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COMPARATIVE ASSESSMENT OF BIOCHEMICAL AND IMMUNOLOGICAL MARKERS IN TUBERCULOSIS

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Abstract

Introduction: Pulmonary tuberculosis is major public health problem in developing countries like India.Millions of people have died from tuberculosis. Many times it is difficult to get sputum sample from the patients. Some tests lack specificity, some other lack sensitivity. Hence, there is need of precise and faster diagnosis for patients attending hospitals. In this study, we compared the detection potential of biochemical and immunological markers(ADA, LDH and Mycobacterium tuberculosis H_{37}Ra ES-31 & EST-6 antigens & antibodies based ELISAs) in pulmonary tuberculosis.

Methods: 50 pulmonary tuberculosis cases confirmed by sputum examinationfor acid fast bacilli (AFB) and 50 age and sex matched control subjects were included in this study.ADA & LDH were estimated by using commercial kits. Tubercular antigens and antibodies were detected by ELISA method.

Results: SerumADA detected pulmonary tuberculosis with sensitivityand specificityof 94%. Sensitivity and specificity of serum LDH in detecting pulmonary tuberculosis was found to be 94% and 36% respectively.Serumtubercular antigens detected pulmonary tuberculosis with sensitivity and specificity of 88%. Sensitivity and specificity of serum tubercular antibodies in detecting pulmonary tuberculosis was found to be 80% and 90% respectively.

Conclusion: Adenosine deaminase has better detection potential over other markers in pulmonary tuberculosis.

Keywords: Adenosine deaminase, Lactate dehydrogenase, Tuberculosis antigen-antibody

INTRODUCTION:

TB infection has been dated back a long journey in the course of history and the contribution of TB to the misery of mankind had been immeasurable. Millions of people have died from tuberculosis and it remains an important determinant of morbidity and mortality. The disease still continues its ravenous journey across the world; it is the second most cause of death from infectious disease after those due to HIV/AIDS [(1)]. The survey by WHO framework for effective control has stated that the highest priority for effective TB control is the rapid identification and cure of the disease, but the main problem is a lack of standardized diagnostic criteria in early cases of TB infection [(2).]

Several attempts have been made to improve the sensitivity and speed of detection of tubercle bacilli or its components by techniques such as radiometric determination of bacterial growth, [(3)] gas chromatography / mass spectroscopy, [(4)] DNA hybridization, [(5)] Interferon γ release assay [(6)] and PCR using 1S6110 insertion element of Mycobacterial tuberculosis [(7)]. All these tests also have limitation of either sensitivity or cost. Tuberculin skin tests must be interpreted with a scientific understanding of the possibilities of false positive and false negative tests [(8)]. Also, newer modalities are less available and require trained personnel which are not possible in developing countries [(9)].

Hence, there is need of precise and faster diagnosis for patients attending hospitals. Accordingly, there develops a considerable interest in the development of tests based on humoral immune response and biochemical response of body towards tuberculosis infection. These tests appear to be a promising approach for the diagnosis of pulmonary as well as extra pulmonary TB.

Estimation of adenosinedeaminase activity in serum is an indirect biochemical test. It has been demonstrated that, the activity of adenosine

deaminase is increased in tuberculosis. Measurement of adenosine deaminase activity is very simple. It is a rapid test for early diagnosis of tuberculosis. It can differentiate pulmonary tuberculosis from other lower respiratory tract infections. However, very few studies have been carried out on adenosine deaminase[10]. Serum LDH activity is found to be increased in pulmonary tuberculosis[11]. However, to confirm the results from these studies, further studies are required in Indian context.

In the last three decades several immunological methods have been explored in the diagnosis of TB [12]. Several immunological assays with different modalities have been developed and tried for the detection of antigens, antibody, and circulating immune-complexes (CIC) in the body fluids.

In this study, it has been attempted to evaluate the detection potential of biochemical markers: Adenosine deaminase& Lactate dehydrogenase and immunological markers: Mycobacterium tuberculosis H_{37}Ra antigen & antibody assays in serum samples of pulmonary tuberculosis cases (confirmed by AFB smear) and respective controls.

Aim:

To compare the detection potential of biochemical and immunological markers: ADA, LDH and Mycobacterium tuberculosis H_{37}Ra ES-31 & EST-6 antigens & antibodies based ELISAs in pulmonary tuberculosis.

Materials & Methods:

The present study is considered as observational and non-interventional, case-control study carried out in the department of Biochemistry at Tertiary Care Hospital. The study was approved in institutional ethical committee meeting.

Inclusion criteria:

A total of 100 subjects were recruited in this study after obtaining their written consent in regional languages. The study was approved in institutional ethical committee meeting.

50 pulmonary tuberculosis cases were included in this study. All these cases were sputum positive for acid fast bacilli (AFB). A total of 50 age and sex matched control subjects were included in this study. Age and sex matched 30 diseased controls (pneumonia, malignancy and empyema) were included for comparison.20 were healthy controls, without past or present history of pulmonary or extra pulmonary TB or any other chronic ailments and with normal skiagram chest were included as healthy controls in this study. 30 diseased controls having similar clinical features are included in this study.

Exclusion criteria: Those having immune compromised status, heart disease, liver disease, typhoid, leprosy, lymphocytic lymphoma, Q fever pneumonia, extensive muscular injury, diabetes mellitus, infectious mononucleosis, kidney diseases, chronic malnutrition, organ transplantation and those on corticosteroid treatment were excluded from this study.

Collection of blood, processing and its storage: Blood samples about 5 ml was collected from the antecubital vein in a plain bulb from each individual after taking informed consent and kept for settling to coagulate for at least 10 minutes. The sera were separated by centrifuging the coagulated blood at 3000 rpm for 10 minutes and samples were processed as soon as possible.

Estimation of adenosine deaminase (ADA)

ADA was estimated by using commercial ADA-MTB kit manufactured by MICROXPRESS division of Tulip Diagnostics (P) Ltd. Procedure in a kit was followed.

Estimation of LDH

Total LDH was estimated by using commercial Erba Mannheim XL System Pack for LDH reagent. LDH was measured on XL-360 autoanalyser by Transasia Company in Clinical Biochemistry Laboratory of the Hospital.

Detection of MtbH37Ra ES-31 & EST-6 antigens and their antibodies

The *M. tuberculosis* H37Ra strain used in the study was procured from Tuberculosis Research Centre (TRC) Chennai, India. Tubercular antigens were detected by sandwich penicillinase ELISA and tubercular IgGantibodies were detected by indirect penicillinaseELISA method.

Results:

The analysis was done by using SPSS 17.0 version and graphpad prism 5.0 and the results were tested at 5% level of significance.

Out of the 50 patients of PTB serum ADA levels were above cut off (40 IU/L) in the 47 cases of pulmonary TB. Out of 30 disease controls in 3 cases serum ADA

levels were above cut off. No healthy control showed serum ADA above the cut off (Table 1).

Table 1: Detection potential of serum ADA in pulmonary tuberculosis

Group	No of samples assayed	No showing ADA levels above 40 IU/L
Pulmonary tuberculosis (PTB)	50	47 (96)
Disease control (DC)	30	3 (10)
Healthy controls (HC)	20	0 (0)

Figures shown in parenthesis are in per cent.

Out of the 50 patients of PTB, serum LDH levels were above cut off (450 U/L) in 47 cases. All 30 disease controls showed serum LDH levels above cut off.Out of 20 healthy controls 2 cases showed serum LDH above the cut off (Table 2).

Table 2: Detection potential of serum LDH in pulmonary tuberculosis

Group	No of samples assayed	No showing LDH levels above 450U/L
Pulmonary tuberculosis (PTB)	50	47 (94)
Disease control (DC)	30	30 (100)
Healthy controls (HC)	20	2 (10)

Figures shown in parenthesis are in per cent.

For $MtbH_{37}Ra$ ES-31 & EST-6 antigens & their antibodies, only those cases were considered positive which showed positive reaction in 1:300 & 1:600 dilutions respectively. Out of the 50 patients of PTB, serum $MtbH_{37}Ra$ anti ES-31 & anti EST-6 antibodies werepositively detected in 44 cases. Out of 30 disease controls sera of 4 cases showed $MtbH_{37}Ra$ anti ES-31 & anti EST-6 antibodies. Out of 20 healthy controls sera of 2 cases showed $MtbH_{37}Ra$ anti ES-31 & anti EST-6 antibodies (Table 3).

Table 3: Detection potential of serum $MtbH_{37}Ra$ anti ES-31 & anti EST-6 antibodies using $MtbH_{37}Ra$ ES-31 & EST-6 antigens

Group	No of samples assayed	Sera showing positive reaction in 1:600 dilutions
Pulmonary tuberculosis (PTB)	50	44 (88)
Disease control (DC)	30	4 (13.33)
Healthy controls (HC)	20	2 (10)

Figures shown in parenthesis are in per cent.

Out of the 50 patients of PTB, serum $MtbH_{37}Ra$ ES-31 & EST-6 antigens were positively detected by using respective antibodies in 40 cases. Out of 30 disease controls sera of 3 cases showed $MtbH_{37}Ra$ ES-31 & EST-6 antigens. Out of 20 healthy controls sera of 2 cases showed $MtbH_{37}Ra$ ES-31 & EST-6 antigens (Table 4).

Table 4: Detection potential of serum $MtbH_{37}Ra$ ES-31 & EST-6 antigens using $MtbH_{37}Ra$ anti ES-31 & anti EST-6 antibodies

Group	No of samples assayed	Sera showing positive reaction in 1:300 dilutions
Pulmonary tuberculosis (PTB)	50	40 (80)
Disease control (DC)	30	3 (10)
Healthy controls (HC)	20	2 (10)

Figures shown in parenthesis are in per cent.

Both sensitivity and specificity of serum ADA in detecting pulmonary tuberculosis were found to be 94%. Sensitivity and specificity of serum LDH in detecting pulmonary tuberculosis were found to be 94% and 36% respectively. Both sensitivity and specificity of antigens were found to be 88%. Sensitivity and specificity of antibodies were found to be 80% and 90% respectively. (Table 5)

Table 5: Comparative evaluation of biochemical and immunological markers in detection of pulmonary tuberculosis

	Biochemical Markers		Immunological Markers	
	ADA	LDH	Antigen	Antibody
Sensitivity (%)	94	94	88	80
Specificity (%)	94	36	88	90

Discussion:

Recent years have seen increased incidence of tuberculosis in both developing and industrialized counties [(13)]. In 1993, 111 years after Koch's epoch-making discovery, WHO took the unprecedented step of declaring tuberculosis a global emergency in an attempt to heighten public and political awareness.

Immunological markers appear to be a promising approach for the diagnosis of tuberculosis. Biochemical markers are easy to carry out, relatively faster & cheap and do not require specialized laboratory facilities. In this study we carried out ADA & LDH assays (biochemical markers) and Indirect

ELISA for detection of anti $MtbH_{37}Ra$ ES-31 & ES-6 antibodies and Sandwich ELISA for detection of $MtbH_{37}Ra$ ES-31 & ES-6 antigens (immunological markers) as to evaluate their comparative detection potential in pulmonary tuberculosis.

Demographic parameters (age and sex) were comparable in controls and cases included in this study; hence differences in other parameters in this study could not be attributed to differences either in age or gender of study population.

In this study, serum ADA levels were found to be significantly high in patients with pulmonary tuberculosis compared to healthy and diseased control group. Agarwal MK et al, 1991 [(14)] and Jhamaria JP et al, 1988 also found increased serum ADA level in patients with pulmonary tuberculosis.

Paliwal R et al, 1998 [(15)] evaluated the efficiency and usefulness of serum ADA activity for diagnosis of pulmonary tuberculosis and other non-tuberculosis respiratory conditions. The sensitivity and specificity of serum ADA, as a diagnostic test for pulmonary tuberculosis were found to be 100% and 88.6% respectively which can be considered as an important investigation to arrive at diagnosis. In our study, taking 40 U/L as cut off point, the sensitivity and specificity of serum ADA level was 94% and 97.14% respectively. However, Jhamaria P et al, 1988 [(16)] using 33 U/L as cut off limit had found 100% specificity and 98% sensitivity of serum ADA level for detection of pulmonary tuberculosis. Serum ADA was found as selective marker of immune stimulation in tuberculosis but not in other non tuberculous conditions in comparison to the serum ADA level of pulmonary tuberculosis patients with the patients of non tuberculous pulmonary diseases and healthy individuals [(17)]. In a similar study carried out by Lakshmi et al. 1992 with cut off values of 30 U/L the sensitivity and specificity of serum ADA in PTB was found to be 87% and 90% respectively [(18)].

However, elevated levels of ADA have been reported in other diseases involving stimulation of cell mediated immunity. According to Giblett et al. 1972, [(19)] a fully functioning cell mediated immune response is dependent on normal lymphocyte metabolism which is, in part, regulated by the purine salvage enzyme, adenosine deaminase. Therefore we also get increased ADA activity in disease controls in which cell mediated immunity was stimulated. But the increase in ADA was not as high as in tuberculosis.

To our knowledge, LDH in tuberculosis has not received much emphasis in the literature. Tissue necrosis, a characteristic feature of tuberculous granulomas, provides a ready explanation for elevated LDH values [(20)]. Serum lactate dehydrogenase activity is increased in pulmonary and numerous extra pulmonary diseases [(21)]. In this study, we did not get significant difference between LDH activity in PTB, other non-tubercular conditions included as disease controls compared to each other but the serum LDH levels were comparatively raised when compared to healthy controls. These results were similar to study carried out by Quist J & Hill A[(22)]. Taking 450 IU/L of serum LDH as an upper normal limit in healthy individual, serum LDH was able to detect pulmonary tuberculosis with a sensitivity of 94% and specificity of 51.43%. Above results indicate that serum LDH has good sensitivity in detecting PTB cases but with compromised specificity. These findings strengthen the established general view that LDH is a non-specific biochemical marker [(23)].

Vast data is available in the literature on immunodiagnosis of pulmonary tuberculosis reporting the sensitivity ranging from 31-81% and specificity 78-100% using PPD antigen [(24)].Various studies carried out earlier have reported the potential of $M.tbH_{37}Ra$ excretory secretory antigens in diagnosing pulmonary tuberculosis [(25)] [(26)]. The proteins of low molecular weight 3-9 and 26-35 kDa have been reported to possess marked stimulatory properties [(27)]. Tuberculosis patients produce a wide spectrum of humoral response at different stages of the disease. These variations in antibody response may be due to differential antigen expression in different disease condition. In the present study the immunological response to ES-6 antigen and its affinity purified anti ES-6 antibody was analyzed in pulmonary TB.

In the present study, we attempted to evaluate the comparative detection potential of biochemical (ADA & LDH) and immunological markers ($MtbH_{37}Ra$ ES-31 & EST-6 antigens and their antibodies) in pulmonary tuberculosis which in fact has not been reported earlier. Results in our study clearly showed that ADA has the greater sensitivity and specificity over the other markers in detecting pulmonary tuberculosis. LDH has good sensitivity but it lacks specificity, hence considered as a non-specific marker for tubercular detection. $MtbH_{37}Ra$ ES-31 & EST-6 antigens & their antibodies have sensitivity and specificity in between other two markers under this study.

Conclusion:

Comparing all four markers, it could be envisaged that ADA has better detection potential over other markers in pulmonary tuberculosis, especially in cases where it is difficult to obtain sputum specimens. This study is carried out at tertiary care hospital in India. Further such studies are required at different locations and over different population to generalize the results of this study.

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