Indian Journal of Clinical Biochemistry, 2002, 17 (1) 5-8

ANALYSIS OF SEVA TB ES-31 ANTIGEN SPECIFIC IMMUNOGLOBULINS IgM, IgA AND IgG IN SERA OF SPUTUM AND CULTURE POSITIVE PULMONARY TUBERCULOSIS

SONIKA GUPTA, NIRAJ SHENDE, SWATI BANERJEE, SATISH KUMAR,
M. V. R. REDDY and BHASKAR C. HARINATH

Jamnalal Bajaj Tropical Disease Research Centre
and Department of Biochemistry
Mahatma Gandhi Institute of Medical Sciences
Sevagram, Wardha-442 102, Maharashtra, INDIA

ABSTRACT

Tuberculosis remains major health problem in India and developing countries. Immunodiagnosis has important role in screening, diagnosis and management of tuberculosis. SEVA TB ES-31 antigen has shown potential in detecting tuberculous IgG antibody in earlier studies from our laboratory. In the present study we have analysed **SEVA TB ES-31** antigen specific immunoglobulins **IgM, IgA and IgG** in clinically and bacteriologically confirmed pulmonary tuberculosis cases to determine the usefulness of specific immunoglobulin class in the diagnosis of patients attending the hospital.

Of the 30 cases of pulmonary tuberculosis 25 (83.3%) were positive for IgG, 19 (63.3%) for IgM and 16 (53.3%) for IgA. On combining IgG and IgM positivity, sensitivity was increased to 93.3%. While combining IgG and IgA positivity, sensitivity increased to 90%. However specificity was decreased to 66.6% and 70% for both of these combinations respectively. It could be envisaged from this study that IgG antibody detection against ES-31 antigen showed acceptable sensitivity (83.3%) and specificity (86.6%) compared to IgM or IgA alone or in combination. When immune responses were analysed according to degree of sputum positivity, IgG response was observed to be predominant in all grades, compared to IgM or IgA antibody. The addition of IgM or IgA as an adjunct test increases the sensitivity but at the cost of specificity. Hence the detection of IgG alone is more useful compared to IgM or IgA assay, in detecting tuberculosis disease cases coming to the hospital.

KEY WORDS :
Immunoglobulins, SEVA TB ES-31 antigen, ELISA, Pulmonary tuberculosis

INTRODUCTION

Tuberculosis is one of the major health problem and has been declared as a global emergency by WHO in 1993. Accurate and rapid diagnosis of tuberculosis patients is essential in control of this disease. The conventional techniques for the diagnosis of tuberculosis like acid fast staining and culture are slow and insensitive. Sensitive and reproducible assay like enzyme linked immunosorbent assay has been explored for serological diagnosis of tuberculosis. The SEVA TB ES-31 antigen isolated from culture filtrate of *M.tb* $H_{37}Ra$ bacilli was shown to be immunodiagnostically useful in our earlier studies (1).

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

In this communication, we report the status of SEVA TB ES-31 antigen specific IgG, IgM and IgA immunoglobulins in patients with bacteriologically confirmed pulmonary tuberculosis (PTB).

MATERIALS AND METHODS

Sera samples:

Blood samples were collected from 30 bacteriologically confirmed smear positive, culture positive (S+ C+) pulmonary tuberculosis cases. According to Revised National Tuberculosis Control Programme (RNTCP) classification sputum positivity was graded as 1+, 2+, 3+. For normal controls, blood samples were collected from 30 healthy individuals of this locality without any evidence of tuberculosis infection. Sera were separated and stored at -20°C with 0.01% sodium azide as preservative.

Isolation of ES-31 antigen:

Mycobacterium tuberculosis $H_{37}Ra$ excretory-secretory SEVA TB ES-31 antigen, a 31 kDa glycoprotein with protease activity was isolated by affinity chromatography using affinity purified anti ES-31 antibody coupled sepharose 4B column, as described by Nair *et al* (2, 3).

Stick indirect penicillinase ELISA for detection of anti tuberculous IgG, IgM and IgA antibody:

Stick indirect penicillinase ELISA was carried out as described by Nair *et al* for anti tuberculous IgG antibody detection (4). Similarly detection of anti tuberculous IgM and IgA antibody assay were carried out. The optimum concentration of ES-31 antigen protein 1 ng/ stick was coated on sticks. The optimally diluted sera (1:600) and optimally diluted antihuman IgG, IgM and IgA penicillinase conjugate (1:1000) were used. The test sample at a serum dilution of 1:600 showing complete decolorization of blue colour substrate at least 4-5 min earlier than the negative control is considered as positive for tuberculous antibody.

RESULTS AND DISCUSSION

In this preliminary study, the tuberculous specific IgG, IgM and IgA antibody responses were analyzed in smear and culture positive pulmonary tuberculosis sera and healthy controls using SEVA TB ES-31 antigen by stick indirect penicillinase ELISA.

A total of 60 sera including pulmonary tuberculosis cases and healthy controls were screened. The results are summarized in Table-1. This study showed sensitivity of 83.3% (25/ 30) for IgG, 63.3% (19/ 30) for IgM and 53.3% (16/ 30) for IgA antibody. When the combinations of IgG and IgM classes were tried, increased sensitivity of 93.3% (28/ 30) was observed, however specificity was found to be decreased to 66.6% compared to 86.6% for IgG alone. Similarly considering IgG and IgA positivity, sensitivity was increased to 90% (27/ 30) and specificity decreased to 70%. Gupta *et al* showed sensitivity of 98.3% and specificity of 90% of combined IgG and IgA test using antigen A60 in adult pulmonary tuberculosis cases (5). Al Hajjaj *et al* showed sensitivity of 87% and specificity of 95% for combined IgG and IgM estimation using A60 antigen (6), while Charpin *et al* showed sensitivity of 68% for combined IgG and IgM estimation (7). The wide variations in sensitivity of different combination of antibody types could be due to the different geographical areas, different antigen used, as well as severity of disease in different studies. The IgM antibody response is mainly found in primary infection or in reactivation cases (5), while production of IgA antibodies occurs in some anergic cases where immune anergy has depressed the synthesis of IgM or IgG antibodies (8). Addition of IgM or IgA as an adjunct test increased the sensitivity but at the cost of specificity. The IgM positivity was observed in increased number of healthy normals compared to IgG or IgA may be due to the latent or inapparent infection or presence of rheumatoid factor, contact with mycobacterial focus or environmental mycobacteria (9). The presence of IgA antibodies in the serum of healthy people has been correlated with a frequent contact with infectious foci (10).

On analyzing immune response in tuberculosis cases with different grade of sputum positivity or bacillary load, IgG antibody response compared to IgM and IgA was found to be predominant in all grades in particular grade 3+ (Table-2). This predominance of IgG antibody response may be due to the establishment of infection and continuous antigenic stimuli secreted from live bacilli.

There is considerable confusion about the ideal immunoassay test based on the specific immunoglobulin class detected in tuberculosis. Anti IgG, IgM and IgA based commercial tests

(ERBA LISA TEST and PATHOZYME MYCO) are available without clear data on specificity and sensitivity of testing each immunoglobulin, thus making the clinicians to request for all the tests. This situation of doing all the tests (IgG, IgM and IgA) many a time leads to no definite conclusion on the positivity or negativity of a particular test sample. Hence in this study an attempt is made to determine sensitivity and specificity of individual immunoglobulins versus combination of immunoglobulins using ES-31 antigen in known cases of tuberculosis.

From this study it is observed that amongst SEVA TB ES-31 antigen specific immunoglobulin classes, IgG alone with considerable sensitivity and specificity is more useful for detection of tuberculosis compared to IgM or IgA alone or in combination in hospital patients.

ACKNOWLEDGEMENTS

This work was supported in part by a Tropical Disease Research grant of Kasturba Health Society, Sevagram. We thank Late Dr. Sushila Nayar, Founder Director, MGIMS, Shri Dhirubhai Mehta, President, KHS and Dr. O. P. Gupta, Dean, MGIMS for their keen interest and encouragement in this work. Technical Assistance of Mrs. S. Ingole and Miss Aparna Gupta are appreciated.

TABLE-1

Detection of SEVA TB ES-31 antigen specific IgG, IgM and IgA antibodies by indirect penicillinase ELISA.

Group	No. Screened	No. showing positive reaction at 1:600 sera dilution					
		IgG	IgM	IgA	IgG/ IgM	IgG/ IgA	IgG/ IgM/ IgA
Pulmonary tuberculosis	30	25 (83.3)	19 (63.3)	16 (53.3)	28 (93.3)	27 (90)	30 (100)
Healthy Control	30	4 (13.3)	8 (26.6)	6 (20)	10 (33.3)	9 (30)	14 (46.6)

Note: Figures in parenthesis are percentage positivity.

Table-2:

Percentage positivity of SEVA TB ES-31 specific IgG, IgM and IgA antibodies according to degree of sputum positivity

Grading of Sputum positivity	No.	No. (%) showing positive reaction* for IgG	IgM	IgA
1+	11	8 (72.7%)	8 (72.7%)	6 (54.5%)
2+	9	8 (88.8%)	5 (55.5%)	5 (55.5%)
3+	10	9 (90%)	6 (60%)	5 (50%)
Total	30	25 (83.3)	19 (63.3)	16 (53.3)

*Sera showing positive reaction at 1:600 sera dilution.

REFERENCES

1) Nair, E. R., Banerjee, S., Kumar, S. and Harinath, B. C. (2000) Isolation and characterization of a 31 kDa Mycobacterial antigen from tuberculous sera and its identification with *in vitro* released culture filtrate antigen of *M.tb* $H_{37}Ra$ bacilli. Scand. J. Infect. Dis. 32, 551-556.

2) Nair, E. R., Banerjee, S., Kumar, S., Reddy, M. V. R. and Harinath, B. C. (2001) Isolation of *Mycobacterium tuberculosis* 31 kDa antigen protein of diagnostic interest from culture filtrate using anti ES-31 antibody by affinity chromatography. Ind. J. Clin. Biochem. 16 (1), 132-135.

3) Nair, E. R., Banerjee, S., Kumar, S., Reddy, M. V. R. and Harinath, B. C. (2001) Purification and characterization of a 31 kDa Mycobacterial excretory-secretory antigenic protein with a diagnostic potential in pulmonary tuberculosis. Ind. J. Chest Dis. Allied. Sci. 43, 81-90.

4) Nair, E. R., Kumar, S., Reddy, M. V. R. and Harinath, B. C. (1998) *Mycobacterium tuberculosis* $H_{37}Ra$ *ESAS-VII* an excretory-secretory antigen fraction of immunodiagnostic potential in pulmonary tuberculosis. Ind. J. Clin. Biochem. 13 (2), 98-105.

5) Gupta, S., Kumari, S., Banwalikar, J. N. and Gupta, S. K. (1995) Diagnostic utility of the estimation of mycobacterial antigen A60 specific immunoglobulin IgM, IgA and IgG in sera of cases of adult human tuberculosis. Tuberc. Lung. Dis. 76, 418-424.

6) Al-Hajjaj, M. S., Gad-El-Rab, M. O., Al-Orainey, I. O. and Al-Kassimii, F. A. (1999) Improved sensitivity for detection of tuberculosis cases by a modified Anda-Tb ELISA test. Tuberc. Lung. Dis. 79 (3), 181-185.

7) Charpin, D., Herbault, H., Gevaudan, M. J., Saadijan, M., Micco, ph. de., Arnaud, A., Vervloet, D. and Charpin, J. (1990) Value of ELISA using A 60 antigen in the diagnosis of active pulmonary tuberculosis. Am. Rev. Respir. Dis. 142, 380-384.

8) Zou, Y. L., Zhang, J. D., Chen, M. H., Shi, Q. G., Prignot, J. and Cocito, C. (1994) Serological analysis of pulmonary and extra pulmonary tuberculosis with enzyme linked immunosorbent assays for anti A-60 immunoglobulins. Clin. Infect. Dis. 19, 1084-1091.

9) Agrawal, A. and Maudgil, K. D. (1988) Enzyme immunoassay based study of IgG and IgM antibody response to antigens of *M. tuberculosis* ($H_{37}Rv$) in patients with pulmonary tuberculosis. Ind. J. Tub. 35, 12-16.

10) Bogers, W., Stad, R. K., Van, Es. L. and Daha, M. (1991) Immunoglobulin A: Interaction with complement, phagocytic cells and endothelial cells Complement. Inflamm. 8, 347-358.