificities with those found by other workers (3,7,10,12-14), IgG antibody detection assay to ES-31 antigen was found to be superior in all with 92% sensitivity and 94.7% specificity. In two cases, IgG antibodies could not be detected, may be due to an anergic state, where there is absence of IgG antibodies (15), but could be confirmed by circulating free and/ or CIC-Ag assays. Thus the combination of antibody and antigen detection assays definitely detect larger number of TB infected cases.

It could be concluded from this study that detecting IgG antibody to ES-31 antigen has a good potential in confirmation of tuberculosis cases. Thus IgG assay can be preferred where choice has to be made amongst antibody and circulating free and CIC-Ag assay systems. However, in these cases the assay of circulating free and CIC-Ag may definitely serve as adjunct tool for confirming TB cases, as shown in this study.

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