DIAGNOSTIC CUT-OFF OF PLEURAL FLUID ADENOSINE DEAMINASE (ADA) VALUE IN TUBERCULOUS PLEURAL EFFUSION

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ABSTRACT

BACKGROUND

Among the various aetiologies of exudative pleural effusion, tuberculosis still remains high in the list, especially in developing countries like India. Tuberculous pleural effusion is the second most common form of extrapulmonary tuberculosis, just behind lymph node tuberculosis. Establishing tuberculosis as the aetiology of pleural effusion is hence very important. Though demonstration of acid fast bacilli in pleural fluid by smear/culture or caseating granuloma in the histopathological examination of pleural biopsy are diagnostic, these are not feasible in all cases. In this context comes the diagnostic value of adenosine deaminase (ADA) in pleural fluid. This is a biochemical test done in pleural fluid, which can give a clue towards the diagnosis of tuberculous aetiology. Several studies have shown different cut-off values of ADA, above which it is considered as positive. Hence, this study was planned to find out the best cut-off value of ADA in diagnosing tuberculous pleural effusion in a tertiary care centre in Trivandrum, Kerala.

The aim of this study is to determine the best cut-off value of ADA that can diagnose tuberculous pleural effusion.

MATERIALS AND METHODS

Settings and Design- This was a descriptive study done in the Department of Pulmonary Medicine, GMC, Trivandrum. Study population included consecutive cases of exudative pleural effusion, attending the Department during the period from January 2015 to April 2016. All exudative pleural effusions during the period were taken and a total of 175 cases (80 tuberculous) were studied. A case was taken as tuberculous using defined criteria. Patients with empyema and post-traumatic effusions were excluded from the study.

Statistical Analysis- Analysis was done using SPSS version 22.0. Sensitivity and specificity of pleural fluid ADA in diagnosing tuberculosis were determined. An ROC curve was plotted to find the optimum cut-off value of ADA to predict a diagnosis of tuberculosis. This was further verified using the Youden's index.

RESULTS

A cut-off value of pleural fluid ADA of 38.3 U/L had a sensitivity of 93% and a specificity of 97%. Youden's index was maximum (0.89) for this value, which was taken as the optimum cut off.

CONCLUSION

Pleural fluid ADA levels were significantly higher in tuberculous pleural effusion when compared to the non-tuberculous group. The cut-off value of 38.3 U/L with the maximum Youden's index was comparable with previous studies published.

KEYWORDS

Adenosine Deaminase, Tuberculosis, Pleural Effusion.

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BACKGROUND

Pleural effusion is a common clinical problem encountered by physicians globally. They are mainly classified as exudative and transudative. Transudative effusions are often secondary to some other systemic illnesses like cardiac failure, nephrotic syndrome, cirrhosis of liver, hypoproteinaemia etc. Exudative effusions occur secondary to infections, malignancy, collagen vascular diseases etc. Among the causes of exudative pleural effusions, tuberculosis still ranks high in the list, especially in developing countries like India. Tuberculous pleural effusion

is the second most common extrapulmonary tuberculosis just behind lymph node tuberculosis. Hence, it is very important in diagnosing tuberculosis as the aetiology of pleural effusion. Tuberculous effusions are mainly lymphocytic pleural effusions and the main differential is a malignant pleural effusion. A definitive diagnosis can be obtained if acid fast bacilli are demonstrated in pleural fluid/ pleural biopsy specimen by direct smear/ culture or if a histopathological evidence of caseating granuloma is available. This is not yielding in all cases, as the positivity of AFB in pleural fluid is very low (5% - 10%).¹ Culture is time consuming (Yield around 36% for mycobacterium tuberculosis) and pleural biopsy is an invasive procedure.¹ So it will be of great use, if there is a reliable molecular marker that can rapidly and more or less accurately differentiate tuberculous pleural effusion from non-tuberculous effusions, mainly malignant. In this context comes the value of adenosine deaminase (ADA), which is a biochemical investigation done in pleural fluid that can give a clue towards the diagnosis of tuberculosis as the aetiology of pleural effusion. Several meta-analyses have

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shown that ADA has a favourable diagnostic value in tuberculous pleural effusions.^{2,3,4}

ADA is an enzyme which catalyses the conversion of adenosine to inosine. It has a major role in differentiation of lymphoid cells. Its activity is high in conditions where the cellular immunity is stimulated.⁵ ADA has two isoforms, ADA-1 and 2. ADA-1 is found in all cells including lymphocytes and monocytes, whereas ADA-2 is seen exclusively in monocytes. ADA-2 is the one which increases in tuberculous pleural effusion. But for practical purposes in clinical field, total ADA activity assessment is enough. Usefulness of ADA in diagnosing tuberculosis depends on the prevalence of disease in the population. In the West, where the prevalence is low positive predictive value of the test decreases, but negative predictive value is high. Hence, this can be used as an effective screening test. In India, where there is high tuberculosis prevalence, the sensitivity and specificity of ADA is high.⁶ Several studies have shown different cut-off values for ADA (Ranging from 30 - 100 IU/L), above which it is taken as positive.⁷ Hence, a study was planned in the Department of Pulmonary Medicine, Government Medical College, Trivandrum, to find out the optimum cut-off value of ADA that can diagnose tuberculous pleural effusions.

MATERIALS AND METHODS

This was a descriptive study done in the Department of Pulmonary Medicine, Government Medical College, Trivandrum, during the period from January 2015 to April 2016. The study population included all patients with exudative pleural effusion (according to Light's criteria), who attended the Department during the study period. Patients with frank empyema and post traumatic effusions were excluded from the study. After history and clinical examination, radiological evaluation with a chest x-ray (USG chest and CT chest only in selected cases) was done in all. Pleural fluid aspiration was done in all cases and fluid analysis was done (biochemistry including ADA, microbiology and cytology). Percutaneous blind pleural biopsy was done in a few selected patients. Thoracoscopy was not done as the facility was not available in the hospital during the study period. Pleural fluid ADA was done using diazyme method in all patients to ensure uniformity in the results. Sputum for AFB/cytology, lymph node aspiration cytology etc. were done in indicated patients. A case was diagnosed as tuberculous pleural effusion if any one of the following was present;

- Sputum for AFB positive.
- Pleural fluid AFB smear/ culture positive.
- Histopathological or cytological evidence of tuberculosis from pleural biopsy, lymph node or other sites.
- If all were negative, a clinical and radiological picture suggestive of tuberculosis with definite improvement on anti-tuberculosis treatment was taken as diagnostic of tuberculous pleural effusion.

All the 175 cases of exudative pleural effusions who attended the Department during the study period were taken for the study, which included 80 cases with tuberculous aetiology. Institutional Ethics Committee clearance was obtained prior to the conduct of the study.

Statistical analysis was done using SPSS version 22.0. Quantitative variables were expressed as mean and standard deviation. Categorical variables were expressed as proportion.

A p-value < 0.05 was considered statistically significant. Sensitivity and specificity of pleural fluid ADA in diagnosing tuberculosis were found out. An ROC curve was plotted to find out the optimum cut-off value of pleural fluid ADA to predict tuberculosis diagnosis. This value was further verified using Youden's index.

RESULTS

Of the 175 cases studied, 80 were diagnosed as tuberculous effusion.

The following table shows the baseline characteristics of the two groups, tuberculous and non-tuberculous. The male: female ratio was 2.3:1 among the tuberculous group and 1.7:1 among the non-tuberculous group.

	Tuberculous Effusion (N=80)	Non-Tuberculous Effusion (N=95)
Male: Female	56:24	60:35
Mean age $\pm$ 2 SD	41.46 $\pm$ 34.26 years	54.82 $\pm$ 28.4 years
History of Diabetes Mellitus	19	49
History of Smoking	38	54
Smoking Index >500	0	4

Most of the cases who were smokers had a smoking index between 300 and 400. Among the non-tuberculous group 4 cases had a smoking index more than 500, whereas none in the tuberculous group had the index > 500. A diagnosis of tuberculosis was made in three cases each with sputum AFB positivity and a diagnostic histopathology in blind pleural biopsy specimen. Five cases had an aspiration cytology from cervical lymph node, suggestive of tuberculosis.

The table below shows the aetiological distribution of non-tuberculous pleural effusion.

Diagnosis	Number of Cases
Syn-pneumonic effusion	60
Malignancy	33
Connective tissue disease	1
Pancreatic effusion	1

The split up of malignant effusions was as follows:

Diagnosis	Number of Cases
Metastatic adenocarcinoma	26
Non-Hodgkin's lymphoma	2
Plasma cell myeloma	1
Poorly differentiated squamous cell carcinoma	1
Poorly differentiated adenocarcinoma	1
Small cell carcinoma	1
Metastasis from poorly differentiated carcinoma	1

The pleural fluid was straw coloured in all cases of tuberculous pleural effusion. In non-tuberculous group, 37 had a haemorrhagic pleural fluid macroscopically. The pleural fluid differential counts showed a mean lymphocyte count of 0.906 (SD 0.106) among tuberculous pleural effusions.

The mean ADA value was 60.09 ± 38.36 IU/L among the cases with tuberculous effusion and 21.01 ± 22.82 IU/L in the other group. An ROC curve plotted for pleural fluid ADA values for tuberculous pleural effusions showed an AUC of 0.977. At a cut-off value of 38.3 IU/L, pleural fluid ADA showed a sensitivity of 93% and a specificity of 97% in diagnosing tuberculosis aetiology. This was further verified using Youden's index, which was maximum (0.89) at the above cut-off value.

ROC Curve

Area	Standard Error	P	95% CI
0.977	0.012	0.000	0.954 - 1.000

Youden's Index

ADA Value (IU/L)	Sensitivity (Sn)	Specificity (Sp)	Youden's Index = (Sn+Sp) - 1
>15	100%	33%	0.33
>20	100%	57%	0.57
>25	100%	77%	0.77
>30	99%	86%	0.84
>35	96%	91%	0.87
>38.3	93%	97%	0.89
>40	90%	97%	0.86
>45	77%	98%	0.74
>50	66%	98%	0.64
>55	51%	98%	0.48
>60	42%	98%	0.39
>65	32%	98%	0.30
>70	24%	99%	0.23
>75	20%	99%	0.19
>80	17%	100%	0.17

The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of pleural fluid ADA as a diagnostic test is given below.

Pleural Fluid ADA (IU/L)	Tuberculous Effusion (N=80)	Non-Tuberculous Effusion (N=95)	Total
≥ 38.3	74 (True positive)	3 (False positive)	77
< 38.3	6 (False negative)	92 (True negative)	98

Sensitivity = $74/80 \times 100 = 92.5\%$

Specificity = $92/95 \times 100 = 97\%$

Positive predictive value = $74/77 \times 100 = 96\%$

Negative predictive value = $92/98 \times 100 = 94\%$

Diagnostic accuracy = (True positive + True negative)/ Total cases $\times 100 = 166/175 \times 100 = 95\%$

DISCUSSION

The major diagnoses among non-tuberculous pleural effusions in our study were syn-pneumonic effusions and malignant effusions. One case each of connective tissue associated effusion and pancreatic effusion were also encountered. Literature also reveals a similar pattern of aetiology of pleural effusion in clinical practice. If we narrow down the cases to lymphocyte predominant pleural effusions, then the major differential will be malignant effusions. A study by Sahn SA et al says that if the fluid lymphocyte percentage is more than 85%, it is very much suggestive of tuberculous pleural effusion.⁸ Another study by Berger HW et al shows that among tuberculous pleural effusions, only around 10% had a lymphocyte count in pleural fluid of less than 50%.⁹ The mean lymphocyte ratio in tuberculous pleural effusions in our study was 0.906 (SD 0.106) and that of the non-tuberculous group was 0.204.

The mean ADA of tuberculous pleural effusion in our study was 60.09 (SD 19.18) IU/L. Moreover, when a cut-off value of 38.3 IU/L of ADA was used, there was maximum sensitivity and specificity for the diagnosis of tuberculosis. Various studies have taken cut-off values ranging from 30 to 100 IU/L of pleural fluid ADA in diagnosing tuberculosis with varying sensitivity and specificity.⁷ The difference in the values may be due to the different methods used for ADA analysis, prevalence of tuberculosis in different study groups and also difference in study population characteristics. For ensuring uniformity in results, we had used the same method for analysis of ADA (diazyme method) in our study. A study done by Tay, Tee et al concluded that the cut-off level of ADA for diagnosing tuberculous pleural effusion was different in different age groups (72 IU/L in < 55 years and 26 IU/L in > 55 years' age group).¹⁰ The same study also analysed other variables that can affect the pleural fluid ADA level. Though the strongest correlation was with age, other factors like pleural fluid protein, LDH and fluid lymphocyte count also were found to influence the level of ADA.

Most studies use a cut-off value of ADA of > 40 IU/L for diagnosing tuberculous pleural effusions. Our study revealed a cut-off value of 38.3 IU/L. Another Indian study by Sharma SK et al had the cut-off of 35 IU/L for ADA, which is comparable to our value.¹¹ When ADA values are combined with a pleural fluid lymphocyte ratio more than 50%, the chances of making a diagnosis of tuberculosis are even higher. In a study done by Rahman et al in Bihar, India, pleural fluid ADA value > 50 IU/L showed a sensitivity of 97.6% and a specificity of 87.5% in diagnosing tuberculous pleural effusion. When this was combined with a pleural fluid lymphocyte ratio > 0.75 , the sensitivity increased to 100% and specificity to 92.8%.¹² This

was earlier established in other studies too using it as a tool for diagnosing tuberculous effusion.^{13,14,15} Combined use of ADA and lymphocyte- neutrophil ratio in pleural fluid was found to be more useful in diagnosing tuberculous effusion than ADA alone. The study by Antin et al showed that the pleural fluid lymphocyte ratio > 0.75 was a significant indicator of tuberculous pleurisy when compared to other lymphocyte predominant exudative effusions.¹⁴

Making a definitive diagnosis with a gold standard is often difficult in tuberculous pleural effusion. Though we had put forward the criteria for diagnosing tuberculosis, a gold standard diagnosis was available only in eleven cases. Most of the cases had a clinical diagnosis. It is in these situations that ADA plays a helpful role. Searching the literature, we found that many of the other studies also have taken diagnostic criteria similar to ours and were not based on gold standard diagnosis alone. Lack of thoracoscopy to take pleural biopsy was a limitation of our study. Valdes L et al in his study reiterates that with the high diagnostic yield of pleural fluid ADA in regions with high prevalence of tuberculosis it would be possible to diagnose tuberculous pleural effusions without pleural biopsy. Biopsy should be reserved for patients with low ADA, a negative fluid cytology and a high suspicion of malignancy.¹⁶

The following table compares the cut-off value taken for ADA in some previous studies with ours, and the sensitivity, specificity, positive predictive value and negative predictive value for each. The mean ADA value for tuberculous effusions in these studies are also shown.

Author (Year)	Mean ADA Value	ADA Cut-Off	Sensitivity	Specificity	PPV	NPV
Gupta BK (2010) ⁶	67.34 ± 22.85	40 U/L	92.8	90	92.86	90
Sonone KK (2014)	82.61 ± 12.03	33.3 U/L	93.93	96.69	96.87	94.11
Verma SK (2008) ¹⁷	---	36 U/L	100	77	---	---
Dave KK (2013) ¹⁸	---	60 U/L	69.2	92	90	74
Helmy NA (2012) ¹⁹	83.5 ± 50.3	30 U/L	80	85	84.2	81
Chen ML (2004) ²⁰	---	55.8 U/L	87.3	91.8	82.1	94.4
Sharma (2001) ¹¹	95.8 ± 57.5	35 U/L 100 U/L	83.3 40	66.6 100	---	---
Mehta A (2014) ²¹	64.11 ± 32.33	40 U/L	85.7	80.8	75	89.5
Nayak M (2017) ²²	84.99 ± 45.45	40 U/L	90	96.67	96.43	90.62
Riantawan P (1999) ²³	---	60 U/L	95	96	96	95
Yu YX (2010) ²⁴	---	28.7 U/L	75.5	95.2	---	---
Current Study	60.09 ± 38.36	38.3 U/L	92.5	97	96	94

CONCLUSION

The mean ADA values were significantly higher in tuberculous pleural effusions when compared to the non-tuberculous group. At the cut-off value of 38.3 U/L, ADA had a sensitivity of 93% and specificity of 97% in diagnosing tuberculosis. The above value had the maximum Youden's index (0.89) also.

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