Journal of Cardiovascular Disease Research

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

Role of Adenosine Deaminase Activity in Serum and Pleural Fluid for Diagnosis of Pulmonary Tuberculosis: A Cross-Sectional Study among Tribal and Non-Tribal Population of Udaipur, Rajasthan

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ABSTRACT

Background and Objective: Tribal populations are the least well-off groups in society, and because of their ignorance, they are ill-equipped to take advantage of government possibilities. Hence, the study aimed to evaluate the level of ADA in Serum and Pleural Fluid and find its role in early diagnosis of Pulmonary Tuberculosis (PTB) among Tribal and Non-Tribal Populations.

Method: 76 participants in the study were split into two groups. Group-I includes 35 healthy control participants, and Group-II consists of 41 PTB patients, with 28 sputum-negative (-ve AFB) patients and 13 sputum-positive (+ve AFB) patients between the age of 18 to 65. In both patient groups, ADA levels were determined in serum and pleural fluid at the Pacific Institute of Medical Sciences in Umarda, Udaipur.

Results: The ADA level in Serum and pleural fluid were significantly higher in sputum positive (+ve AFB) patients, 35.62±2.41 IU/L and 29.27±3.20 IU/L as compared to sputum negative (-ve AFB) patients, 57.40±32.14 IU/L and 55.94±18.60 IU/L. Further, serum ADA and Pleural Fluid ADA levels among tribal males were higher as compared to tribal females and among non-tribal females, it was higher as compared to non-tribal males.

Conclusion: In the area of Udaipur where tuberculosis is quite prevalent among Tribal Population, ADA level in serum and pleural fluid is helpful as a quick and precise test for the early detection of PTB as ADA is an extremely sensitive, precise, affordable, quick, and easily accessible.

Keywords: Adenosine deaminase, pulmonary tuberculosis, Pleural Fluid, Acid Fast Bacilli

INTRODUCTION

TB continues to be a significant source of morbidity and mortality globally. Globally, an increase in morbidity and mortality from tuberculosis (TB) is predicted in the near future.[1] India continues to have the greatest incidence of TB in the world, making up one-fifth of the total.[2] Every year, India is thought to diagnose 1.9 million new cases of tuberculosis (TB). Infectious new sputum positive TB (NSP TB) cases make up 0.8 million of these.[3] The causing agent of tuberculosis, Mycobacterium tuberculosis, typically affects the lungs, but it

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

can also spread to the colon, meninges, bones and joints, lymph glands, skin, and other bodily tissues. [4] People with active tuberculosis exhibit various symptoms, such as coughing, occasionally with sputum or blood, chest pain, weakness, weight loss, fever, and night sweats.[5] It is already evident that India is a nation that is rich in diversity. People from various communities live in different ways. According to statistics, almost 111 million people in India are from tribal communities, or almost 8.6% of the country's total population. Information on TB in this group is sparse, especially about the prevalence of the disease among them and the behavior of those who exhibit symptoms suggestive of TB in terms of seeking medical attention. [5] Further, despite the diversity of tribal communities, they have several things in common, like poor health indicators, higher rates of morbidity and mortality, and very restricted access to healthcare service.[7]

Social and cultural factors have been found to increase the risk of tuberculosis in the tribal community. The primary factor appears to be a deeply ingrained conviction in using conventional treatments rather than going to hospitals. Furthermore, they know little about TB and government relief program.

Additionally, they were more likely to develop tuberculosis (TB) due to their poor nutrition, usage of tobacco (Gutka), smoking, drinking (especially country liquor), and smoking.[6]

According to a recent WHO report, with a projected incidence of 26.9 lakh cases in 2019, India is the country with the greatest TB burden in the world. Another significant year for India's TB monitoring program is 2019, with a record-high notification of 24 Lakh cases, an increase of almost 12% from the previous year. 90% (N=21.6 lakhs) of the 24 lakh cases of tuberculosis (TB) were incident cases (new and relapse/recurrent). In contrast to the estimated incidence rate of 199 cases per lakh population, this translates to an incident notification rate of roughly 159 cases per lakh. This reduces the difference between the estimated and notified incident cases to just 40 cases per lakh population, or roughly 5.4 lakh missing cases across India.[8]

Hence, the presence of acid-fast bacilli on the patient's sputum smear examination allows for the definitive diagnosis of pulmonary tuberculosis. The results of a chest X-ray can be vague since many non-tubercular lung diseases look like pulmonary tuberculosis. Due to the high cost of other tests including ELISA, PCR, and gamma interferon, a specialized diagnostic test that is less expensive, trustworthy, and high in specificity is needed for the early identification of pulmonary tuberculosis.[9]

Although TB is a serious health concern for tribal groups and research on these populations has been limited in Southern Rajasthan. Hence, the present study is planned to diagnose the efficiency of serum and pleural Fluid ADA activity in pulmonary tuberculosis Patients among Tribal and Non-Tribal population.

Material and Method

The participants in this case-control study included both healthy controls and patients with Pulmonary Tuberculosis. The study was conducted in a Pacific Institute Of Medical Sciences, Umarda, Udaipur, in the biochemistry department in conjunction with the TB & chest department. All of the participants in this study received thorough explanations of the study in their native languages, and their written consent was obtained.

The study was carried out in 76 subjects of both the sexes between the age group of 25-83 years and was divided into two groups. **Group-I** includes 35 subjects as healthy individuals control and **Group-II** includes 41 cases of active pulmonary tuberculosis patients. The patients of the study group were further divided into two groups:

A) **Sputum Smear Positive cases (AFB+ve)** which include patient with at least two sputum specimens positive for acid-fast Bacilli

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

B) Sputum Smear negative cases (AFB-ve) which include two sputum specimens negative for acid-fast bacilli and radiographic abnormalities consistent with pulmonary TB.

Based on the following inclusion and exclusion criteria, patients were chosen for the study:

- Inclusion Criteria: Cases identified as a "new case" of tuberculosis must have at least two positive sputum smear tests positive for acid-fast bacilli and radiographic abnormalities consistent with pulmonary tuberculosis.
- Chronic pulmonary TB (getting at least two cycles of anti-TB treatment for more than six months), HIV organ transplantation, diabetes mellitus, and treatment with corticosteroids are excluded from the study. Extra-pulmonary TB and/or patients needing surgical intervention are also eliminated.

A thorough physical examination and standard laboratory tests were done after taking a comprehensive medical history. Clinical assessment, chest x-ray, sputum smear, pleural fluid for AFB by ziehl-Neelson staining, and other laboratory studies were used to determine the diagnosis of tuberculosis.

Blood samples were collected from each patient and control under aseptic conditions when TB was diagnosed, and they were then submitted right away to the lab.

ADA kit produced by Diazyme Laboratories Company was used to measure serum ADA leve ls. Giusti and Galanti colorimetric method was used with the ADA assay kit to evaluate the se rum adenosine deaminase activity (Diazyme Kit, USA). ADA activity in the blood was measured in IU/L. According to the manufacturer's protocol, every 1 IU/L of ADA corresponds to the production of one micromole of ammonia per litre of serum every minute at 37°C. REF DZ 117.Con and REF DZ 117 Cal, respectively, performed the kit's validity and calibration. Then the data were analysed by using Statistical package for Social Sciences (SPSS) software.

Results

The study comprised of 41 cases of pulmonary tuberculosis, which included 13 Sputum Positive Cases and 28 Sputum Negative Cases. The control Group had 35 individuals. The Serum ADA activity was in the range of 22.20 IU/L to 60.10 IU/L in AFB +ve subjects and it was 18.8 IU/L -51.8 IU/L in AFB –ve subjects. A level of Pleural Fluid ADA ranges lies between 23.10 IU/L to 130.90 IU/L in AFB +ve whereas as in TB –ve ranges lies between 28.90 IU/L to 91.50 IU/L. According to the results showed that 100 % of Sputum Positive participants had Pleural Fluid levels above 40 IU/L, while 82% of TB subjects had levels below 40 IU/L and the remaining 18% had values between 30-40 IU/L.

Table 1 represents the mean serum ADA levels in control group and study group. The mean Serum ADA level in Control Group was 16.90±5.30 IU/L whereas in Study Group it was 58.13±12.56 Statistical analysis showed that in comparison to the control group, the study group's mean serum ADA level was considerably greater (P<0.0001).

Table-1 Distribution of Serum ADA levels in control and study group

GROUP	NO OF CASES	SERUM ADA MEAN ± SD (IU/L)	t- VALUE	P-VALUE
CONTROL	35	16.90±5.30	18.081	P<0.0001
STUDY GROUP	41	58.13±12.56		

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Further, the Tuberculosis patients (n =41) were sub grouped as Sputum Positive (AFB+ve) and Sputum Negative (AFB-ve) where,

Sputum Positive AFB +ve (n= 13)

Sputum Negative AFB –ve (n=28)

Table 2 represents mean serum ADA levels in control group and in Sputum Positive Patients. The mean Serum ADA level in Control Group was 16.90±5.30 IU/L whereas in Sputum Positive Patients it was 35.62±2.41 Statistical analysis showed that the mean serum ADA level in Sputum Positive Patients as compared to Healthy control group was significantly higher (P<0.0001).

Table- 2 Statistical comparison of Serum ADA levels in controls and Sputum positive cases

GROUPS	NO. OF CASES	SERUM ADA LEVEL		
		Mean ± SD	t- Value	P-Value
CONTROLS	35	16.90±5.30	12.211	P<0.0001 statistically significant
SPUTUM POSITIVE GROUP	13	35.62±2.41		

Table- 3 Statistical comparison of Serum ADA levels in controls and Sputum negative cases

Table 3 represents the mean serum ADA levels in the Sputum Negative Patients and Control Group. In the control group, the mean serum ADA level was 16.90±5.30 IU/L, whereas in patients with negative sputum tests, it was 29.27±3.20 IU/L. According to statistical analysis, the mean serum ADA level in patients with sputum negative results was substantially greater than in the healthy control group (P<0.0001).

GROUPS	NO. OF CASES	S. ADA LEVEL		
		Mean ± SD	t Value	P Value
CONTROLS	35	16.90±5.30	10.858	P<0.0001 statistically significant
SPUTUM NEGATIVE GROUP	28	29.27 ± 3.20		

Table- 4 Comparison of Serum ADA levels between Sputum Positive and Sputum Negative Group

The comparison between patients with positive sputum and those with negative sputum is shown in Table 4, and it was found that the mean serum ADA among patients with positive sputum was 35.62 2.41 IU/L, which was considerably higher than that of patients with negative sputum, who had 29.27 3.20 IU/L and were Statistically significant with a P<0.001Similar outcomes were observed for Pleural Fluid ADA levels, where the mean P. Fluid ADA levels were 57.4032.14IU/L and were greater in Sputum Positive reports.

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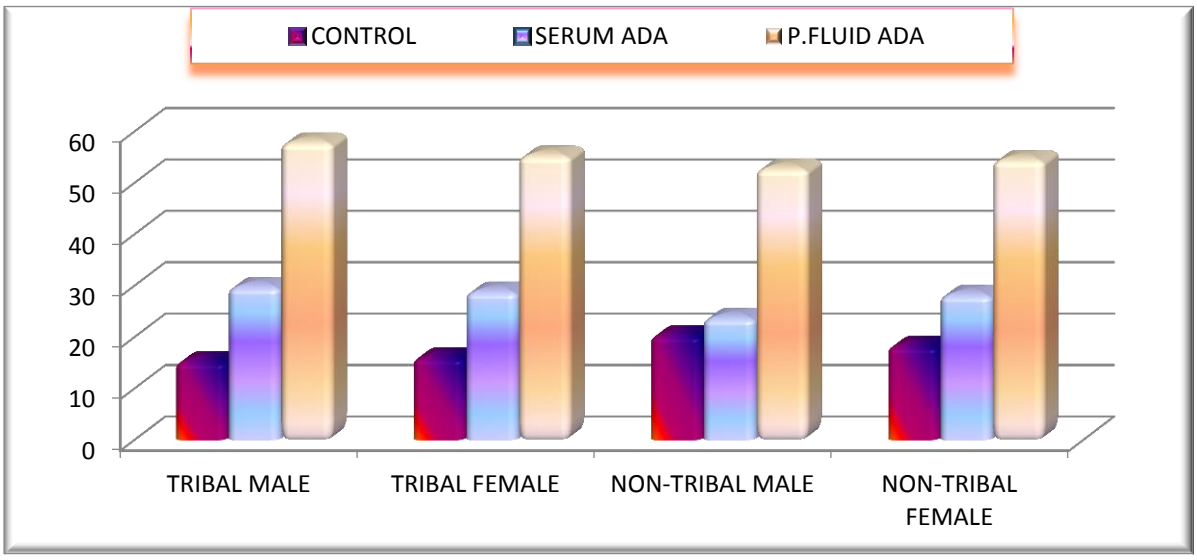
GROUPS	NO. OF CASES	SERUM ADA LEVEL			PLEURAL FLUID ADA LEVEL		
		Mean ± SD	t-Value	P-Value	Mean ± SD	t-Value	P Value
Sputum Positive Group	13	35.62±2.41	6.48	P<0.0001	57.40±32.14	0.966	P=0.8450
Sputum Negative Group	28	29.27 ± 3.20			55.94±18.60		

Additionally, the investigation was conducted based on the residential status among tribal and non-tribal population:

Graph I shows the comparison between Tribal and Non-Tribal Population on the basis of their sex. It was shown that the level of pleural fluid ADA across the entire population was twice as high as the level of serum ADA. Additionally, a sex-by-sex analysis reveals that tribal males and non-tribal females had higher levels of pleural fluid ADA than tribal females and non-tribal males.

Graph 1. Comparison between Serum ADA and Pleural Fluid ADA among Tribal and Non-Tribal population



Discussion

Pulmonary tuberculosis primarily affects the lungs but can also affect other body organs. With more than 9 million new cases and 3 million fatalities each year, tuberculosis is still one of the biggest health issues in the world. Pleural effusion frequently results from tuberculosis, particularly in nations like India. Moreover, the prevalence of tuberculosis is rising globally. [10]

Due to lifestyle choices and imbalances, tuberculosis appears to be steadily rising in Rajasthan, especially among the tribal people. Additionally, Rajasthan struggles with a persistent water shortage issue. Lakes provide the majority of the water supply, which is

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

typically stagnant, dirty, and unclean. People can contract viruses and infectious diseases by using stagnant water.[11]

It has been determined that the enzyme ADA, which is part of the purine metabolic pathway, is made up of two isoenzymes, ADA1 and ADA2. The enzyme is widely distributed throughout the human body, with T lymphocyte differentiation and proliferation being its primary physiological role. T-cells have an enzyme level that is 5–20 times higher than that of B-cells.[12] In our study, we evaluated total 76 patients out of which 35 were healthy control group (Group I) and 41 were patients (Group II) out of which 13 belong to Sputum Positive group and 28 belong to Sputum Negative group.

ADA Levels in PTB cases and Control group

In our study, the mean serum ADA level in study group and control group was 58.13±12.56 IU/L and 16.90±5.30 IU/L. Highly significant level of Serum ADA (P<0.0001) was observed as compared to control. Sanjay Varma and Archana Toppo 2015 observed a mean ADA of 36.74±2.83 U/L in their study of 50 cases. [13]

In a study by Trupti G. Lende et.al., 2019, there is significant rise in the serum ADA level of Patients suffering from active pulmonary tuberculosis compared to healthy controls. [14] Another study in 2004 carried out by Amniafshar et al. [14] with 50 cases of active PTB and 50 healthy controls found the serum ADA levels in new cases of TB (42.4 ± 21.5 IU/L) which were higher than that for healthy controls (26.6 ± 8.21 IU/L) (P < 0.0001).

Effect of Sputum Positivity

We have observed that the level of Serum ADA among Tuberculosis AFB +ve was higher than AFB –ve and their mean ± SD were 35.62 ± 2.41 and 29.27 ± 3.20. Similar, results were shown in the study given by Mishra et al have also shown raised ADA activity under antigenic stimulation is found in infection such as tuberculosis and typhoid fever where cell mediated immunity is stimulated.[11]

In our study, finding seems to confirm that ADA activity is a useful marker for diagnosis of tuberculosis and tubercular effusion. As the mean level of Pleural fluid ADA in Sputum positive patients (57.40±32.14) were significantly higher as compared to Pleural Fluid ADA levels in Sputum Negative Patients (55.94±18.60). This is in accordance with the studies of Y.C.Gary Lee et al, in 2001 studied 106 cases of lymphocytic pleural effusion origin of different etiologies and concluded saying that ADA levels in TB pleural fluid exceeds than that in other non-tuberculosis lymphocytic pleural fluid[16]. In 2004, Kaisemann et al.[17] came to the conclusion that ADA determination in pleural fluid is a sensitive and specific approach for diagnosing pleural TB and that its usage can eliminate the necessity for pleural biopsy in the first assessment of pleural effusion in patients.

The Mean ± SD level of Pleural Fluid ADA among Sputum Positive and Sputum Negative was 57.40 ± 32.14 IU/L and 55.94 ± 18.60 IU/L and it was more in AFB +ve as compared to AFB-ve Patients. This is in accordance with the study of Sonone Kanchan K.et. al.,[18] 2014 stated that in both tuberculous (p<0.0001) and non-tuberculous (p<0.0008) pleural effusions, the levels of pleural fluid ADA were considerably more significant than serum ADA levels, pointing to a localized intra-pleural release of ADA**.**

Based on the sex and residential status study was conducted on tribal and non-tribal population and their results were tabulated.

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The level of ADA was relatively low in tribal areas, which appeared to be primarily owing to their lack of knowledge on food choices. They still give vegetables and fruits relatively little thought, which is largely to blame for their inferior vitamin and mineral intake and consequently low immunity to infectious diseases. The present study indicated tuberculosis as a major health problem amongst tribal population of Udaipur region. Our finding also reveals that the prevalence of Tuberculosis among Tribal male was more compared to Tribal females. Similar studies have also been reported amongst the tribal Population from Madhya Pradesh region of central India.[20]

In the current study, we have observed that the level of Pleural fluid ADA among tribal males was higher as compared to tribal females and among non-tribal females it was higher as compared to non-tribal males and this could be due to the kind of lifestyles preferred by men and women may account for the differences between them. Male tribal members mostly work outside in forests or herd animals on farms. They consume pan masala or smoke frequently. But women tend to stay at home and prepare meals. Another explanation could be the inherent disparities between men's and women's vulnerability to TB. Apart from potential biological explanations, there may be a number of other factors contributing to the gender imbalance in TB prevalence, including access to healthcare facilities, smoking, drinking, and exposure to indoor dust and air pollution.[14]

Acknowledgement

I want to express my sincere gratitude towards my Guide Dr. Suman Jain for enabling me to accomplish my research and a Heartfelt thankyou to Dr. Rekha Chaudhary for volunteering her valuable time to review this article.

Limitations

A small sample size may make it difficult to determine if a particular outcome is a true finding

Conclusion

Most of the participants in this study had some level of familiarity with TB. Tuberculosis is equally prevalent in both tribal and non-tribal populations, and it has been discovered that both men and women have a similar understanding of the disease. The serum ADA levels and sputum status did not significantly correlate in the current investigation since they increased significantly in both sputum-positive and Sputum-negative individuals, and this increase was statistically significant. An even higher level of ADA in patients may serve as an accessible, accurate, and direct indicator of tuberculosis and may aid in early detection and treatment. It is now crucial for the government to improve the health system and raise public awareness in order to slow the spread of tuberculosis among tribal and non-tribal populations.

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