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ORIGINAL ARTICLE

Association Of Serum Adenosine Deaminase with Sputum Conversion at the End of Second Month and at the End of The Anti-Tuberculous Drug Treatment among New Smear Positive Pulmonary Tuberculosis Patients

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Abstract:

Background: *Tuberculosis (TB) is global public health problem. Sputum microscopy for AFB is a well-known method for treatment monitoring in case of smear positive PTB patients. But it is not always easy to obtain sputum samples at the end of treatment. Many studies have proved the role of ADA in diagnosis of tuberculosis in effusion fluids and a decrease in ADA activity at the end of treatment. But association between serum ADA level with treatment monitoring in sputum smear positive PTB cases is not widely studied.*

Objective: *To determine the association of serum adenosine deaminase with sputum conversion at the end of second month and at the end of the treatment among new smear positive pulmonary tuberculosis patients.*

Materials & Methods: *This prospective observational study was conducted in the department of respiratory medicine of National Institute of Diseases of the Chest and Hospital (NIDCH) from June 2020 to September 2021. Ninety-eight new smear positive pulmonary tuberculosis patients were enrolled in this study according to inclusion and exclusion criteria of the study. Sputum sample was collected from each subject for microscopic examination at initial, at the end of the 2nd month and at the end of 6th month. Blood was collected from each subject for measurement of serum ADA level at initial, at the end of 2nd month and at the end of 6th month. Serum ADA was measured by enzymatic photometric method using MICROEXPRESS ADA-MTB reagent and result was expressed as U/L. Data analysis was done through Statistical Package for Social Science (SPSS) version 23.*

Results: *Mean age of the study subject was 39.7±13.0 years with male (82.8%) predominance. Ultimately 10 patients were lost to follow-up and 1 died during the study period. Rest of the study subjects (87 out of 98) showed that their sputum were converted at the end of 2nd month and remained negative at the end of 6th month. Mean serum ADA level was significantly decreased at the end of 6^{th} month (21.8±5.7 U/L) than 2nd month (25.1±8.3 U/L) and baseline (29.8±11.5 U/L) (P value = 0.001). To see the association between serum ADA level and sputum smear for AFB, Analysis of Variance test was done and it revealed that at the*

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end of 2nd month and 6th month mean serum ADA level was significantly higher among patients with higher bacillary load (P value =0.001). Multivariate regression analysis was done which found independent relationship between serum ADA and sputum for AFB 3+.

Conclusion: *This study showed there is significant association between serum ADA level and sputum smear conversion among the new smear positive pulmonary tuberculosis patients.*

Keywords: *Smear positive pulmonary tuberculosis, Serum ADA, Sputum smear conversion*

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Introduction:

Tuberculosis (TB) is one of the most ancient diseases of mankind, with molecular evidence going back to over 17,000 years. Inspite of newer modalities for diagnosis and treatment of TB, unfortunately, people are still suffering, and worldwide it is among the top 10 killer infectious diseases, second only to HIV[1].

TB is an airborne bacterial infection caused by M. Tuberculosis which affects any part of the body and most commonly the lungs[2].

Globally, an estimated 10.0 million (range, 8.9–11.0 million) people fell ill with TB in 2019, a number that has been declining very slowly in recent years. There were an estimated 1.2 million (range, 1.1– 1.3 million) TB deaths among HIV-negative people in 2019 (a reduction from 1.7 million in 2000), and an additional 208000 deaths (range, 177 000–242 000) among HIV-positive people (a reduction from 678000 in 2000)[3].

Epidemiological studies have shown that tuberculosis is a disease that endangers the health of a community with increasing incidence rate. In diagnosis of tuberculosis microbiologic, genetic, immunologic and biochemical methods are used[4].

When sputum smear positive patients are initiated on multidrug anti-tuberculosis treatment, there is a multifold reduction in bacillary load expelled in sputum. Patients, who respond are likely to become smear and culture negative during the course of treatment. It is expected that 80 to 90% of patients will undergo smear conversion within two to three months of treatment[5].

The World Health Organization (WHO) recommends sputum smear follow-up tests at two or three, five, and six or eight months[6].

Adenosine deaminase (ADA) is an enzyme of purine and is needed for the breakdown of adenosine from food and for the turnover of nucleic acids in tissues. Moreover, it regulates lymphocyte metabolism and is important for lymphocytic differentiation and growth. It is present in lymphocytes in high concentration[7].

Its activity appears to be necessary for an effective immune response as shown by many studies, such as in combined immunodeficiency disease[8].

Increased serum ADA activity can be seen in diseases associated with cellular system stimulation, such as typhoid fever, infectious mononucleosis, liver disease, sarcoidosis, leukemia, brucellosis, acute pneumonia, rheumatoid arthritis, malignancies and tuberculosis[9].

The significance of ADA level in diagnosis of Tuberculosis is known in effusion due to pleural, pericardial, meningeal and peritoneal tuberculosis specially in countries with high tuberculosis prevalence[4].

This study was conducted to find out the association of serum ADA levels with sputum conversion in new smear positive PTB cases, so as to assess its role as a possible treatment monitoring method in PTB.

Materials and methods:

This prospective analytical study was conducted in the department of respiratory medicine of National Institute of Diseases of the Chest and Hospital, Mohakhali, Dhaka during the period from July 2020 to September 2021.

Inclusion criteria were:

Newly diagnosed sputum smear positive pulmonary tuberculosis patients and age e”18 years.

Exclusion criteria were:

1. Extra-pulmonary tuberculosis, previous history of PTB, DR-TB patient
2. HIV infected/AIDS patient
3. Any malignancy
4. DM, CLD, CKD, rheumatological diseases, typhoid fever

5. Pregnant and lactating women.
6. Patient who refused to be part of study

Ninety eight (98) patients were included in the study using purposive sampling method. All newly smear positive pulmonary tuberculosis patients with inclusion & exclusion criteria were selected for the study, full information regarding nature of study, possible outcome, and importance of follow up, written consent was obtained from the patient. Enrolled patients were supplied with a predesigned questionnaire in locally understandable language. Blood sample was collected from each participant for measurement of serum adenosine deaminase level and treatment was started. Serum adenosine deaminase level and sputum smear examination were done at the end of second month and at the end of treatment. All the data was recorded systematically in a preformed data collection sheet and analyzed using appropriate statistical formula

Results:

Total 98 patients were preliminary enrolled in the study, among them 11 (10 patients were lost to follow up and 1 died) were excluded. Finally 87 patients were included for analysis. The majority (26.4%) patients belonged to the age group 21-30 years with mean age was 39.7±13.0 years. Male patients were predominant 72(82.8%) with male female ratio was 4.8:1. Most of the patients 48(55.2%) from urban background. (Table I).

Table-I

Demographic characteristics of the study patients (n=87)

Demographic characteristics	Number of patients	Percentage
Age (years)		33
≤20	4	4.6
21-30	23	26.4
31-40	18	20.7
41-50	22	25.3
51-60	19	21.8
>60	1	1.1
Mean±SD	39.7	±13.0
Range (min-max)	18.0	-70.0
Sex		
Male	72	82.8
Female	15	17.2
Geographical location		
Urban	48	55.2
Rural	39	44.8

Initially majority 51(58.6%) patients had BMI 18.5-24.9 kg/m^2, at the end of 2nd month more than three fourth (75.9%) patients had BMI 18.5-24.9 kg/m^2 and at the end of 6th month 80(92.0%) patients had BMI 18.5-24.9 kg/m^2.(Table II).

Table-II

BMI in different follow up (n=87)

BMI (kg/m^2)	Number of patients	Percentage
Initial		
<18.5	35	40.2
18.5-24.9	51	58.6
>25.0	1	1.1
At the end of 2nd month		
<18.5	19	21.8
18.5-24.9	66	75.9
>25.0	2	2.3
At the end of 6th month		
<18.5	5	2.3
18.5-24.9	80	92.0
>25.0	5	5.7

At the time of diagnosis, 40.2% patients had 3+ sputum AFB. At the end of the 2nd month and 6th month there was complete sputum conversion. (Table III).

Table-III

Sputum for AFB in different follow up (n=87)

Sputum for AFB	Number of patients	Percentage
Initial		
1+	22	25.3
2+	30	34.5
3+	35	40.2
At the end of 2nd month		
Negative	87	100.0
At the end of 6th month		
Negative	87	100.0

Mean serum ADA at the start of treatment was 29.0±11.5 U/L, at the end of 2nd month 25.1±8.3 U/L, at the end of 6th month 21.8±5.7 U/L. Mean serum ADA level was significantly decreased at the end of 6th month than initial. (Table IV)

Twenty two patients had 1+ AFB initially and their mean serum ADA level was significantly decreased at the end of 2nd month (19.1±3.4 U/L) and 6th month (17.7±3.2 U/L) than initial (21.1±4.0 U/L). (Table V)

Thirty patients had 2+ AFB initially and their mean serum ADA level was decreased at the end of 2nd

month (23.7±5.6 U/L) and 6th month (21.7±4.6 U/L) than initial (26.3±7.2 U/L) which was statistically significant (P value 0.001%). (Table V)

Thirty five patients had 3+ AFB initially and their mean serum ADA level was significantly decreased at the end of 2nd month (30.2±9.4 U/L) and 6th month (24.6±6.1 U/L) than initial (36.4±13.2 U/L). (Table V)

A significant relationship was found between sputum conversion with serum ADA level initially, at the end of 2nd month and 6th month. Mean serum ADA level was higher in patients with higher bacillary load in sputum. (Table V)

In multivariate analysis, baseline low BMI and serum ADA were found to be independent predictors for bacillary load in sputum. It was evident that patients who had high serum ADA had 18.02 times risk of having smear positive PTB. Again, 2.50 times risk prevailed among the patient who had low BMI. However, no association was found between sputum result and patient demographics (e.g. age, sex, geographical location). (Table VI)

Table-IV

Serum ADA in different follow up (n=87)

Serum ADA (U/L)	Mean±SD	*P value (Initial vs at the end of 6th month)*
Initial	29.0±11.5	0.001s
Range (min-max)	16.0-67.1	
At the end of 2nd month	25.1±8.3	
Range (min-max)	14.3-54.0	
At the end of 6th month	21.8±5.7	
Range (min-max)	11.9-38.5	

s= significant

P value reached from paired t-test

Table-V

Association between initial sputum for AFB with serum ADA level (n=87)

Serum ADA (U/L)	Initial sputum for AFB			F value	df	P value
	1+ (n=22) Mean±SD	2+(n=30) Mean±SD	3+(n=35) Mean±SD			
Initial	21.1±4.0	26.3±7.2	36.4±13.2	18.84	2	0.001s
Range (min-max)	16.0-32.0	17.7-55.0	19.3	-67.1		
At the end of 2nd month	19.1±3.4	23.7±5.6	30.2±9.4	17.73	2	0.001s
Range (min-max)	14.3-28.6	16.4-46.6	16.6-54.0			
At the end of 6th month	17.7±3.2	21.7±4.6	24.6±6.1	12.95	2	0.001s
Range (min-max)	11.9-25.0	15.18-37.1	15.7-38.5			

s= significant

P value reached from ANOVA test

Table-VI

Multivariate regression analysis for sputum for AFB 3+ (n=87)

	Adjusted OR	95% CI		P value
		Lower	Upper	
Age	0.454	0.141	1.456	0.184ns
Sex	0.293	0.061	1.416	0.127ns
Geographical location	1.557	0.504	4.812	0.442ns
Low BMI	2.509	1.067	4.869	0.039s
Serum ADA	18.022	5.321	61.041	0.001s

s= significant, ns= not significant

p-value reached from multivariate analysis by binary logistic regression analysis

OR=Odd's Ratio

Discussion:

This prospective observational study was carried out in the department of respiratory medicine, NIDCH, Dhaka from June 2020 to September 2021. 98 patients were included for the study among them 11 (10 patients were lost to follow-up and 1 died) patients were excluded in this study. Finally 87 patients were analyzed.

We observed that the majority 23(26.4%) patients belonged to the age group 21-30 years with mean age was 39.7±13.0 years. Almost similar study conducted by Lende et al.[10] where they found the mean age of the participants was 40 years. Soedarsono et al.[11] consisted of 42.85 years.These findings are consistent with the present study.

In this present study it was observed that male patients were predominant 72(82.8%) and female was 15(17.2%) with male female ratio was 4.8:1. Similarly, Soedarsono et al.[11] had observed that men were 21(80.8%) and females were 5(19.2%).These findings are consistent with my study.

Regarding body mass index (BMI) our observation showed that initially significant proportion of study population (40.2%) were underweight (BMI <18.5). On successful treatment there was improvement of nutritional status i.e. BMI >18.5 in 75.9% and 92% patients on 2nd and 6th month of therapy respectively. In a study done by Phan et al.[12] showed that BMI increased significantly after 2 months of treatment.

In this study it was observed that initial serum ADA was 29.0±11.5 U/L, at the end of 2nd month mean serum ADA 25.1±8.3 U/L, at the end of 6th month mean serum ADA was 21.8±5.7 U/L. Mean serum ADA level was significantly decreased at the end of 6th month than initial. In a study of Kartaloglu et al.[13] reported that the mean serum ADA levels at one month was 45.1±10.6 U/l, two months 34.6±10.1 U/l, and six months 24.6±4.7 U/l in the patients. The differences in serum ADA levels between the first measurement and that at one month, one month and two months, and one month and six months were statistically significant (p=0.005, p=0.016, and p<0.001, respectively). Soedarsono et al.[11] had observed that all patients experienced sputum conversion at the end of 2nd month of TB treatment. Examination of serum ADA levels showed that the mean value of serum ADA levels before treatment was higher than after receiving anti-tuberculous drug with (26.40 IU/L vs. 19.67 IU/L). A significant difference in serum ADA levels before and after the intensive phase of TB treatment with $P < 0.001$. Serum ADA levels in PTB patients decreased with TB treatment and suggest that serum ADA levels can be used as a prognostic marker. Higher AFB sputum smear shows the severity of the disease based on the number of bacterial loads. Saini et al.[14] stated that the average level of serum ADA levels increased significantly in PTB patients. A study by Pandey et al.[15] reported that the increase in serum ADA levels along with the increase of the sputum AFB grading, and the increase in serum ADA level was caused by stimulation of cell mediated immunity.

All participants in their study experienced sputum conversion. The results of the examination of serum ADA levels after the end of the intensive phase of TB treatment have decreased. This indicates that examination of serum ADA levels can be used in monitoring TB therapy response. Serum ADA levels were expected to increase twice in PTB patients at the time of diagnosis and subsequently experienced a significant decrease in the mean value of serum ADA levels after treatment in PTB patients[4]. Serum ADA levels decreased to normal levels after 1 month of effective treatment in patients with PTB. A decrease in serum ADA levels can be caused by changes in the number of lymphocytes induced by *M. tuberculosis*[16]. Significant differences were obtained in ADA serum activity before and after treatment and also from older TB patients and healthy control patients, indicating that serum ADA activity was increased in PTB patients[17].

Rao *et al.*[18] reported the decrease in serum ADA levels during PTB treatment. They found significant difference of serum ADA level before and after the 2nd month of TB treatment and concluded that measurement of serum ADA could help the evaluation of therapy response. Other study reported the same results that serum ADA levels decreased during TB treatment[19,20].

In this study, twenty-two patients had 1+ AFB, thirty patients had 2+ AFB and initially and thirty-five patients had 3+ AFB initially with baseline ADA their mean serum ADA level (17.7±3.2 U/L),

(26.3±7.2 U/L) and (36.4±13.2 U/L) respectively. At the end of 2nd month was (19.1±3.4 U/L), (23.7±5.6 U/L), (30.2±9.4 U/L) and at the end of 6th month it was (17.7±3.2 U/L), (21.7±4.6 U/L), (24.6±6.1 U/L) respectively. In every aspect mean serum ADA level was significantly decreased after the end of intensive phase and at the end of 6th month. The results were consistent with the study done by Soedarsono et al.[11] Patients with 3+ AFB have a higher mean value of serum ADA level before and after the end of the intensive phase of TB treatment. Previous study reported the same results that the mean value of serum ADA level was higher among 3+graded sputum-positive patients. This is due to the stimulation of cell-mediated immunity[15].

In this study it was observed that initially, at the end of 2nd month and 6th month mean serum ADA level was significantly higher in 3+sputum for AFB than 2+AFB and 1+ AFB. Saini et al.[14] showed that that irrespective of the sputum smear status ADA can help determine the patients who can be suffering from PTB, but difficult to diagnose on the basis of sputum smear examinations. Levels of serum ADA in sputum positive TB patients were correlated with severity of disease. Severity of disease was determined on the basis of acid fast bacilli (AFB) grading on sputum microscopy and was categorized as scanty, 1+, 2+ and 3+.

Multivariate regression analysis was done to evaluate the relationship among the different variables. Here we found that low BMI and high serum ADA level were independent predictors of sputum bacillary load. It was evident that patients who had high serum ADA had 18.02 times risk of having smear positive PTB. Again, 2.50 times risk prevailed among the patients who had low BMI. However, age, sex, geographical location were not found to be independent predictor for sputum smear positive PTB.

Conclusion:

Mean serum ADA level was independently associated with sputum smear conversion at the end of the second month and at the end of anti-tuberculous drug treatment from baseline in sputum smear positive PTB patients. Monitoring of Serum ADA level have a potential utility to evaluate the therapeutic response of anti TB treatment in sputum smear positive PTB patients. Further studies may be done to validate our findings by measuring the cut-off value, sensitivity, specificity, PPV and NPV of serum ADA level through further studies.

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