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Assessment of pleural fluid adenosine deaminase and alkaline phosphatase in tubercular and non-tubercular effusions

Santanu Mondal[1], Swati Bhattacharyya[2], Subhra Mitra[3]

[1]Final Year Postgraduate Resident, [2]Professor and Head, Department of Biochemistry, [3]Professor and Head, Department of Chest Medicine, Calcutta National Medical College and Hospital, Kolkata, West Bengal, India

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ABSTRACT

Background: Many studies have showed that adenosine deaminase (ADA) and alkaline phosphatase (ALP) values were highly supportive in the diagnosis of tuberculosis but comparison between ADA and ALP has not been much documented in pleural fluid (PF) in eastern India. Hence, the main aim of the study was to identify the relation between ADA and ALP in diagnosis of tuberculosis if any. **Aims and Objectives:** This study was conducted to estimate and compare the levels of ADA and ALP of PF in tubercular and non-tubercular diseases. **Materials and Methods:** This study was hospital-based, cross-sectional, and observational study during the period of 1 year. A total of 90 patients have been analyzed among them after excluding few outliers, 82 patients had been taken whose ADA and ALP were measured in PFs. **Results:** Kolmogorov–Smirnov and Shapiro–Wilk tests for statistical analysis were used to determine whether continuous variables were normally distributed or not. In the comparison of tubercular and non-tubercular effusion groups, P-value for ADA in independent t-test was 0.000, and P-value for ADA and ALP in Mann–Whitney U-test was 0.000 and 0.000, respectively. Receiver operating characteristic (ROC) curve showed area under the curve (AUC) of 0.965 and 0.751 in ADA and ALP for diagnosing tuberculosis. Cutoff values of 31.87 IU/L for ADA were determined by ROC curve analysis with 100% of sensitivity and 82.3% of specificity and cutoff values of 40.5 IU/L for ALP were also determined by ROC curve analysis with 75.6% of sensitivity and 68.3% of specificity for diagnosis of tuberculosis. **Conclusion:** Although ADA is the best parameter for diagnosing tubercular pleural effusions, analysis of ALP is cost-effective.

Key words: Adenosine deaminase; Alkaline phosphatase; Pleural fluids; Area under the curve; Receiver operating characteristic curve

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INTRODUCTION

Adenosine deaminase (ADA) value is considered highly supportive in the diagnosis of tuberculosis.[1] In many studies, it has been seen that alkaline phosphatase (ALP) can be used as an effective marker of tubercular pleural effusion, along with ADA.[2,3] ALP which can be used as a supportive diagnostic marker in tubercular and non-tubercular disease will be explored in the present study. However, comparison between ALP and ADA has not been much documented in pleural fluid (PF) in Indian and especially in Eastern India. Therefore, the aim of the present study would be to explore the two enzyme markers while differentiating Tubercular (TB) from Non Tubercular (Non-TB) pleural effusion.

In Indian scenario, ADA value more than 36 IU/L and ALP value more than 71 IU/L in PF were indicative of tubercular pleural effusion.[4,5] The mean ADA in the PF of non-tubercular pleural effusion patients was significantly lower compared to those with tubercular pleural effusion.[6]

Aims and objectives

This study was conducted to estimate and compare the levels of ADA and ALP of PF in tubercular and non-tubercular diseases.

Address for Correspondence:
Santanu Mondal, 8486, Santipally, Santra Para Road, North 24 Parganas, Kolkata - 700 119, West Bengal, India.
Mobile: +91 9073482453, **E-mail:** santanumondal109@gmail.com

MATERIALS AND METHODS

In the Department of Biochemistry and Department of Chest Medicine in Calcutta National Medical College, Kolkata, this hospital-based, cross-sectional, and observational study has been conducted for 1-year duration (May' 2021–April' 2022) with 46 tubercular and 44 non-tubercular subjects with comparable distribution of age and sex.

Inclusion criteria

Inclusion criteria for this study were patients diagnosed with tubercular pleural effusion (reverse transcription polymerase chain reaction or *Bacillus* or Radiological) admitted in Indoor of Chest Medicine in Calcutta National Medical College and Hospital (CNMC) and such patients visiting the outpatient department of Chest Medicine in CNMC.

Exclusion criteria

Exclusion criteria for this study were: Patients who did not give consent, pyogenic tap-frank pus, terminal illness, and acute illness including fever and chylous tap were excluded from the study and patients diagnosed with multidrug-resistant TB were also excluded from the study.

Data were collected by interviewing patients and their spouses and parents, clinical examination and laboratory investigation, and radiological evidences. Informed consent was taken from patients or their spouses or parents for cases. Pre-structured and pre-tested format had been used to collect the data. Baseline data including age, sex, detailed medical history, and clinical examination had been included as a part of methodology. PF had been collected in in-house setup after proper asepsis as and when advised by the clinician. The sample had been centrifuged in the laboratory centrifuge machine and cell-free fluid had been collected in separate sterile vial and utilized for estimation of all parameters. No invasive interventions had been done for the sake of the study itself.

PFADA has been measured by purine nucleoside phosphorylase-xanthine oxidase (PNP-XOD) Method (general system wavelength was 546 nm) in autoanalyzer and PFALP has been measured by IFCC Method (general system wavelength was 405 nm) in Auto-analyzer Kone lab PRIME 600i and Kone lab 60i. Values are compared with semi-auto analyzer. Coefficient of variance (CV) did not vary significantly. Routine quality control procedure was validated and CV was calculated for each biochemical parameters for monitoring the precision of methods. The identity of the patients was not revealed.

Principle of ADA estimation: ADA catalyzes deamination of adenosine to inosine which was then converted to hypoxanthine by PNP. Hypoxanthine was then converted to uric acid and hydrogen peroxide by XOD. Hydrogen peroxide was then reacted with 4-Aminoantipyrine in the presence of peroxidase to generate quinone dye.

Principle of ALP estimation: ALP catalyzes the transfer of the phosphate group from p-nitrophenyl phosphate to 2-amino-2-methyl-1-propanol, liberating p-nitrophenol. The rate of p-nitrophenol formation measured photometrically was proportional to the catalytic concentration of ALP present in the sample.

Ethics

It is an observational study where no interference was done. The detailed methodology was submitted to the Institutional Ethics Committee. This research study was approved by the Ethics Committee of Calcutta National Medical college, Kolkata-700014. No violation of International human research ethics has been violated.

RESULTS

In the tuberculosis group, the mean age (mean±standard deviation [S.D.]) of patients was 39.293±16.097. In the non-tuberculosis group, the mean age (mean±S.D.) of patients was 55.122±14.574. Table 1 shows that the most number of tuberculosis patients belonged to 41–50 years of age (22.0%) and the most number of Non-tuberculosis patients belonged to 51–60 years of age (34.1%). Association of age in group with tubercular and non-tubercular effusions was statistically significant (P=0.003) with Chi-square value of 20.019. This table also shows that in the tuberculosis group, 25 (61.0%) patients were female and 16 (39.0%) patients were male and in the non-tuberculosis group, 14 (34.1%) patients were female and 27 (65.9%) patients were male. Association of sex with tubercular and non-tubercular effusions is statistically significant (P=0.015) with Chi-square value of 5.917. This table further shows that in tuberculosis, 9 (22.0%) patients have <40 IU/L ADA and 32 (78.0%) patients have 40 and above IU/L ADA. This also shows that in tuberculosis, 27 (65.9%) patients have <75 IU/L ALP and 14 (34.1%) patients have 75, and more IU/L ALP. Association of ADA and ALP cutoff values with tubercular and non-tubercular effusions both was statistically significant (P-value for ADA reference was 0.000 and P-value for ALP reference was 0.035).

Table 2 shows that in tuberculosis group, the mean ADA (mean±S.D.) of patients was 58.37±26.419 and the mean ALP (mean±S.D.) of patients was 66.73±36.752. In non-tuberculosis group, the mean ADA (mean±S.D.) of patients was 23.47±9.297 and the mean ALP (mean±S.D.) of patients is 43.193±38.934.

Table 3 shows that distribution of ADA and ALP both with tubercular and non-tubercular effusions was statistically significant (P=0.000 which is ≤0.05).

Receiver operating characteristic (ROC) analysis was also done for evaluating the diagnostic sensitivity, specificity, and cutoff values of ADA in diagnosing tuberculosis (Figure 1). ADA preferred as an excellent tool for diagnosing tubercular pleural effusions (The area under the curve AUC=0.965 with 95% of confidence interval 0.933–0.998) and the comparison of tubercular with non-tubercular effusions, a cutoff value of 31.87 IU/L was determined in the ROC analysis to diagnose tuberculosis. This cutoff value of ADA had sensitivity of 100% and specificity of 82.9% to diagnose tuberculosis.ROC analysis was also done for evaluating the diagnostic sensitivity, specificity, and cutoff values of ALP in diagnosing tuberculosis (Figure 2). ALP showed a lower diagnostic performance, they were also an acceptable tool for diagnosing tubercular pleural effusions (AUC=0.751 with 95% of confidence interval 0.646–0.856) and the comparison of tubercular with non-tubercular effusions, a cutoff value of 40.5 IU/L was determined in the ROC analysis to diagnose tuberculosis. This cutoff value of ALP had sensitivity of 75.6% and specificity of 68.3% to diagnose tuberculosis.

DISCUSSION

Another Indian study concluded that ALP is also a helpful marker in separating tubercular from non-tubercular pleural effusions. Result analysis of this study has shown ROC curve of ALP with sensitivity and specificity of 90% and 80%, respectively, for a cutoff value of 71 IU/L for pleural

Table 1: Association between age, sex, reference ADA, and ALP cutoff with tubercular and non-tubercular effusions

Charactrestics	Tuberculosis	Non-tuberculosis	Chi-square value	P-value
Age (in years)				
≤20	6	0	20.019	0.003
21–30	8	2		
31–40	8	6		
41–50	9	6		
51–60	6	14		
61–70	4	8		
>71	0	5		
Sex				
Female	25	14	5.917	0.015
Male	16	27		
ADA				
40 and Above	32	2	45.221	0.000
<40 IU/L	9	39		
ALP				
75 and Above	14	6	4.232	0.035
<75 IU/L	27	35		

ADA: Adenosine deaminase, ALP: Alkaline phosphatase

Table 2: Distribution of mean ADA and ALP with tubercular and non-tubercular effusions

Parameters	Diseases	Mean	SD	Minimum	Maximum	Median
ADA						
Tuberculosis	41	58.37	26.419	32.120	164.700	49.990
Non-tuberculosis	41	23.47	9.297	10.210	46.700	21.730
ALP						
Tuberculosis	41	66.730	36.752	21.000	167.000	55.000
Non-tuberculosis	41	43.193	38.934	10.000	243.900	32.420

ADA: Adenosine deaminase, ALP: Alkaline phosphatase, SD: Standard deviation

Table 3: Mann–Whitney U test for ADA and ALP with tubercular and non-tubercular effusions

Diseases with Parameters	No	Mean rank	Sum of rank	Asymp Sig. (2-tailed)	Z-score
Tuberculosis with ADA	41	60.59	2484.00	0.000	−7.257
Non-tuberculosis with ADA	41	22.41	1279.00		
Tuberculosis with ALP	41	51.80	2124.00	0.000	−3.919
Non-tuberculosis with ALP	41	31.20	1279.00		

ADA: Adenosine deaminase, ALP: Alkaline phosphatase

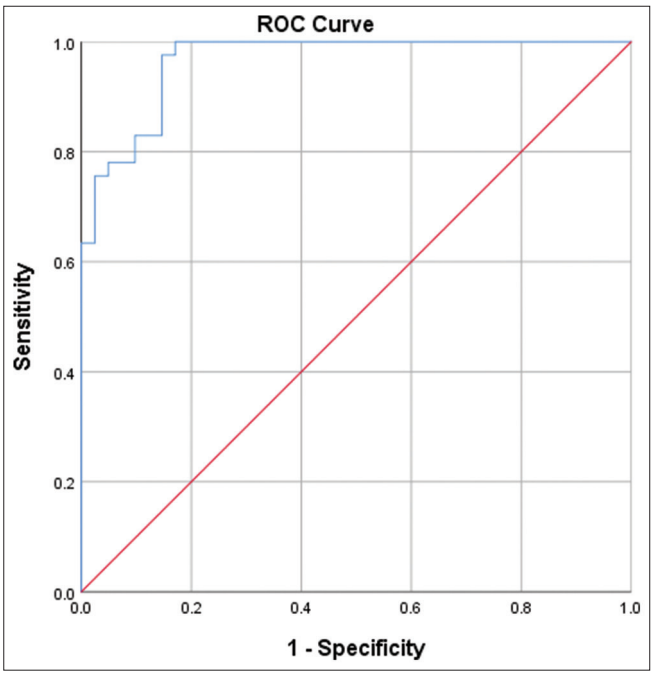

Figure 1: ROC for ADA with tubercular and non-tubercular effusions (The positive actual state is tuberculosis). ROC: Receiver operating characteristic, ADA: Adenosine deaminase

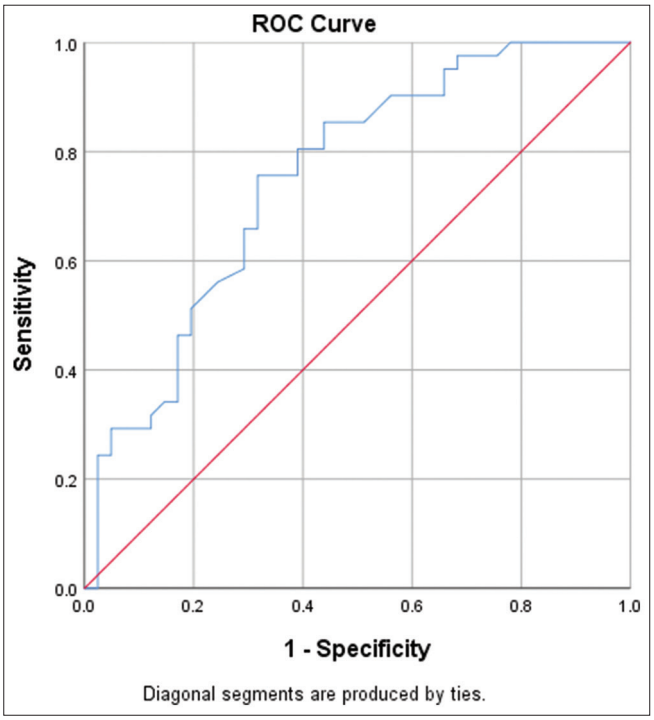

Figure 2: ROC for ALP with tubercular and non-tubercular effusions (the positive actual state is tuberculosis). ROC: Receiver operating characteristic, ALP: Alkaline phosphatase

ALP activity.[4] It has been also observed that PFALP more than 75 IU/L is a useful biochemical marker for separating exudative effusions from transudative effusions.[7] In this present study, it has been found that ROC curve of ALP shows a sensitivity of 75.6% and specificity of 68.3% for a cutoff value of more than 40.5 IU/L in pleural effusions. It has also found that cutoff value of more than 75 IU/L has a sensitivity of 34.1% and specificity of 85.4% for diagnosing tuberculosis. Hence, it can be proposed that a lower cutoff should be considered for separating tubercular from non-tubercular effusions now a day.

Limitations of the study

All the analytes could have been assessed in better analytical instruments for better output.

CONCLUSION

1. ADA alone is still the best criteria to differentiate between tubercular and non-tubercular effusions
2. ALP can be used as the secondary parameter which points toward tubercular effusions (AUC: 0.751). This helps especially in rural and peripheral minimalistic setup where ALP is regularly done as a liver function test parameter. No separate logistics help is required for assessing ALP in diagnosis of tubercular effusions. ALP assessment is also cost-effective than ADA assessment.

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Work attributed to:
Calcutta National Medical College and Hospital, Kolkata - 700 014, West Bengal, India.

ORCID ID:
Dr. Santanu Mondal - https://orcid.org/0009-0005-3129-8537
Dr. Swati Bhattacharyya - https://orcid.org/0000-0003-0563-5518
Dr. Subhra Mitra - https://orcid.org/0000-0001-7161-7014

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