Original Research Article

Sensitivity and Specificity of Pleural Fluid Adenosine Deaminase (ADA) and Pleural Fluid Lymphocyte/Neutrophil Ratio in the Diagnosis of Tubercular Pleural Effusion

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Abstract

Background: Pleural effusion is one of the most prevalent extrapulmonary manifestations of tuberculosis. The conventional culture method of diagnosis suffers from sensitivity. Hence, the current research was commenced with the primary aim to suggest a better diagnostic of pleural effusion of tubercular origin by assessing adenosine deaminase ADA activity in combination with lymphocyte/neutrophil (L/N) ratio than with ADA use alone. **Settings and Design:** Data from 60 pleural effusion study subjects were gathered using a pretested proforma, fulfilling the study objectives. **Materials and Methods:** A physical examination, detailed history and needed inspections were made. The diagnosis of tubercular pleural effusion was done based on the identification of bacilli in pleural fluid or culture of the fluid or radiological & clinical tuberculosis evidence and absenteeism of any other obvious cause related to pleural effusion & those who showed a positive response to antitubercular treatment. ADA was estimated for all exudative pleural fluid specimens. The specificity, sensitivity, PPV (positive predictive value) and NPV (negative predictive value) were determined to distinguish among non-tubercular and tubercular pleural effusion. **Statistical analysis used:** Investigations were analyzed with the clinical profile, and the data were compiled, and the appropriate statistical analysis was performed with the use of SPSS software. **Results:** The majority of study subjects, i.e., 26.70%, belonged to 21-30 years of age group with male predominance (51.70%) compared to females (48.30%). In 26.7% of study subjects, plural culture tested positive for mycobacterial growth. Whereas 70.0% of study subjects were diagnosed based on positive for pleural biopsy at the 50 U/L ADA level, the specificity, sensitivity, NPV, PPV and efficacy for TB identification were computed at 89.8%, 81.8%, 95.6%, 64.3%, and 88.3% correspondingly. When the added requirement of an L/N ratio of 0.75 or greater was comprised, specificity, the sensitivity, NPV, PPV and efficacy for the TB identification were computed at 95.7%, 86.9%, 93.7%, 83.3%, and 91.7%, respectively. **Conclusion:** It was demonstrated through this study that pleural fluid adenosine deaminase in combination with the L/N ratio has remained a valuable test in tuberculous pleuritis diagnosis.

Key-words: Tuberculosis, pleural effusion, ADA, L/N ratio, sensitivity, specificity.

Introduction

Tuberculosis (TB) is still a leading mortality and morbidity cause globally. An approximated ten million individuals contracted tuberculosis in 2019 worldwide. 1.2 million children, 3.2 million women and 5.6 million men. Tuberculosis (TB) affects people of all ages and present in all countries[1]. However, Tuberculosis is preventable and curable. TB (Tuberculosis) is an infectious disease caused by the bacteria Mycobacterium tuberculosis and, less frequently, by other tuberculosis complex organisms. Pleural effusion is one of the most prevalent extrapulmonary symptoms of tuberculosis. It is essential to take into consideration the possible TB pleuritis risks in all study subjects without diagnosing pleural effusion[2]. Step by step Tuberculosis pleural effusion diagnosis is consequently the same as that for any other exudative pleural effusion. A diagnostic thoracocentesis is always recommended as a preliminary indication. Due to the low specificity and sensitivity of non-invasive diagnostic tools, making a definitive TB pleural effusion diagnosis can be challenging.

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Able to detect the bacterium on culture or smear is regarded as the golden standard, but it lacks sensitivity as well as takes a long time to provide results. Therefore AFB (acid-fast bacilli) staining is positive in only 10-25 per cent of cases, whereas AFB culture is positive in less than 25% of cases[3]. Many pleural fluid markers were evaluated for the diagnosis of TB pleural effusion; even so, none has been found to be ideal due to lack of specificity or sensitivity, availability, or difficulty in executing etc.

In as many as 65 per cent of study subjects, failing to diagnose as well as treat pleural tuberculosis can lead to progressive illness with other organ involvement. The enzyme ADA (adenosine deaminase), which is involved in the metabolism of purine, is 10 times highly concentrated in lymphocytes compared to that in erythrocytes. Adenosine deaminase is a strong predictor of cellular immunity that is active. The pleural fluid activity of adenosine deaminase is one of the finest, offering a reliable basis for treatment decision, especially in exclusion of tuberculosis diagnosis, as it is highly sensitivity[4]. Even though several research has shown that high adenosine deaminase is diagnostically significant in tuberculous pleurisy; however, other investigations have demonstrated that adenosine deaminase is of limited use because elevated levels are also related to numerous other disorders which include bacterial infections (fever, brucellosis),[5,6] malignancies (particularly those of haematologic origin), empyemas as well as other collagen vascular illness namely rheumatoid arthritis viz. SLE (systemic lupus erythematosus). Pleural effusions might

cause systemic or secondary pulmonary disease, and their progression is typically related to inflammatory cells' influx into the pleural space[7]. In tubercular and malignant pleural effusions, there is a predominance of lymphocytes[8]. With this viewpoint, we endeavoured to find out the specificity and sensitivity of pleural fluid ADA, and pleural fluid L/N ratio & their combination for the tubercular pleural effusion diagnosis will offer more effective means for TB pleurisy diagnosis than using adenosine deaminase levels alone.

Materials and methods

A case-control research was carried out at a tertiary care hospital from August 2018 to August 2019. Overall, 60 specimens of pleural fluid from study subjects admitted to the pulmonary & medical ward were examined. Study subjects were categorized as transudative & exudative pleural effusions on the basis of the Light's criteria[9]. Ethical approval was obtained from the institutional ethics committee before start of study. All cases of exudative effusion were included in the study, and written informed consent was obtained. Study subjects with transudative pleural effusion, malignant pleural effusion, immunodeficient states like HIV/AIDS, those on chemotherapy, study subjects having hemothorax or empyemas too turbid for analysis were omitted from the study. Radiographs of the Lateral and Posteroanterior chest were performed for all cases. ADA was estimated for all exudative pleural fluid specimens as per the description of the Giusti and Galanti method, and the ADA activity was determined by using conversion factor, i.e., 50[10]. The specificity, sensitivity, NPV (negative predictive value) and PPV (positive predictive value) were estimated to distinguish between non-tubercular & tubercular pleural effusion.

After obtaining the study subject's consent, a skin test was carried out with 2% xylocaine to determine if the patient is sensitive to xylocaine. The site for pleural biopsy was selected by noting the area of maximum dullness over the intracapsular area near the posterior axillary line, which is usually the 8th intercostal space. The area was prepared by painting with tincture iodine followed by spirit. The area was infiltrated with xylocaine, and a skin incision of 3-4 mm was made. Abraham's biopsy punch was introduced into the pleural cavity with rotator movements. The inner tube was turned anti-clockwise, and the stylet was withdrawn, which causes the pleural fluid to come out, indicating that the needle tip is in the pleural space. The notch was directed along with the intercostal space with the help of an indicator knob situated on the front of the hexagonal grip. The punch was withdrawn slowly, & pressure on sideways was maintained in the direction of notch till it was felt to engage in the chest wall. Then, the punch was firmly held against the chest wall, the back of the hexagonal grip was sharply twisted clockwise, and the punch was withdrawn. The finger pressure was applied at the biopsy site for 2-3 minutes to stop bleeding. The specimen was either found inside the cutting cylinder or in the hollow point. The tissue was preserved in 10% buffered formalin solution and subjected to histopathological examination. A single suture was put at the biopsy site, and then dressing was applied. A pleural fluid sample was delivered to microbiology for bacterial tuberculosis culture, and a differential cell count also was performed on the additional sample, which was sent to cytology. All study subjects with exudative effusions had their medical records examined, & diagnoses were made using the preceding predetermined criteria.

Tuberculous pleuritis was diagnosed on the basis of:

1. The granulomas presence in pleural biopsy tissue, or identifying bacilli in biopsy specimen or pleural fluid by culture or by a stain.
2. Radiologic and clinical evidence for tuberculosis in the absence of any other clear cause is related to pleural effusions & linked to a positive response to anti-tuberculous treatment.

Identification of Infective effusions

Pneumonic effusions correlated to purulent sputum, pulmonary pneumonic infiltrates, acute febrile illness, and responsiveness to antibiotic therapy or identifying organism in the pleural fluid; septicaemia, defined by radiological indications of pulmonary infiltrates as well as multi-system involvement in the presence of positive blood cultures; and other evident infective conditions in the absence of any other factor linked to pleural effusions. Empyematous effusions, which are identified by the presence of frank pus in the pleural cavity, are involved if the turbidity of the specimen did not hinder the related examinations.

Diagnosis Neoplastic effusions were done when one of the following requirements was met:

1. The presence of cytological or histological indication of a malignant pleural effusion
2. Cases of pleural effusion with a known malignancy at other sites
3. Histopathological confirmation with the exclusion of any other cause found to be linked to pleural effusions.

Other exudates were classified as effusions evidently caused by Dressler's syndrome, pancreatitis, collagen vascular disease, pulmonary embolus or infarction, & a variety of other rare but well documented exudative pleural effusion causes. There were no signs of pulmonary infiltrates, malignancy as well as illnesses that caused transudates in any of the cases. Study subjects that had several superimposed illnesses or effusion of unclear aetiology were classified as "undiagnosed". In all the study subjects with exudative pleural effusions, the diagnosis was confirmed by pleural fluid culture and/or pleural biopsy, and these study subjects were treated with antitubercular treatment and were followed up. Data was collected by using a structured proforma. Descriptive analysis was conducted by the mean, standard deviation for quantitative variables and frequency & proportions for qualitative variables. Correlation among two qualitative variables was evaluated by using the Chi-square test. Cut-off values for all variables were calculated using the receiver operating characteristic curve (ROC) curve. The calculation of specificity, sensitivity, NPV and PPV was done at a specific cut off for the adenosine deaminase and lymphocyte/neutrophil ratio. Statistical significance was determined when $p<0.05$.

Results

The majority of study subjects, i.e., 26.70%, belonged to the group 21 to 30 years of age with male predominance (51.70%) as compared to females (48.30%) [Table 1].

Table 1: Distribution of study subjects based on age and gender

Age group in years	Frequency	Percent
< 20	2	3.30
21-30	16	26.70
31-40	11	18.30
41-50	10	16.70
51-60	7	11.70
> 60	14	23.30
Gender	**Frequency**	**Percent**
Female	29	48.30
Male	31	51.70

Among 60 subjects, 20% were smokers, 15% had a history of diabetes, and 13.3% of the study subjects were tested HIV positive. The positive pleural fluid culture was observed in 26.7% of study subjects [Table 2].

Table 2: Distribution of study subjects based on history

		Frequency	Percent
Smoker	Yes	12	20.0
	No	48	80.0
Diabetes	Yes	9	15.0
	No	51	85.0
HIV	Yes	8	13.3
	No	52	86.7
Pleural fluid culture	Positive	16	26.7
	Negative	44	73.3

In 26.7% of study subjects, plural culture tested positive for mycobacterial growth. Whereas 70.0% of study subjects were diagnosed based on positive for pleural biopsy [Table 3].

Table 3: Showing results of Pleural culture and pleural biopsy of study subjects

		Frequency	Percent
Pleural fluid culture with mycobacterial growth	Positive	16	26.7
	Negative	44	73.3
Pleural biopsy	Positive	42	70.0
	Negative	18	30.0

The ADA level of study subjects was >50 and <50 in 76.70% and 23.30% respectively. Similarly, L/N ratio of study subjects was >0.75 and < 0.75 in 71.70% and 28.30% of study subjects correspondingly. Adenosine deaminase and L/N ratio combination i.e., > 50 and >0.75 &< 50 and < 0.75 was observed in 80 % and 20 % of subjects respectively [Table 4].

Table 4: Showing results of ADA, L/N ratio, and combination of ADA, L/N ratio

		Frequency	Percent
ADA level	> 50	46	76.7
	< 50	14	23.3
	Total	**60**	**100.0**
L/N ratio	> 0.75	43	71.7
	< 0.75	17	28.3
	Total	**60**	**100.0**
ADA and L/N ratio combined	> 50 and >0.75	48	80.0
	< 50 and < 0.75	12	20.0
	Total	**60**	**100.0**

ADA levels > 50 U/L and L/N ratio > 0.75 were considered as cut-off values for TB pleural effusion. A total of 46 study subjects had ADA > 50 U/L, of which in 44 tubercular effusion was observed, whereas, in 2 subjects, tubercular effusion was not detected. Lymphocyte/Neutrophil ratio of >0.75 was seen in a total of 43 subjects, of which in 40 tubercular effusion was observed, whereas in 3 subjects, tubercular effusion was not detected [Table 5].

Table 5: Showing results of ADA and L/N ratio grading among study subjects

		Present	Absent	Total
ADA grading	> 50	44 (89.8%)	2 (18.2%)	46
	< 50	5 (11.2%)	9 (81.8%)	14
	Total	49	11	60
L/N ratio	> 0.75	40 (81.6%)	3 (27.8%)	43
	< 0.75	9 (18.4%)	8 (72.2%)	17
	Total	49	11	60

As per the ADA ROC curve, 89.80% was sensitivity, 81.80% was specificity, respectively with PPV (positive predictive value) of 95.60%, NPV(negative predictive value) was 64.30%, & 88.30% was the accuracy. The area under the curve was found to be 0.94 [Figure 1]

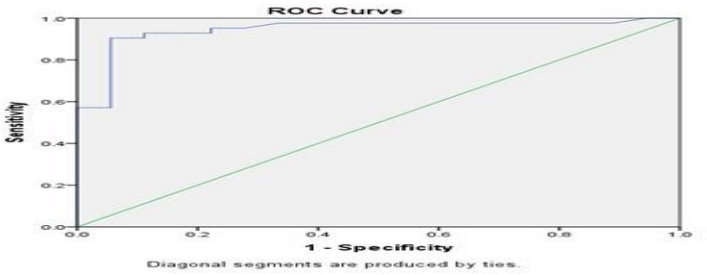

Figure 1: ROC curve showing the sensitivity of ADA

According to the ROC curve of L/N ratio, sensitivity was 81.6%, specificity was 72.7%, PPV was 90.3%, NPV was 47.5% and accuracy-78.3%. The area under the curve was found to be 0.53 [Figure 2].

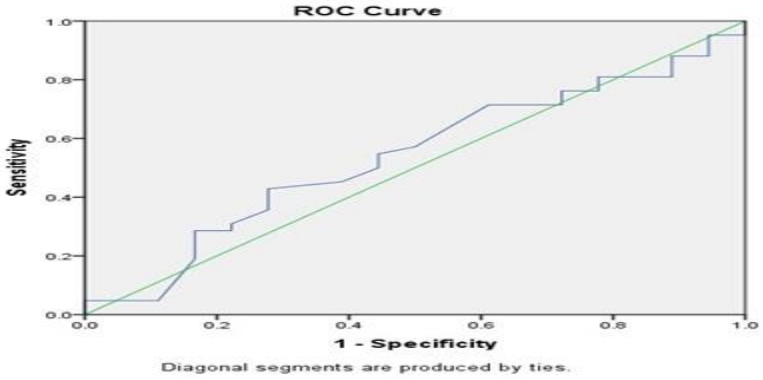

Fig 2: ROC curve showing the Sensitivity of L/N ratio

According to the ROC curve of adenosine deaminase and L/N ratio, sensitivity was 95.7%, specificity was 76.9%, PPV was 93.7%, NPV was 83.3%, and accuracy was 91.7%. The area under the curve was found to be 0.94[Figure 3].

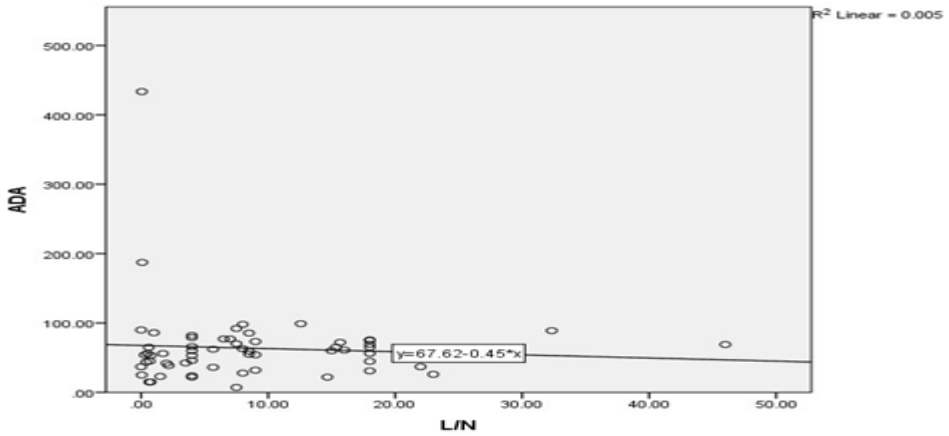

Fig 3: Correlation of adenosine deaminase and L/N ratio interactive dot diagram

The specificity, sensitivity, NPV of ADA, PPV, L/N ratio, combined ADA & L/N ratio were represented in Table 6.

Table 6: Sensitivity, specificity, PPV, NPV of ADA, L/N ratio, Combined ADA and L/N ratio

	Sensitivity	Specificity	PPV	NPV
ADA	89.9%	81.8%	95.6%	64.3%
L/N ratio	81.6%	72.7%	90.3%	47.5%
ADA and L/N ratio	95.7%	76.9%	93.7%	83.3%

Discussion

A common symptom of extrapulmonary tuberculosis is tubercular pleural effusion[11]. It happens when a subpleural focus ruptures, leading to exposure of tubercular antigen to helper T cells, causing delayed-type hypersensitivity[12]. This mechanism is observed in both reactivation & primary tuberculosis cases. As seen clearly, the pleural fluid will either have less bacteria or no bacteria[13]. Since pleural Tuberculosis is a paucibacillary illness, low sensitivity will be observed in cases of direct microscopy (0 to 5 per cent) and mycobacterial culture (25 to 35 per cent), which are gold-standard procedures for effusion diagnosis[14]. High sensitivity is seen only when a pleural biopsy is performed through thoracoscopy. To distinguish between non-tubercular & tubercular pleural effusion, a variety of alternative new tests were investigated. Pleural IFN, serum Cancer Antigen 125 (CA 125), pleural fluid ADA, pleural fluid procalcitonin concentration, pleural fluid PCR, pleural alkaline phosphatase, pleural tumour necrosis factor, pleural fluid Interleukin-1 etc. were also included. As previously discussed, the alternative novel tests' efficiency is typically carried out against the gold standard, which is a mycobacterial culture having low sensitivity. Findings of these tests must be evaluated with caution, considering complete clinical as well as other supportive laboratory factors, & must not be blindly followed. Several authors have reported higher adenosine deaminase and lymphocyte/neutrophil ratio levels in TB pleural effusion; however, the combination of these markers have not been evaluated adequately in the past. Hence, in our study, we endeavoured to assess the specificity as well as the sensitivity of pleural fluid adenosine deaminase and pleural fluid L/N ratio and their combination for the tubercular pleural effusion diagnosis will offer far more effective means for TB pleurisy diagnosis compared to using adenosine deaminase levels alone.

ADA is a polymorphic enzyme that catalyses the deamination of adenosine and deoxyadenosine, producing inosine and deoxyinosine, correspondingly, during purine catabolism. Even though it is detected

in most human tissues, its activity is highest in lymphoid tissues,[15] where it is involved in lymphoid cell differentiation,[16,17] and in monocytes to macrophages maturation[18]. Adenosine deaminase is a non-specific inflammation marker secreted by activated neutrophils, lymphocytes and macrophages. It can be raised in both non-tubercular and tubercular pleural effusion. Both non-tubercular and tubercular pleural effusions can cause it to rise. The ADA2 isoenzyme, produced by macrophages and monocytes, is found to predominant in tubercular pleural effusion and is the most dominant booster of aggregate adenosine deaminase activity[19]. If in pleural effusions, an increased activity is seen, it is typically linked to TB[20-23]. Though, it might happen owing to numerous reasons, which might affect the diagnostic utility of Adenosine deaminase measurements negatively as well as reduce its specificity in TB diagnosis[20]. Our study findings revealed that with a 50 U/L cut-off, Adenosine deaminase was identified to have specificity, sensitivity, PPV, NPV, as well as efficiency of 89.8%, 81.8%, 95.6%, 64.3%, and 88.3%, correspondingly, which is quite comparable to several other research findings[24-28]. It should be observed, though, that this investigation only included exudates. All transudates with low levels of ADA (less than 20U/L) were eliminated. The ADA presence in pleural fluids indicates the pleural cavity's cellular response, particularly lymphocyte activation[29]. Identifying certain pleural fluid adenosine deaminase isoenzyme patterns in various pleural ailments highlights this corelation between adenosine deaminase and various cellular as well as immunological responses that occurs in the pleural cavity When lymphocyte/neutrophil ratio (≥0.75) was deemed along with the activity of adenosine deaminase (≥50U/L),[30] the outcomes considerably improved for diagnosing tuberculous pleuritis. The specificity, sensitivity, NPV, PPV as well as the efficacy were 95.7%, 76.9%, 93.7%, 83.3%, and 91.7% respectively.

It is unclear what causes the elevated activity of ADA reported in TB effusions. Several researchers suggest that the elevated levels are related to the reasons that TB pleurisy is a T-cell driven response[12,31]. Though inconclusive or insignificant outcomes have been attained for study attempts to prove an association between lymphocyte populations or a number of lymphocytes and adenosine deaminase levels[32,33]. A monocyte-macrophage origin of ADA has been hypothesised by several researchers[32,34]. In only certain T-cell lymphoproliferative disorders, adenosine deaminase levels in the blood are likewise elevated, and it could be predicted to spill over into the pleura in conditions like these[28]. In the parainfective effusions, neutrophils or lymphocytes are the probable origins of adenosine deaminase[35].

In the current research finding, the highest activity of adenosine deaminase was seen amongst the TB group. Parainfective conditions were also observed being related to high activities of ADA. The Lymphocytes/Neutrophils ratio or relative cell count might be utilized to differentiate among 2 of these entities[36,37]. A predominant lymphocyte count was typically identified in TB pleurisy patients, leading to a 0.75 or greater Lymphocyte/Neutrophil ratio, while a predominant neutrophil count (Lymphocyte/Neutrophil ratio 0.75) was typically identified in parainfective effusions cases. As mentioned already, malignant effusions might also be related to elevated counts of lymphocytes[38,39]. On the basis of the activity of ADA, it is usually possible to distinguish between TB and malignant effusions. In general, adenosine deaminase levels in malignant effusions are lower than in TB. Though, effusions secondary to leukaemia and lymphomas were commonly linked to elevated activities of ADA compared to non-hematologic malignancies & can be mistaken for TB effusions based on L/N ratios and ADA. Rheumatoid pleuritis could also be a reason for false positives. Rheumatoid pleurisy seems to have been a distinct entity in that it cannot be distinguished from pleural tuberculosis only on the basis of adenosine deaminase activity. Ocana et al. found out differential counts on all these effusions in addition to assessing the activity of ADA in these study subjects[40]. Traditionally, the diagnosis of TB pleurisy is done either by biopsy specimen cultures and/or by identifying M. tuberculosis in pleural fluid or the granulomas' presence in pleural biopsy tissue. 20-30 % of sensitivity is seen in Pleural fluid cultures,[41] pleural biopsy specimens show 50-80% sensitivity,[42] based on proficiency of clinician. Due to the requirement of extended culture periods, therapeutic and clinical decisions are made often before these laboratory outcomes turn out to be available.

Concurrently, blends of these quick approaches on pleural liquid may be used to test symptomatic productivity in varied prevalent conditions. The measurement of L/N ratio and ADA is simple, and it is believed that the findings of this research delineated that each of these tactics can offer a methodology to get analytic expertise as well as distinguish among non-tubercular and tubercular effusions. However, the combination of these tests results in an increase in specificity. Jethani et al. conducted research on the assessment of adenosine deaminase, IFN & L/N ratio in pleural fluid for differential pleural TB diagnosis in 90 pleural effusion study subjects. For ADA, they considered >40 U/L as a cut off and 0.75 for L/N ratio as sensitivity for assay detection. Combining the adenosine deaminase levels with L/N ratio activity exhibited a sensitivity of 77.78% & a specificity of 91.11%[43]. Although in our research, Lymphocyte/Neutrophil ratio and adenosine deaminase combination offer sensitivity of 95.7% & specificity of 86.9 depicted that these findings were comparable to results reported by Jethani et al. Adenosine deaminase level use particularly in combination with Lymphocyte/Neutrophil ratio, is, hence, a useful diagnostic tool with regards to this, as it offers a quick and precise means to detect Tuberculosis pleurisy. Our study had the limitation of having the 50U/L as cut-off values for ADA taken as 50U/L, which is much higher compared to the cut-off values taken in studies conducted in an endemic area (ADA 3U/L)[28].

Conclusion

This study demonstrated that at the 50 U/L ADA level, the specificity, sensitivity, NPV, PPV as well as efficacy for identifying TB were assessed at 89.8%, 81.8%, 95.6%, 64.3%, and 88.3%, correspondingly. When the added requirement of 0.75 or greater L/N ratio was included, specificity, sensitivity, NPV, PPV as well as efficacy for TB identification were computed at 95.7%, 86.9%, 93.7%, 83.3%, and 91.7%, respectively. Hence, when combining with the L/N ratio, ADA persists to be valuable test in TB pleuritic diagnosis.

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