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Estimation of serum adenosine deaminase level in patients of pulmonary tuberculosis in a tertiary care hospital in Chhattisgarh

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Abstract:

Introduction: Adenosine deaminase (ADA) is one of the major enzymes in purine metabolism. There are 2 isoforms of ADA: ADA1 and ADA2. The principal action of this enzyme is in immune system cells, the level of ADA in T-cell is 5-20 fold more than B-cell. The level of ADA elevates as the lymphocyte (T-cell) activity increase. Tuberculosis has been studied extensively with relevance of ADA levels and apart from serum, various body fluids as pleural, peritoneal, cerebrospinal fluids of patients of Pleural effusion, Ascitis and Tubercular Meningitis, has also its raised levels. Measurement of the level of (ADA) enzyme in body fluids is a helpful diagnostic tool. **Aim:** To study the serum Adenosine Deaminase Activity in patients of Pulmonary Tuberculosis and to evaluate the diagnostic significance of ADA activity in serum in these patients. **Material and Methods:** Present study was carried out in fifty patients of both the sexes with different ages suffering from Pulmonary Tuberculosis attending OPD and admitted in Medicine Wards in Pt. J.N.M.Medical College Hospital, Raipur C.G. 20 normal healthy individuals were included as control subjects. The diagnosis of Pulmonary Tuberculosis was established by clinical history, physical examination, suggestive radiological changes and presence of AFB on sputum smear examination. Sputum positivity is taken as diagnostic of Pulmonary Tuberculosis however sputum negative cases with suggestive radiological signs were also included in the study. Estimation of serum ADA of study and control groups was done by Galanti and Guisti methods by Spectrocolorimeter in Biochemistry Lab. **Results:** 50 cases of different types of pulmonary tuberculosis and 20 controls were studied for serum ADA levels. The mean serum ADA level in controls was 9.88 ± 0.47 U/L which was taken as the base line values. The mean serum ADA level in study group was 36.74 ± 2.83 U/L which is highly significant ($p< 0.001$). These values are significantly higher than the corresponding value obtained in the control group. Mean serum ADA levels in sputum positive cases was 38.32 U/L and in negative cases was 35.99 U/L. In Group I Infiltrative lesions 22 cases and mean serum ADA levels was 35.95 ± 3.051 U/L. Group II of Fibrocavitary tuberculosis 21 cases and mean serum ADA levels was 37.96 ± 2.167 U/L. Group III Tubercular consolidation there were 7 cases and mean serum ADA levels was 35.70 ± 3.009 U/L. Significantly higher serum ADA level ($p< .001$) was observed in each group as compared to controls. Radiologically in Group I minimal disease 20 cases and mean serum ADA level was 35.76 ± 3.109 U/L. Group II moderate advance disease 23 cases and mean serum ADA level was 37.07 ± 2.624 U/L. Group III far advance disease 7 cases and mean serum ADA levels was 38.45 ± 1.643 U/L. We observed that all radiologically positive cases of pulmonary tuberculosis had significantly ($p <.001$) higher serum ADA level as compared to control. **Conclusions:** There was no variation in the serum ADA levels of pulmonary Tuberculosis cases belonging to various age groups and either sex. In present study significantly higher values of serum ADA level was observed in presence of illness more than one month duration. It was raised significantly in both sputum positive as well as negative cases. ADA was raised significantly in all clinical groups of pulmonary Tuberculosis. It was observed that ADA was raised significantly in different radiological extent of disease. It was concluded that the serum ADA level estimation is a reliable and specific diagnostic test in various forms of Pulmonary Tuberculosis.

Key words: ADA- Adenosine Deaminase, AFB-Acid Fast Bacilli, TB-Tuberculosis

Introduction:

Tuberculosis (TB), which is caused by bacteria of the Mycobacterium tuberculosis complex, is one of the oldest diseases known to affect humans and a major cause of death worldwide [1]. An estimated 1.7 billion persons, one-third of the world's population, are infected with M. Tuberculosis [2]. A definite diagnosis of pulmonary tuberculosis can be made with the presence of acid fast bacilli on sputum smear examination of a patient. Problem arises when sputum smear result is repeatedly negative for acid fast bacilli. Chest skiagram provides only a probable diagnosis and culture for tubercle bacilli is a sophisticated and time consuming process. To overcome this difficulty, biochemical tests as ADA estimation in serum can be one which may help to confirm the diagnosis of pulmonary tuberculosis.

Adenosine Deaminase or ADA or adenosine aminohydrolase is an enzyme involved in purine metabolism. Adenosine deaminase (ADA) is a protein that is produced by cells throughout the body and is associated with the activation of lymphocytes, a type of white blood cell that plays a role in the immune response to infections [3]. Conditions that trigger the immune system, such as an infection by **Mycobacterium tuberculosis**, the bacteria that causes tuberculosis (TB), may cause increased amounts of ADA to be produced in the areas where the bacteria are present. There are 2 isoforms of ADA: ADA1 and ADA2. The principal action of this enzyme is in immune system cells, the level of ADA in T-cell is 5-20 fold more than B-cell. The level of ADA elevates as the lymphocyte (T-cell) activity increase [4].

In present study diagnostic usefulness of estimation of ADA activity in serum was planned by us in patients of parenchymal Pulmonary Tuberculosis.

Material and Methods:

Study was carried out in 50 patients of both the sexes with different ages suffering from Pulmonary Tuberculosis attending OPD and admitted in Medical Wards in Pt. J.N.M.Medical College Hospital , Raipur C.G. and 20 normal healthy individuals were included as control subjects.

Groups included:

1. Studygroup-50 cases of active pulmonary tuberculosis confirmed by positive sputum smears for AFB and in association with relevant clinical and radiological findings.
2. Control group-20 normal healthy individuals were included as control subjects.

The patients of the study group are divided into two group:

(A) Sputum smear positive cases

(B) Sputum smear negative cases

Each of these group is further divided depending upon the radiological extent of the disease according to the criteria laid down by national tuberculosis association of USA 1961.

(A) Sputum Smear Positive Cases.

(i) Minimal disease

(ii) Moderately advanced disease

(iii) Far advanced disease

B) Sputum Smear Negative Cases

(i) Minimal disease

(ii) Moderately advanced disease

(iii) Far advanced disease

Minimal Disease :- Minimal lesion are those which are non cavitary and do not exceed in total extent the area of one lung above the second chondrosternal junction and the spine of the fourth or body of the fifth vertebra.

Moderately Advance Disease :- Moderately advance disease is that which does not exceed the area of one lung and which has total cavitation diameter of less than 4 cm.

Far Advanced Disease :- Is more extensive than moderately advanced lesion.

Method:

The diagnosis of Pulmonary Tuberculosis was established by clinical history, physical examination, suggestive radiological changes and presence of AFB on sputum smear examination. Sputum positivity is taken as diagnostic of Pulmonary Tuberculosis however sputum negative cases with suggestive radiological signs were also included in the study.

Estimation of serum ADA of study and control groups was done by Galanti and Guisti methods by Spectrocolorimeter in Biochemistry Lab after separation of serum from blood of study group and control. This test is based on the release of ammonia during deamination of adenosine to inosine. This ammonia liberated during the deamination is chemically converted to Sodium hypochlorite and phenol in alkaline medium which has an intense blue color and its level is estimated by spectrocolorimeter which are proportional to the levels of ADA activity.

Results:

The study comprised of 50 cases of pulmonary tuberculosis, which included 33 males and 17 females. The control group had 20 individuals which included 12 males and 8 females belonging to same age as study groups.

Table 1 Showing distribution of 50 cases of pulmonary tuberculosis and controls

S.No.	Groups	Males		Females		Total	
		No.	%	No.	%	No.	%
1	Sputum positive group	12	24	4	8	16	32
2	Sputum negative group	21	42	13	26	34	68
	Total	33	66	17	34	50	100
	Controls	12	60	8	40	20	100

Table 2 Showing statistical comparison of serum ADA levels in control and study group

S.No	Group	No of cases	Serum ADA level				
			Mean U/L	S.D	Z	P	Significance
1	Control group	20	9.88	0.47			
2	Study group	50	36.74	2.83	64.72	<0.001	Highly significant

Statistically comparison of serum ADA levels in control group with study group. There was statistically significant (p <0.001) higher value of serum ADA levels in study group was observed as compared to control group.

Table 3 Showing statistical comparison of serum ADA levels in controls and sputum positive cases irrespective of radiological extent of disease

S.No	Group	No of cases	Serum ADA level				
			Mean U/L	S.D	Z	P	Significance
1	Controls	20	9.88	0.47			
2	Sputum positive group	16	38.32	1.65	66.8	<0.001	Highly significant

There was higher serum ADA level observed in sputum positive group as compared to control group which is (p <0.001) statistically highly significant.

Table 4 Showing statistical comparison of serum ADA levels in controls and sputum negative cases irrespective of radiological extent of disease

S.No	Group	No of cases	Serum ADA level				
			Mean U/L	S.D	Z	P	Significance
1	Controls	20	9.88	0.47			
2	Sputum negative group	34	35.99	2.98	50.00	<0.001	Highly significant

There was higher serum ADA levels observed in sputum negative group as compared to control group which is (p <0.001) statistically highly significant.

Table 5 Showing statistical comparison of serum ADA levels between sputum positive and sputum negative cases

S.No	Group	No of cases	Serum ADA level				
			Mean U/L	S.D	Z	P	Significance
1	Sputum positive group	16	38.32	1.65			
2	Sputum negative group	34	35.99	2.98	1.87	>10	Insignificant

The mean serum ADA level was insignificantly higher in sputum positive group as compared to sputum negative group. Difference of serum ADA levels between positive and negative group was (p>10) highly insignificant.

Table 6 Showing statistical comparison of mean serum ADA levels in relation to total duration of symptoms

S.No	Duration of symptoms	No of cases	Serum ADA level				
			Mean U/L	S.D	Z	P	Significance
1	< 1 month	19	35.26	1.28			
2	>1 month	31	37.02	2.18	3.57	< 0.01	Highly significant

Statistically highly significant (p <.01) level of serum ADA was observed in total duration of illness of >1 month as compared to total duration of illness of < 1 month.

Table 7 Showing statistical comparison of serum ADA levels in controls and various clinical groups of pulmonary tuberculosis

S.No	Groups	No of cases	Serum ADA level				
			Mean U/L	S.D	t	P	Significance
1	Infiltrative lesions	22	35.95	3.05	38.96	<.001	Highly significant
2	Fibrocavitary tuberculosis	21	37.96	2.17	56.88	<.001	Highly significant
3	Consolidation	7	35.70	3.00	30.94	<.001	Highly significant
	Control	20	9.88	0.47			

In statistical comparison of mean serum ADA level in various clinical groups of pulmonary tuberculosis in all three groups, there were(p< .001) highly significant serum ADA levels was observed as compared to control group.

Table 8 Showing statistical comparison of serum ADA levels in different groups of radiological extent of disease irrespective of sputum status

S.No	Groups	No of cases	Serum ADA level				
			Mean U/L	S.D	t	P	Significance
1	Minimal disease	20	35.76	3.10	26.31	<.001	Highly significant
2	Moderately advanced disease	23	37.07	2.62	45.84	<.001	Highly significant
3	Far advanced disease	7	38.45	1.64	73.04	<.001	Highly significant
	Control	20	9.88	0.47			

In statistical comparison of mean serum ADA level in different groups of radiological extent of disease irrespective of sputum status in all three groups there were (p< .001) statistically highly significant serum ADA levels was observed as compared to control group.

Discussion:

Adenosine deaminase is an enzyme of the purine catabolic pathway which catalyses the irreversible deamination of adenosine into inosine. Its major physiological role is related to the proliferation and differentiation of lymphocytes . Increased ADA levels in pulmonary tuberculosis may be due to a stimulation of cell mediated immunity. According to Giblett and Anderson, 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 [5]. Increased serum ADA activity, therefore, is also found in other diseases involving stimulation of cell mediated immunity such as typhoid fever, infectious mononeucleosis and bronchogenic carcinoma [6]. Other mechanisms may also be playing a part in tuberculosis to cause increase in serum ADA levels.

The present study consists of 50 cases and 20 controls and was carried out in Department of Medicine, Pt J.N.M. Medical College & Medical College Hospital, Raipur (C.G).

ADA Levels in PTB Cases and Control group

In our study mean serum ADA level in study group was 36.74 ± 2.83 U/L as compared to 9.88 ± 0.47 U/L in controls. Highly significant level of serum ADA ($p< 0.001$) was observed as compared to control. Bansal S K et al 1991 and Nagraja M V et al 1992 observed a mean ADA of23.38 ± 4.47 and 41.98 ±17.33 in their study of 86 and 30 cases respectively [7,8]. In a study by Saeed Aminiafshar et al there is significant rise in the serum ADA level of patients suffering from active pulmonary tuberculosis compared to the healthy controls with a P value of <0.0001, consistent with the results of our study [9]. Mishra et al also noticed raised serum adenosine deaminase activity in a group of 51tuberculosis cases compared to 20 healthy individuals [10]. Similarly in a study done by Agarwal et al the mean serum ADA level in patients of pulmonary tuberculosis was 38.48 ± 1.5634 U/ L in 36 cases of pulmonary tuberculosis as compared with mean value for serum ADA levels, in 38 healthy controls was 15.30 ± 0.2290 U/Lit. This value was significantly higher than the mean value for healthy controls ($p < .001$) [11].

Age and Sex Variation of ADA Levels

No variation of ADA Levels was observed in relation to age and sex in control as well as study group. Aliasghar et al 2013 also had similar observation [12].

Effect of duration of illness on ADA levels

In present study significantly higher serum ADA levels37.02 ± 2.18 U/L ($p< 0.01$) was observed in which there was total duration of illness more than one month.

Effect of Sputum positivity

In the study mean serum ADA levels in sputum positive cases was 38.32 U/L and in negative cases was 35.99 U/L. Similarly Jhamaria J et al also reported mean serum ADA level in sputum positive group was 43.95 and in sputum negative group was 42.09 U/L.[13] These results showed no difference with the observation of the present study in comparison of sputum positive to sputum negative. Mean serum ADA level were similar in both groups and no significant difference was observed, so presence of detectable A.F.B in sputum has no effect on serum ADA level.

Effect of different clinical type of pulmonary tuberculosis

Group I (Infiltrative lesions) 22 cases and mean serum ADA levels was 35.95 ± 3.051 U/L. Group II (Fibrocavitary tuberculosis) 21 cases and mean serum ADA levels was 37.96 ± 2.167 U/L. Group III (Tubercular consolidation) there were 7 cases and mean serum ADA levels was 35.70 ± 3.009 U/L. Significantly higher serum ADA level ($p< .001$) was observed in each group as compared to controls. So it was concluded that there was no relation of serum ADA levels in different clinical types of pulmonary tuberculosis. Conde et al evaluated serum ADA in active pulmonary tuberculosis and other pulmonary infections and showed no significant difference of ADA levels between them [14].

Relation of serum ADA level to radiological extent of disease

In our sudy Group I (minimal disease) was seen in 20 cases and mean serum ADA levels was 35.76 ± 3.109 U/L. Group II (moderate advance disease) was found in 23 cases and mean serum ADA levels were 37.07 ± 2.624 U/L. Group III (far advance disease) was seen in 7 cases and mean serum ADA levels were 38.45 ± 1.643 U/L.

Jhamaria J et al reported in sputum positive groups serum ADA level was 42.09 ± 1.46 U/L in minimal disease, 40.22 ± 2.5 U/L in moderately advanced disease, 39.57 ± 3.90 U/L in far advanced disease. Sputum negative group serum ADA level was 43.94 ± 2.48 U/L in moderately advanced disease, 44.25 ± 1.95 U/L in far advanced disease. Our study was supported by study of Jharmaria J et al [13]. We observed that all radiologically positive cases of pulmonary tuberculosis had significantly ($p <.001$) higher serum ADA level.

Conclusion

There was no variation in the serum ADA levels of Pulmonary Tuberculosis cases belonging to various age groups and either sex. In present study significantly higher values of serum ADA level was observed in presence of illness of more than one month duration. There was no significant correlation between sputum status and serum ADA levels. It was raised significantly in both sputum positive as well as negative cases. There was no significant correlation between serum ADA levels and different clinical groups of pulmonary tuberculosis. It was raised significantly in all clinical groups of Pulmonary Tuberculosis. There was no significant correlation between serum ADA levels and different radiological extent of disease. It was raised significantly in different radiological extent of disease.

We conclude that the serum ADA level estimation is easy, reliable, specific diagnostic test in

various grades of pulmonary Tuberculosis. It helps in early start of specific antitubercular treatment and decreases the time consuming expensive and invasive investigations. Measurement of the level of (ADA) enzyme in body in serum body fluids is a helpful diagnostic tool in diagnosing Tuberculosis.

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