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Role of serum ADA in diagnosis of pulmonary tuberculosis

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Abstract

Around the world, TB is one of the best 10 reasons for death and the main source from a solitary infection agent which is above HIV/AIDS. A large number of individuals keep on falling debilitated with TB every year. Here we estimated the serum ADA levels in pulmonary tuberculosis to uncover any association of high serum ADA levels with PTB for diagnosis. The data is entered in the Excel spreadsheet, then statistically analyzed using SPSS 16.0 software. The tests like, t-tests, ANOVA etc are applied to analyse the data. The significant level was evaluated for p- value of less than 0.05. we got the mean serum adenosine deaminase level in pulmonary tuberculosis (25.78± 5.71 IU/ml) and in sputum smear negative patients (20.65 ± 3.41 IU/ml) was significantly more than controls (19.9 ± 8.71 IU/ml). The mean serum ADA was found to be maximum (25.78 +/- 8.71 U/L)for sputum positive tuberculosis and minimum (19.9 +/- 3.41 U/L) in healthy control group and this difference of serum ADA is statistically significant(p value = 0.012). The cut-off value for serum ADA is 25 U/L, Serum ADA sensitivity was 53.33%, Serum ADA specificity was 52.51%, and Serum ADA positive predictive value was 45.71 %. This data revealed that increase in serum ADA level increases in active pulmonary tuberculosis is more than sputum smear negative patients but due to poor sensitivity and specificity it cannot be used in establishing diagnosis in sputum smear negative patients. So this could be an useful strategy in diagnosis of pulmonary tuberculosis.

Keywords: Active pulmonary tuberculosis, sputum smear negative PTB , Serum adenosine deaminase.

Introduction

Millions of people continue to fall sick with TB each year. In 2017, TB caused an estimated 1.3 million deaths (range, 1.2–1.4 million) among HIV-negative people and there were an additional 300 000 deaths from TB (range, 266 000–335 000) among HIV-positive people. Globally, the best estimate is that 10.0 million people (range, 9.0–11.1 million) developed TB disease in 2017: 5.8 million men, 3.2 million women and 1.0 million children[1]. As per the Global TB report 2017 the estimated incidence of TB in India was approximately 28,00,000 accounting for about a quarter of the world's TB cases [2]. A definite diagnosis of pulmonary tuberculosis can be made with the presence of acid fast bacilli on sputum smear examination of the patient. Chest X – Ray is non specific as many non-tubercular pulmonary disease mimics pulmonary tuberculosis . Problem arises when there is high clinical

suspicion of pulmonary tuberculosis but there is difficulty in establishing the diagnosis of PTB. ELISA, PCR and gamma interferon are very expensive tests, hence cannot be routinely used [3].

Adenosine deaminase (ADA) an enzyme involved in purine metabolism, is found ten times higher in concentration in lymphocytes than erythrocytes. ADA is a significant indicator of active cellular immunity . The level of serum ADA increases in various diseases in which cell mediated immunity is stimulated such as tuberculosis [4]. The level of ADA in tuberculosis is higher than any other non tubercular pulmonary diseases. Its sensitivity and specificity are very high. The serum ADA value is sufficiently useful in identifying those patients in whom the diagnosis of pulmonary tuberculosis should be actively considered [5,6]. Estimation of serum ADA is cost effective ,easily available and less time consuming, hence can be used in diagnosing pulmonary tuberculosis where it is difficult to establish the diagnosis by conventional methods [7]. In this study we have used the serum ADA levels in sputum smear positive pulmonary tuberculosis patients and sputum smear negative patients with clinical features suggestive of PTB to prove high levels of serum ADA in PTB patients and differentiate diseases mimicking PTB.

Materials and Methods

The present study was carried out in Department of Pulmonary Medicine, V.S.S. Medical College & Hospital, Burla. Patients included in the study were selected from OPD & IPD of Pulmonary Medicine department of V.S.S Medical College, Burla from september 2011 to August 2013. This study was an prospective case control study, where the inclusion criteria was bacteriologically or histologically proven TB patients, whose sputum smear was found negative comparison with a healthy control . Similarly the exclusion criteria was patient having HIV, organ transplanted patients, diabetes mellitus, Chronic kidney disease, treatment with corticosteroids, patients requiring surgical intervention (as it may introduce new infection and alter the result), Patient not giving consent.

A detailed clinical history was taken with emphasis on age, sex, caste, occupation, duration of symptoms and specific complaints like fever, chest pain, shortness of breath, expectoration, loss of appetite, weight loss and other constitutional symptoms. A clinical diagnosis was done after through physical examination and investigations like- CXR PA view, Lateral view if needed, sputum smear for AFB, routine laboratory investigations(CBC, DC, LFT ,RFT,FBS or RBS,HIV tests), ADA in serum, USG and CT in selected patients, blood culture in selected cases, Gram stain and culture of sputum in selected cases, histopathological studies as and when required. The Serum adenosine deaminase estimation was done by the spectrophotometry method described by Constine et al[8] . The data is entered in the Excel spreadsheet, then statistically analyzed using SPSS 16.0 software. The tests like, t-tests, ANOVA etc are applied to analyze the data.

Results

Age and gender

It was found that in sputum smear positive tuberculosis group (n = 30) majority of patients i.e 8 patients(26.6 %) belong to age group 56 – 65 years. In sputum smear negative group (n = 30) , majority of patients i.e 10 patients (33.3%) of patients belong to the age group 26 – 35 years. In healthy group (n = 10) majority of patients i.e 4 patients(40 %) belong to age group 46 – 55 years. In case of gender, majority of patients were males in all three groups . In sputum smear positive group (n = 30) 17 patients (56.6%) were males and 13 patients (43.3%)were females In Sputum smear negative (n = 30) 24 patients (80%) were males and 6 patients (20%) were females. In

healthy control (n = 10) 9 patients (90%) were males and 1 patient each (10 %) was a female.

Distribution of patients with respect to symptoms and clinical signs

In sputum positive tuberculosis group cough was the most common symptom in 24 patients (80%) , followed by expectoration in 21 patients(70%),weakness in 17 patients (56.6%) and loss of weight in 14 patients(46.6%). In Sputum smear negative group all the patients presented with fever i.e 30 patients (100%),followed by cough in 24 patients(80%),expectoration in 21 patients(70%) and chest pain in 17patients (56.6%). (Table. 1)

In my study , pallor was the most common clinical sign in in sputum positive tuberculosis group(93.3%) and group of nontubercular pulmonary diseases (90%) . In sputum positive tuberculosis group edema in 19 patients (63.3%), lymphadenopathy in 13 patients (43.3%) and icterus and clubbing in 5 patients(16.6%) were other common symptoms. In Sputum smear negative group other common symptoms were found to be cyanosis in 10 patients (33.3%) , edema in 8 patients (26.6%)and clubbing in 5 patients (16.6%).

Table 1: Distribution of patients with respect to symptoms.

	Sputum Positive Tuberculosis			Sputum Smear Negative		
SL.NO	SYMPTOMS	NO.OF PTS (30)	%	SYMPTOMS	NO.OF PTS (30)	%
1	FEVER	14	46.6	FEVER	30	100
2	COUGH	24	80	COUGH	24	80
3	EXPEXTORATION	21	70	EXPEXTORATION	21	70
4	CHEST PAIN	2	6.6	CHEST PAIN	17	56.6
5	BREATHLESSNESS	2	6.6	BREATHLESSNESS	11	36.6
6	HAEMOPTYSIS	5	16.6	HAEMOPTYSIS	3	10
7	WEAKNESS	17	56.6	WEAKNESS	7	23.3
8	LOSS OF WT.	14	46.6	LOSS OF WT.	4	13.3
9	NIGHT SWEATS	8	26.6	NIGHT SWEATS	0	0
10	LOSS OF APPETITE	14	46.6	LOSS OF APPETITE	0	0

Correlation and comparisons of mean serum ADA

The serum ADA was high in patients with increased percentage of lymphocyte count in differential count in both the groups. Here we observed that in sputum positive tuberculosis group and in Sputum smear negative group there was a positive correlation between the serum ADA and lymphocyte count (i.e high serum ADA in patients with high lymphocyte count) (Table. 2) .Pearson correlation = 0.227 (0 to 1). The mean serum ADA was found to be maximum (25.78 +/- 8.71 U/L) for sputum positive tuberculosis and minimum (19.9 +/- 3.41 U/L) in healthy control group and this difference of serum ADA is statistically significant(p value = 0.012). (Table. 3)

Table 2: Correlation of serum ADA with lymphocyte count (%) in DC.

Sputum positive tuberculosis		Sputum smear negative	
% of lymphocytes in DC	S.ADA in U/L	% of lymphocytes in DC	S.ADA in U/L
27	21	26	32.3
14	36	23	26.8
28	23.12	29	26.1
21	29	20	22.2
10	16	27	23.1
18	23.3	25	12.6
25	20.18	27	37.4
27	22.54	15	28.81
21	25.42	34	38
21	42.03	24	28
29	8.2	21	28
25	23.51	25	30
24	22.39	27	22.36
27	25.4	21	32.2
29	21.19	13	27.46
26	21.6	23	21.29
22	28.8	13	23
26	54.6	22	19.6
13	22.6	26	36.2
10	29.64	12	26
25	18.39	20	28.66
5	36.8	25	26.85

29	28.36	28	22.33
30	41	14	24.78
27	27.7	28	31.42
27	23.51	23	34.12
30	27	16	23.56
29	29.35	18	22.58
20	25.2	24	19.13
30	29.01	30	26.7

Table 3: Comparison of mean serum ADA

Sl. No	Groups	No .of pts	Mean Serum ADA in U/L	Standard Deviation
1	Healthy control	10	19.9	+/- 8.71
2	Sputum positive pulmonary TB	30	25.78	+/- 5.71
3	Sputum smear negative	30	20.65	+/- 3.41

Table 4: Cut off value of sputum positive tuberculosis and control

Cut off value	Sputum positive tuberculosis (n = 30)	Sputum smear negative and healthy control (n = 40)
Serum ADA >/=25 U/L	16	19
Serum ADA < 25 U/L	14	21

The cut-off value was calculated by taking the median values of all the three groups (70cases): The cut-off value for serum ADA is 25 U/L, Serum ADA sensitivity was 53.33%, Serum ADA specificity was 52.51%, and Serum ADA positive predictive value was 45.71 %. (Table. 4).

Discussion

ADA is an enzyme in the purine metabolic pathway, and is shown to consist of two isoenzymes (ADA1 and ADA2). The enzyme is scattered throughout the human body and its main physiological function is found in T-lymphocyte propagation and differentiation. The enzyme is higher in T-cells compared with B-cells with the ratio of 5-20 folds more [9]. In present study we evaluated total 70 patients were evaluated out of which 30 patients belonged to sputum smear positive group (group 1), 30 patients belonged to sputum smear negative group (group 2) and 10 were healthy control group (group 3). It was observed that in the age range of (16- 65) years , majority of patients(n=8, 26.6%) in group 1 were between (56-65)years ,in group 2 majority of patients(n=10, 33.3%) were between (26 – 35)years and in group 3 majority of patients(n=4,40%) were between(46 – 55) years [10].

In present study it was found that in all three groups majority were males , in group1(56.6%), group 2(80%), and in group3(90%) and this might be because of more exposure of males to the external environment. Our findings were similar to the observations obtained by Williamson et al [11], Jhamaria et al[12], and Woodruff et al (1963)[13]. No significant mean difference was observed in the serum ADA level in relation to age and sex among these patients. Deller et al[14], Jhamaria et al[12] had also found similar observation. Regarding the clinical presentations it was found that in group 1 cough (n=24, 80%) was the most common symptom followed by expectoration(n=21,70%) and weakness(n=17 , 56.6%), in group 2 fever(100%) was the most common symptom followed by cough(n=24,80%) and expectoration (n=21, 70%).

The most common clinical sign was found to be pallor in group 1(n=28,93.3%) and group 2 (n=27,90%). In group 1 other common clinical signs were edema (n=19,63.3%) and lymphadenopathy (n=13,43.3%). In group 2 the other common clinical signs were icterus and cyanosis (n=10,33.3% each)and edema(n=8,26.6%). In the present study it was found that there is a positive correlation (pearson correlation = 0.227) between the lymphocyte count and serum ADA i.e the serum ADA was high in patients with higher lymphocyte count ,as ADA is released mainly from the lymphocytes. Various workers

have found different values for normal human serum ADA level at 37 °c. Martinek, R.G. Clin. Chem.; 1963 - 5.9 ± 17.6 U/Lit[15]. Schwarts and Bodansky. Proc. Soc. Exptl. BioMed.;1959 - 12.49 ±. 2.50 U/Lit [16]. Krawczynski, J. et al -13.14 ± 4.28 U/Lit [17]. Giusti, G. and Gakis, B. Enzyme, 1971- 15.8 ± 3.7 U/Lit [18] .Giusti, G 1974 - 17.05 ± 3.75 U/Lit [19]. Jhamaria, J.P et al 1988 -19.09 ± 2.99 U/Lit [12]. However serum ADA level in control group reported by Guisti et.al (15.8 ± 3.7 U/L)[18] was almost at par with our study. In my study, serum ADA levels were found to be significantly high in patients with pulmonary tuberculosis compared to patients with sputum smear negative patients and control group and findings of our study was in agreement with the study of Agarwal MK et.al[20] and Jhamaria JP et.al[12] who also found increased serum ADA level in patients with pulmonary tuberculosis and patients with non tubercular pulmonary diseases. However the rise was much higher in patients of pulmonary tuberculosis.

Paliwal.R et.al [21] evaluated the efficiency and usefulness of serum ADA activity for diagnosis of pulmonary tuberculosis and other non tuberculosis respiratory condition, serum ADA levels were determined in 10 healthy, 90 patients with 65 pulmonary tuberculosis, 15 patients with suppurative lung disease and 10 with lung carcinoma. 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 a diagnosis. In this study, taking 25 U/L as cut off point, the specificity and sensitivity of serum ADA level was 52.5% and 53.33% respectively. The low sensitivity and specificity of my study might be due to less number of cases taken for the study. As determination of ADA is not costly or time consuming, should be done routinely, particularly if the diagnosis of tuberculosis is in doubt, and also to differentiate pulmonary tuberculosis from non tubercular pulmonary diseases.

Conclusion

In conclusion, serum ADA is significantly raised in pulmonary TB cases in comparison to sputum smear negative patients with clinical features suggestive of tuberculosis. Tuberculosis being an infectious disease is associated with high morbidity and mortality, so a high degree of clinical suspicion is required which needs newer diagnostic methods such as serum ADA estimation, which can be used routinely for early diagnosis and proper initiation of treatment. However to fully assess its diagnostic significance, it requires further clinical research.

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