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Evaluation of Serum Adenosine Deaminase (ADA) Values for Detection of Pulmonary and Extra-pulmonary Tuberculosis

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Abstract

Introduction: There is need of precise and faster diagnosis for pulmonary (PTB) and extra-pulmonary tuberculosis (EPTB). Accordingly, there develops a considerable interest in the development of tests based on biochemical response of body towards tuberculosis infection (i.e. estimation of serum adenosine deaminase). These tests appear to be a promising approach for the diagnosis of pulmonary as well as extra pulmonary TB.

Materials & Method: Total 150 individuals recruited for this study, out of which 50 cases each of PTB and EPTB. 50 age and sex matched controls were included, out of which 15 were healthy controls and 35 were diseased controls. Serum adenosine deaminase (ADA) was estimated by using commercial ADA-MTB kit.

Results: Serum ADA concentration in patients with PTB & EPTB was found to be significantly higher ($p < 0.05$) as compared to diseased and healthy controls.

Conclusion: Our observations suggest that serum Adenosine deaminase (ADA) measurement has a good detection potential for PTB & EPTB.

Keywords: Adenosine Deaminase, Pulmonary and Extra-Pulmonary Tuberculosis.

Introduction

Tuberculosis is one of the oldest and widespread infectious diseases ravaging a public health and creating challenges in the developing countries affecting both the public health and economy. 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.[1] Diagnosis of tuberculosis can be very easily missed as the clinical features of tuberculosis can at times be extremely vague and non-specific. History of contact may not always be elicited due to the fear of stigmatization.[2] However, accurate diagnosis of the infection is necessary to monitor the effective chemotherapy and for preventing further spread of the disease. Although sputum examination is considered as the 'gold standard' and is a simple and relatively quick means of detecting active TB, its sensitivity is compromised because greater than $10^{(4)}$ bacilli per ml of sputum is required for reliable detection and sputum collection is difficult in childhood TB and not an ideal sample in case of extra pulmonary TB.[3,4] Several attempts have been made to improve the sensitivity and speed of detection of tubercle bacilli or its components by various modern techniques.[5-6] All these tests also have limitation of either sensitivity or cost. Also, newer modalities are less available and require trained personnel which are not possible in developing countries.[7] For definitive diagnosis of extra pulmonary tuberculosis, it requires an invasive procedure to get sample and not all patients agree for it.[8]

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 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 'Adenosine deaminase activity in serum is an indirect biochemical test. It has been demonstrated that, population of T lymphocyte increases in tuberculosis and ADA, an enzyme of purine catabolism, is present in lymphocyte (10 times higher than erythrocytes) so eventually the activity of adenosine deaminase is increased in tuberculosis. Some workers have already studied activity of adenosine deaminase in tubercular pleural effusion, tubercular lymphadenitis and tubercular meningitis; showed diagnostic specificity and sensitivity for tuberculosis ranging from 90% - 100%.[9,10] 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 and TBM from pyogenic meningitis and other CNS infections. However, very few studies have been carried out on adenosine deaminase.[11]

In this study, it has been attempted to evaluate the detection potential of biochemical marker, adenosine deaminase, in Serum samples of pulmonary and extra pulmonary tuberculosis cases (confirmed by AFB smear, culture and cytology examinations) and respective controls.

Aim

To assess the potential of serum adenosine deaminase in detection of pulmonary and extra-pulmonary tuberculosis

Materials and Method

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 150 subjects of various age groups attending Tertiary Care Hospital and District Tuberculosis Hospital were recruited in this study after obtaining their written consent in regional languages.

50 pulmonary tuberculosis cases were included in this study. All these cases were sputum positive for acid fast bacilli (AFB). Age and sex matched 20 diseased controls (pneumonia, malignancy and empyema) were included for comparison.

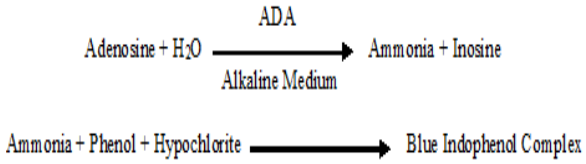
A total of 50 extra-pulmonary tuberculosis cases were included in this study. 20 cases of tubercular meningitis were included, defined on the basis of positivity of CSF microscopy or positive culture for AFB. 15 cases of tubercular pleural effusion were included, based on either positive pleural fluid microscopy or positive pleural fluid culture for AFB**.** 15 tubercular lymphadenitis patients based on their cytology findings were also included in this study.

A total of 50 age and sex matched control subjects were included in this study. Out of which 15 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. 35 diseased controls having similar clinical features are included in this study.

Exclusion criteria: Those having immunocompromised 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 & body fluid samples, processing and its storage: ADA was estimated by using commercial ADA-MTB kit manufactured by MICROXPRESS division of Tulip Diagnostics (P) Ltd.

Principle: Adenosine deaminase hydrolyses adenosine to ammonia and Inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form a blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of blue coloured indophenol complex formed is directly proportional to the amount of ADA present in the sample.



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.

There were no significant differences ($p>0.05$) in age (PTB: 42.28 ± 9.79; EPTB: 42.02 ± 11.34; HC: 42.05 ± 9.56; DC: 43.30 ± 12.01) and sex (PTB, EPTB, & HC: males & females 50% each; DC: males 53.33% & females 46.67%) between study subjects, disease controls and healthy controls.

We estimated serum ADA concentration in all the study subjects (PTB & EPTB) and controls (disease and healthy). We found significantly raised ($p < 0.05$) serum ADA concentration in PTB (44.67 ± 3.22) & EPTB (43.63 ± 3.01) cases compared to disease controls (DC for PTB: 26.20 ± 5.35; DC for EPTB: 28.84 ± 5.78) and healthy controls (13.32 ± 1.97) as shown in Table 1, 2 & Fig. 1, 2.

Out of the 50 patients each of PTB and EPTB, serum ADA levels were above the cut off value of 40 IU/L in the 47 cases in each group. 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 3).

We evaluated the detection potential of serum ADA employing 2X2 table in PTB & EPTB taking 40 IU/L concentration of ADA as detection cut off. The sensitivity and specificity of serum ADA in detecting pulmonary tuberculosis was found to be 94% and 97.14% respectively whereas the positive predictive and negative predictive values were 97.92% and 91.89% respectively. Accuracy of serum ADA in detecting PTB was found to be 95.29%. In detecting extra pulmonary tuberculosis, the sensitivity and specificity, positive and negative predictive values of serum ADA were 94% for each. Accuracy of serum ADA in detecting EPTB was found to be 94% (Table 4 & Fig. 3).

Table 1: Estimation of serum ADA concentration in pulmonary tuberculosis cases and controls Z-Test

Group	No.	Mean	Std. Deviation	Std. Error Mean	z-value	p-value
Pulmonary TB	50	44.67	3.22	0.45	-	-
Disease Controls	15	26.20	5.35	1.38	16.51	S,p<0.05
Healthy Controls	20	13.32	1.97	0.44	40.48	S,p<0.05

S: Significant, when compared to study group

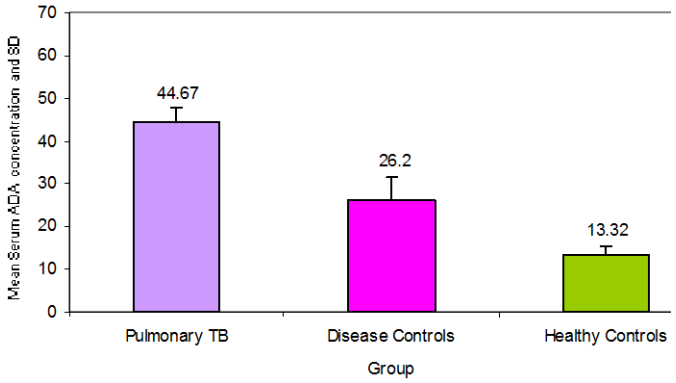


Fig. 1: Estimation of serum ADA concentration in pulmonary tuberculosis cases and controls

Bars represent (Mean ± SD) values in each group,

Table 2: Estimation of serum ADA concentration in extra pulmonary Tuberculosis cases and controls Z-Test

Group	No.	Mean	Std. Deviation	Std. Error Mean	z-value	p-value
Extra Pulmonary TB	50	43.63	3.01	0.42	-	-
Disease Controls	30	28.84	5.78	1.05	15.01	S,p<0.05
Healthy Controls	20	13.32	1.97	0.44	41.40	S,p<0.05

S: Significant, when compared to study group

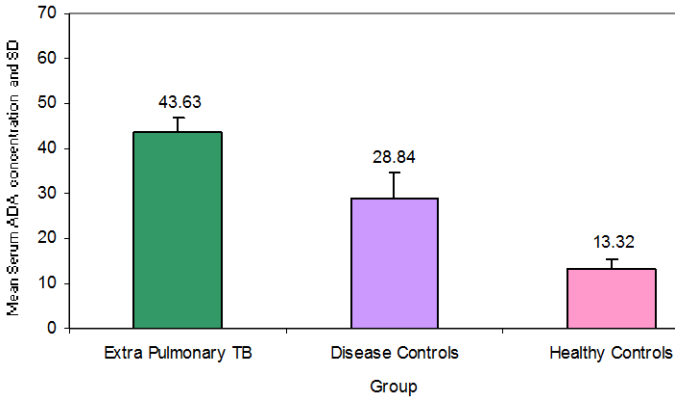


Fig. 2: Estimation of serum ADA concentration in extra pulmonary Tuberculosis cases and controls

Bars represent (Mean ± SD) values in each group,

Table 3: Detection potential of serum ADA in pulmonary and extra pulmonary tuberculosis

Group	No assayed	No showing ADA levels above 40 IU/L
Pulmonary tuberculosis (PTB)	50	47 (96)
Extra-pulmonary tuberculosis (EPTB)	50	47 (96)
Tubercular pleural effusion (TPE)	15	14 (93.33)
Tubercular meningitis (TBM)	20	19 (95)
Tubercular lymphadenitis (TLN)	15	14 (93.33)
Disease control (DC)	30	3 (10)
Empyema chest (EC)	05	1 (25)
Lung malignancy (LM)	05	0 (0)
Pneumonia (PN)	05	0 (0)
Viral meningitis (VM)	08	1 (12.5)
Reactive lymphadenitis (RLN)	07	1 (14.28)
Healthy controls (HC)	20	0 (0)

Table 4: Overall Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), Likelihood ratio (LR) and Accuracy of ADA in detection of tuberculosis

Group	Pulmonary tuberculosis	Extra-pulmonary tuberculosis
Sensitivity	94.00	94.00
Specificity	97.14	94.00
PPV	97.92	94.00
NPV	91.89	94.00
Likelihood Ratio	32.90	15.67
Accuracy	95.29	94.00
Fisher's Exact Test	P<0.0001 Significant	P<0.0001 Significant

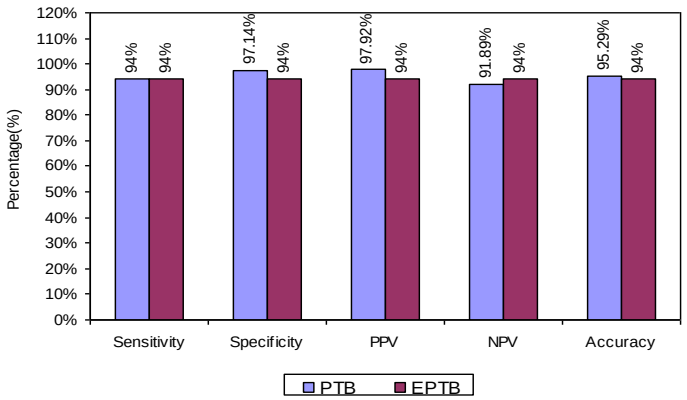


Fig. 3: Detection potential of serum ADA in pulmonary and extra pulmonary tuberculosis

Discussion

Countless millions of people have died from tuberculosis. Despite the availability of effective short course chemotherapy (DOTS) and Bacilli Calmette-Guerin (BCG) vaccine, the tubercle bacillus continues to claim more lives than any other single infectious agent.[12] The diagnosis of tuberculosis largely depends upon clinical, radiological, cytological and bacteriological examinations. Direct microscopy of sputum for bacilli has limitations; insensitive, not helpful in extra-pulmonary TB and difficult to obtain sputum sample in childhood tuberculosis cases. Culture method is cumbersome, time taking and liable to contamination. Biochemical markers are easy to carry out, relatively faster & cheap and do not require specialized laboratory facilities. In this study we estimated serum ADA to evaluate its detection potential in pulmonary and extra pulmonary tuberculosis.

Demographic parameters, age and sex, were not significantly different 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.

Adenosine deaminase (ADA; EC 3.5.4.4) is an enzyme present normally in most mammalian tissue, the activity being highest in organ containing many lymphoid tissues.[13] Its distribution in human organism is ubiquitous, but physiological role is especially important in lymphoid tissue. Its level is ten times higher in lymphocyte than erythrocytes and particularly in T lymphocytes with variation according to cellular differentiation.[14] Adenosine deaminase, called ADA by Spencer is an enzyme of purine catabolism. ADA catalyzes the hydrolytic cleavage of adenosine irreversibly, converting it into inosine and ammonia.

Various studies carried out on ADA in different continents have revealed different values for normal human serum ADA level at 37^0C. Low serum ADA levels have been reported by Schwartz et al, 1959[15] (12.49 ± 2.50 U/L) and Krawezynski et al, 1965[16] (13.14 ± 4.28 U/L). Higher serum ADA level was reported by Giusti[17] (17.05 ± 3.75 U/L) and Jhamaria JP et al, 1988 (19.09 ± 2.99 U/L).[18] However, serum ADA level in healthy control group reported by Krawezynski et al, 1965 (13.14 ± 4.28 U/L) was almost at par with our study. Inter study variation in ADA levels may be attributed to the differences in the ethnicity of study population.

In this study, serum ADA levels were found to be significantly high in patients with pulmonary tuberculosis compared to healthy control group. Agarwal MK et al, 1991 and Jhamaria JP et al, 1988 also found increased serum ADA level in patients with pulmonary tuberculosis and patients with non-tubercular pulmonary diseases.[19] But increase in serum ADA level was much higher in pulmonary tuberculosis cases (44.67 ± 3.22 U/L) compared to non-tubercular pulmonary diseases (26.20 ± 5.35 U/L). Similarly in extra pulmonary tuberculosis, we observed comparatively higher serum ADA activity in EPTB cases (43.63 ± 3.01) compared to healthy controls (13.32 ± 1.97), reason being the same as discussed above in case of the pulmonary tuberculosis.

Our results were similar to those observed in previous studies carried out.[20]

However, elevated levels of ADA have been reported in other diseases involving stimulation of cell mediated immunity. According to Giblett et al. 1972, 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.[21] 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.

Conclusion

The current resurgence of tuberculosis worldwide and particularly in developing countries has created a need for cheaper and more effective diagnostic technique. X-ray and acid fast stain, the two most common methods of diagnosis of tuberculosis in developing world, are not effective in diagnosing paucibacillary and extra pulmonary tuberculosis. Our observations suggest that serum Adenosine deaminase (ADA) measurement has good detection potential for PTB & EPTB. However, further such studies are required at different locations and over different population to generalize the results of this study.

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