Clinico-diagnostic and prognostic markers of pulmonary tuberculosis – A case control study

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Abstract

Introduction: India continues to have the highest number of tuberculosis (TB) cases in the world and around 2 million cases are reported in India every year. Present study was undertaken to assess the usefulness of serum adenosine deaminase (ADA) and C-reactive protein (CRP) as diagnostic and prognostic marker of pulmonary tuberculosis.

Materials and methods: Present study comprises of 72 subjects of which, serum ADA was estimated in 36 confirmed pulmonary TB patients and 36 control group. All 36 patients were treated with anti tubercular therapy for six months and again the serum ADA was estimated. Along with that body mass index (BMI), erythrocyte sedimentation rate (ESR), peripheral blood leukocyte and lymphocyte counts were measured.

Results: In our study serum ADA levels at the beginning of the treatment was 47.83 ± 12.71 IU/L and in controls was 23.60 ± 5.19 IU/L. The levels of serum ADA were significantly higher ($p < 0.001$) in the pulmonary tuberculosis patients than in controls. C-reactive protein was significantly increased ($p < 0.001$) in pulmonary TB cases as compared to controls.

The mean serum ADA levels after completion of treatment was 28.22 ± 6.04 IU/L. The difference in serum ADA levels between before treatment and after treatment was statistically significant ($p < 0.001$). There is also a significant difference in the BMI, CRP, ESR levels and peripheral leukocyte and lymphocyte count.

Conclusion: The estimation of serum ADA can be used as noninvasive and rapid diagnostic and prognostic marker of pulmonary tuberculosis. Estimation of CRP helps as an adjuvant supportive diagnostic tool in pulmonary tuberculosis.

Key words: Serum adenosine deaminase; C-reactive protein; pulmonary tuberculosis.

Introduction

Tuberculosis (TB) continues to be a major public health problem in the world and also in developing countries like India. It is the major cause of mortality and morbidity worldwide. The severity of the diseases can be judged by the fact that it affects all ages, particularly economically productive age groups of 15 to 45 years. No other disease has so much socio-economic health significance as tuberculosis in a country like India. Around 2 million new cases annually, making it the highest TB burden country in the world [1]. According to WHO report reveals that 9.4 million new TB cases including 4 million smear positive cases and 1.4 million HIV positive cases have been reported [2]. It remains the most common serious opportunistic infection and is the leading cause of death in HIV infected adults. It is a chronic infectious disease caused by Mycobacterium tuberculosis [3]. The disease mainly affects lungs and

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cause pulmonary tuberculosis

Early and accurate diagnosis is the prior critical step in controlling TB. Any person with productive cough more than two weeks, evening rise of temperature and weight loss should be evaluated for TB. Despite the presence of standard diagnostic methods, diagnosis of TB is still problematic. Although the diagnostic keys of pulmonary TB are good old methods like based on clinical signs and symptoms, chest radiograph findings, AFB in sputum smears and culture and isolation of bacilli in sputum sample. Even though chest radiograph is done routinely in all institutions it is not the specific for pulmonary TB [4]. The sputum smear is positive only if bacilli should be more than 10,000/ mL of sputum, the sensitivity of sputum smear is only 40-70%. Culture and isolation of bacilli is a more sensitive method among all other methods but it may take up to four to eight weeks, delay is unacceptable in emergency situations [5]. However, molecular or nucleic acid amplification test (NAAT) and polymerase chain reaction (PCR) is a rapid diagnostic methods for pulmonary TB but it is not cost-effective and only few centers use it [6]. Detection of smear positive cases is the highest priority in TB control programme, as these cases are infectious and contribute substantially to the transmission of disease.

Thus it is necessary to find faster methods with higher sensitivity, different methods are tried like biochemical tests, genetic and serodiagnostic tests for rapid and accurate diagnosis of tuberculosis. Among such biochemical tests measurement of serum adenosine deaminase (ADA) activity can be used for rapid and early diagnosis and prognosis of tuberculosis.

The increase in serum ADA levels and its value for early diagnosis has been shown in many studies [7-9], where cell mediated immunity is stimulated. Many studies have confirmed the high sensitivity and specificity of ADA for early diagnosis of extra pulmonary TB such as tuberculous pleurities, pericarditis and meningitis. The studies also showed decrease of serum ADA activity after completion of treatment [10]. C-reactive protein (CRP) a positive acute phase protein. Levels of this protein increased in pulmonary tuberculosis patients and significantly decrease after treatment.

Hence the present study was undertaken to compare serum ADA and CRP levels between the normal healthy controls and pulmonary tuberculosis patients and also to compare the serum ADA and CRP levels in pulmonary tuberculosis patients before and after treatment.

Material and methods

Study was conducted in the department of Biochemistry at Navodaya medical college and Hospital research centre, Revised National Tuberculosis and Control Programme a Directly Observed treatment-Short course Chemotherapy centre, Raichur, Karnataka, India in the year 2010. Present study comprises of 72 subjects out of which 36 healthy controls and 36 pulmonary tuberculosis patients. Informed consent was taken from all the study participants. The study was approved by the institutional ethical committee.

History and clinical examination of all the participants was done. Clinically, microbiologically and radiographyically confirmed new cases of pulmonary tuberculosis were included in the study. Healthy individuals as controls were selected from volunteers among blood donors and hospital staff. Patients suffering from extra pulmonary tuberculosis, enteric fever, leprosy, infectious mononucleosis, HIV, diabetes, hypertension, chronic diseases and other respiratory disorders are excluded from the present study. About 5 mL of venous blood was collected under aseptic precaution from cubital vein. 3 mL is used for biochemical parameters and 2mL with EDTA sample used for ESR and cell count. All the parameters are estimated on the same day.

Serum ADA, CRP, ESR, peripheral blood leukocyte and lymphocyte count was carried out for all participants. Serum was separated by centrifugation and the serum ADA estimated within 2-4 hours. Serum ADA was estimated by Giusti and Galanti method of enzymatic analysis [11] (E) using ADA-MTB kit from Microexpress a division of Tulip diagnostics (P) Ltd. Optical density (OD) measured at 570-630 nm in spectrophotometer, intensity of blue color directly proportional to the amount of ADA present in the given sample and expressed as IU/dL. C-reactive protein (CRP) by Turbidimetric immunoassay [12] from Erba.

All 36 patients came for follow up study, who had taken antitubercular therapy (ATT) for six month according to RNTCP, DOTS regimen and again

serum ADA, CRP, ESR and peripheral blood leukocyte and lymphocyte count was estimated.

Stastical analysis: All the results were expressed as mean ± SD and student't' test (paired) applied for quantitative data using OPEN EPI software. The p value ($p < 0.001$) was considered as statistical significance.

Results

The demographic characters are shown in table-1. Serum ADA, CRP, ESR, leukocyte and lymphocyte count significantly increased in cases than controls where as BMI was significantly decreased in cases than controls table-2.

Table-3 shows the mean values of different parameters before and after treatment. In cases there was a significant decrease in the serum ADA activity after the completion of antitubercular therapy ($p < 0.0001$) as compared to before treatment. There is decrease in CRP, ESR and leukocyte count and a slight increase in BMI and lymphocyte count.

It was observed that there was a difference in the serum ADA levels between after treatment (six month) therapy and control group (p=0.01).

Table No 1. Table showing the demographic characters of controls and cases

Characters		Controls	Cases
No of subjects		36	36
Sex	Male	23	29
	Female	13	07

Table No 2. Comparison of biochemical parameters in controls and cases using unpaired 't' test

Parameter	Controls	Cases	t	P	
BMI in Kg/m^2	21.7 ± 2.3	17.7 ± 1.8	8.49	0.0001	HS
ADA in IU/L	23.6 ± 5.19	47.8 ± 12.71	10.58	0.0001	HS
CRP in mg/L	8.56 ± 1.51	72.97 ± 11.4	33.5	0.0001	HS
ESR after 1hr	14.3 ± 4.54	59.5 ± 9.78	25.13	0.0001	HS
Leucocytes $/mm^3$	8544 ± 1239	11034 ± 1619	7.32	0.0001	HS
Lymphocytes $/mm^3$	1696 ± 310	1718 ± 266	10.96	0.001	S

HS = Highly sensitive S = Sensitive

Table No 3. Change in biochemical parameters after ATT in cases using paired 't' test

Parameter	Before treatment	After treatment	t	P	
BMI in Kg/m^2	17.7 ± 1.8	19.58 ± 2.32	5.16	0.0001	HS
ADA in IU/L	47.83 ± 12.71	28.22 ± 6.04	11.86	0.0001	HS
CRP in mg/L	72.97 ± 11.4	9.79 ± 2.84	32.49	0.0001	HS
ESR after 1 hr	59.5 ± 9.78	22.69 ± 6.38	20.02	0.0001	HS
Leucocytes /mm^3	11034 ± 1619	8171 ± 1033	14.31	0.0001	HS
Lymphocytes /mm^3	1718 ± 266	1813 ± 331	6.81	0.001	S

HS = Highly sensitive S = Sensitive

Discussion

In present study there was significant increase ($p < 0.001$) in serum ADA levels in pulmonary TB cases as compared to controls. The findings are in accordance with the study of Mishra OP [13], Kuyaku N [14] and Meena verma [15]. Adenosine deaminase an enzyme of purine catabolism belongs to hydrolase group (E C: 3.5.4.4 - Adenosine aminohydrolase). It is involved in the maturation, propagation and differentiation of lymphocytes mainly T-lymphocytes, hence high concentration of ADA activity is found in lymphocytes. The serum ADA level increases in diseases result from the intracellular microorganisms as well. ADA is produced after alveolar macrophages are infected by M. tuberculosis and is determined in serum during the active pulmonary TB. Lymphocytes, particularly T-lymphocytes have significant roles in the control of tuberculosis infection, in which lymphocytes turnover increases [16].

ADA is an enzyme that increases in TB because of stimulation of T-cell lymphocytes by mycobacterial antigen. It is a sensitive marker of cell mediated delayed immune response. As it is well known that increase in the serum ADA levels in disease is as a result of lymphocytes and macrophages turnover [17].

According to Mishra OP [13] evaluated serum ADA levels in children with confirmed tuberculosis (pulmonary, peritoneal, meningeal and bone) and healthy controls showing significant increase in serum ADA in tuberculosis compared to controls ($p < 0.001$). Kuyucu [14] estimated ADA level in the serum of children with tuberculosis was significantly higher than that of healthy children and concluded that serum ADA activity is a useful diagnostic tool in childhood pulmonary tuberculosis and in the tuberculosis diagnosis the cut-off value of serum ADA level was declared as 53.76 IU/L.

The association between TB and malnutrition has been recognized for long time. The malnutrition may predispose to TB and in turn TB also causes malnutrition [18]. The nutritional indicator BMI is significantly deficit in patients. In the present study BMI was significantly lower in cases than compared to controls ($p < 0.0001$).

Serum acute phase reactants CRP and ESR significantly increase ($p < 0.001$) in patients with pulmonary TB than compared to healthy controls. Sukesh R [19] assessed the serum CRP in 100 pulmonary tuberculosis patients and reported that serum CRP levels were significantly higher in smear positive group compared with smear negative group.

The total leukocyte counts were significantly higher in cases compared to controls ($p < 0.0001$) these are in favor of other studies Zafer Kartaloglu [20] whereas total lymphocyte count in cases and controls not that much differ.

In our study all 36 pulmonary TB cases were followed after completion of six month of antitubercular therapy. There was a significant decrease ($p < 0.0001$) in the serum ADA levels. Our findings are in accordance with the study of Collazos [21], Altas [10] and Zafer kartaloglu [20].

Collazos [21] performed a prospective follow up study of 25 cases of pulmonary and/or pleural tuberculosis with a normal immune response for a period of six months after initiation of treatment. There was a significant decline in the serum ADA values during the first two months in the patients as a whole followed by stabilization of the serum ADA activity. Perhaps this decrease might reflect the normalization of the altered lymphocyte turnover induced by tuberculosis. This indicates the response to the therapy. Altas [10] emphasized the importance of serum ADA levels in diagnosis and follow up of pulmonary tuberculosis and monitoring the efficiency of therapy. Zafer Kartaloglu [20] studied 35 patients with smear positive pulmonary tuberculosis. The levels of serum ADA were significantly higher in patients than in controls. They have observed serum ADA levels in tuberculosis patients showed a slight elevation in the first month but decreased during treatment in parallel with the effectiveness.

Other parameters like BMI increases slightly with the improvement of general health after the completion of the treatment. The incidence of pulmonary TB decreased with increasing BMI. Studies reported a slight decreasing mortality of TB seen with increasing BMI [22]. Acute phase reactants like CRP, ESR decreased significantly ($p<0.0001$) after the completion of the treatment. Our findings are in statement with that of Immanuel C [23] and Bajaj G [24]. Fall in these levels correlated with clinical response to therapy. They concluded that CRP can serve as a sensitive indicator of activity of the disease. In 1966, Lotfali H [25] evaluated CRP in pulmonary tuberculosis patients and observed the increased CRP in pulmonary TB patients and concluded that it is a proper test for evaluation and prognosis of pulmonary TB. CRP returns to normal faster than an elevated erythrocyte sedimentation rate (ESR).

The peripheral smear abnormalities reverted to normal with antitubercular treatment [26]. There is significant decrease ($p < 0.001$) in total leukocyte count and total lymphocyte count significantly increased after the completion of treatment.

Limitations of the present study were the small sample size and the follow up of all the patients was not possible. To trace the transfer TB cases from one centre to another health centre. Further studies are required with large sample size so that the serum ADA cut-off value can be determined for this ethnic group.

Conclusion

Assessment of serum ADA levels help in the early diagnosis of pulmonary tuberculosis. Estimation of serum ADA activity is a simple, rapid, non-invasive and relatively less expensive method and particularly helps in the diagnosis of smear negative AFB cases. So it should find a place in routine laboratory investigation. Estimation of serum ADA before and after treatment helps to evaluate the prognosis and response to therapy and also aids in objective assessment of the efficiency of chemotherapy used in the treatment.

References

1.World health organization (WHO). Global tuberculosis control Geneva, 2008: Surveillance, Planning, Financing. WHO/HTM/TB/2008.393.

2.Park K. Epidemiology of Communicable Diseases, Tuberculosis: In: Park's textbook of Preventive and social medicine, 20th edition. Banarsidas Bhanot publishers, 2009:159-176.

3.Betty A. Forbes, Daniel F. Sahm, Alice S. Weissfeld, Bailey and Scott's Diagnostic Microbiology, 11th edition. Mosby, An affiliate of Elsevier, 1998: 538-571.

4.Stead WW, Lofgren JP, and Warren E, Thomas C. tuberculosis as an endemic and nosocomial infection among the elderly in nursing homes. N. Engl. J Med 1985; 312: 1483-1487.

5.Kayacan O, karnak D, Delibalta M, Beder S, Karaca L, Tatkak H. Adenosine deaminase activity in bronchoalveolar lavage in Turkish patients with smear negative pulmonary tuberculosis. Respir Med 2002; 96(7): 536-541.

6.Richeldi L, Barnini S, Saltini C. Molecular diagnosis of tuberculosis. Eur Respir J Suppl 1995; 20: 689-700.

7.Lakshmi V, Rao RR, Joshi N, Rao PN. Serum Adenosine deaminase activity in Bacillary or Paucibacillary pulmonary tuberculosis. Indian Journal of Pathology and Microbiology 1992; 35(1): 48-52.

8.Ungerer JPJ, Oosthizen HM, Retiez JH. Significance of ADA activity and its isoenzymes in tubercular effusions. Chest 1994; 106: 33-77.

9.Balasaniants GS, Titarenko OT, D'iakova MN. Diagnostic and prognostic significance of adenosine deaminase in acutely progressive pulmonary tuberculosis.Article in Russian. Probl Tuberk 2001; (8): 46-49.

10.Altas F, Uslu S, Moral H, Aiatas O, Metinatas M, Erginel S, Uegun I. Serum adenosine deaminase activity in pulmonary tuberculosis. Tuberk toraks 2003; 51(3): 277-281.

11.Giusti Galanti. Adenosine deaminase. In: Bergmayer HU. Methods of Enzymatic analysis. 2nd edition. Newyork: 1092-1099.

12.Lars-Olof Hanson et al. Current opinion in infectious Diseases. 1997; 10: 196-201.

13.Mishra OP, Ali Y, Nath G, Das BK. Adenosine deaminase activity and lysozyme levels in children with pulmonary tuberculosis. Journal of Tropical pediatrics 2000; 46: 175-178.

14.Kuyucc N, Karakurt C, Bilaloglu E, Karacan C, Tenzic T. Adenosine deaminase in childhood pulmonary tuberculosis: Diagnostic value in serum. Journal of Tropical Pediatrics 1999; 45: 245-247.

15.Meena verma, Sanjeev narang, Ashish Moonat and Akshara Verma. Study of Adenosine deaminase activity in pulmonary tuberculosis. Indian Journal of Clinical Biochemistry. 2004; 19(1): 129-131.

16.Conde MB, Marinho SR, Pereira F, Silva LJR, Sand MHF, Sales CL et al. the usefulness of serum ADA_2 activity in adults in the diagnosis of pulmonary TB. Respiratory Medicine 2002; 56: 607-610.

17.Moss DW, Henderson R. Clinical Enzymology. In: Burtis CA, Ashwood ER, Tietz textbook of clinical enzymology. 3rd edition. 1998; 617-721.

18.Macallan DC. Malnutrition in tuberculosis. Diagnostic microbiology and Infectious Disease. 1999; 34:153-157.

19.Sukesh Rao, Bernhrdt, Vidya. Serum C-reactive protein in pulmonary tuberculosis: correlation with bacteriological load and extent of disease. Infectious diseases in Clinical Practice 2009; vol 17: issue 5, 314-316.

20.Zafer Kartaloglu, O Okutan, E Bozkanat, M Harun, Ahmet Ilvan. The course of serum adenosine deaminase levels in patients with pulmonary tuberculosis. Med Sci Monit 2006; 12(11):CR476-480.

21.Julio Collazos, Pedro E, Jose M, Eduardo M and Fernando I. Sequential evaluation of serum adenosine deaminase in patients treated for tuberculosis. Chest 1998; 11: 432-435.

22.Tvedal A. Body mass index and incidence of tuberculosis. Eur J Respir Dis. 1986 Nov; 69(5):355-362.

23.Immanuel C, Acharyulu GS, Kannapiran M, Segaran R, Sarma GR.Acute phase proteins in tuberculous patients. Indian J Chest Dis Allied Sci. 1990 Jan-Mar; 32(1):15-23.

24.Bajaj G, Rattan A, Ahmad P, Prognostic value of C reactive protein in tuberculosis. Indian Pediatr. 1989 Oct; 26(10):1010-1013.

25.Lotfali H, Jalal YD, Shiraz. C-reactive protein in pulmonary tuberculosis. Diseases of Chest. Dec 1966; Vol 50 (6).

26.Singh KJ, Ahluvalia G, Sharma SK et al. Significance of haematological manifestations in patients with tuberculosis. J Assoc Physicians India. 2001; 49: 790-794.

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