Original article

Diagnostic accuracy of combined sonographic findings and pleural fluid adenosine deaminase levels for tuberculous pleural effusions in endemic areas - A study of 415 patients

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ABSTRACT

Background: Pleural fluid adenosine deaminase (ADA) level is a useful test for differentiating tuberculous from malignant pleural effusions. Trans-thoracic ultrasound (TUS) is currently being used to identify pleural effusions, septations and safely conduct invasive procedures. Recently sonographic septations are reported as significant predictor of tuberculosis. By combining these two entities, diagnostic yield can be increased tremendously for tuberculosis.

Objectives: To evaluate the combined effect of sonographic findings of septations and pleural fluid ADA levels on the diagnostic yield for tuberculous pleural effusions.

Patients and methods: This prospective study was conducted at the Pulmonology Outpatients Department of Gulab Devi Chest Hospital, Lahore from January 2016 till June 2018. Total 415 cases with age 14 years and above, having radiological evidence of pleural effusion and willing for ADA level estimation were included in the study. Detailed history, physical examination, X-ray chest, transthoracic ultrasound (TUS), haematological and biochemical reports were evaluated. Pleural fluid (PF) cytology, microbiology and biochemical analysis including pleural fluid ADA estimation was done. Data entered and analyzed in SPSS version 21.

Results: Out of 415 cases, 380 (91.56%) were exudates. Statistical analysis of results after combining sonographic septations and PF ADA levels results showed sensitivity of 97.21%, specificity 91.22%, positive prediction 98.43%, and negative prediction of 85.24% for diagnosis of tuberculous effusion.

Conclusion: Combining sonographic septations with PF ADA levels has high diagnostic yield and is recommended as effective tool for initial diagnosis of tuberculous pleural effusions.

Keywords:
Pleural Fluid, ADA levels, Sonographic Septations, Diagnosis, Tuberculous effusion

INTRODUCTION

Pleural effusions (PE) are classified as exudative or transudative, depending upon the protein content.[1] Discrimination between tuberculous and malignant pleural effusions is a practical challenge because both have exudative lymphocytic type. The differential diagnosis between TB and malignancy becomes even more problematic in elderly because this age favors malignancy, reactivation tuberculosis and pyogenic infections in immuno-compromised individuals. Tuberculous and malignant PE present as exudative lymphocytic type. TB is endemic in Pakistan and is considered the sole cause of an exudative-lymphocytic PE until proven otherwise.[2] Cancers and pyogenic infections are other significant contributors.[3] Evidence based diagnosis of tuberculosis is made by identifying acid fast bacilli (AFB), *Mycobacterium tuberculosis* (MTB), or a caseating granuloma. However, failure to identify Mycobacterium TB in the smear or culture and absence of a caseating granuloma on histopathology does not exclude tuberculosis, especially in endemic areas like Pakistan.[4] The reported yield of AFB in pleural fluid is less than 20% and efficacy of biopsy is around 40-80%.[5-7] Pleural fluid (PF) adenosine deaminase (ADA) level gives diagnostic yield around 80-90% for tuberculosis in high prevalence populations.[8-10] Transthoracic chest ultrasound (TUS) identifies pleural effusions and septations with high sensitivity and specificity and is a useful diagnostic predictor for tuberculosis in high prevalence areas.[11-12] When sonographic-septation results are combined with pleural fluid ADA result, better diagnostic yield is obtained for tuberculosis, in high burden populations.[13] This study aims to evaluate the combined yield of these two tools (sonographic septations and PF-ADA level) in the initial diagnosis of tuberculous pleural effusion.

Competing interest: The authors have declared no competing interests exist.
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PATIENTS AND METHODS

This prospective study was conducted at the Out-Patient Department of Pulmonary Medicine, Gulab Devi Teaching Hospital Lahore from January 2016 till June 2018. Consecutive Patients with age 14 years and above having imaging evidence of pleural effusion on chest X-ray and ultrasonography, and willing for ADA level estimation of pleural fluid were included. After detailed history and physical examination, PE suspects on chest X-ray, were subjected to trans-thoracic ultrasonography. Four sonographic patterns were identified; (i) anechoic: pleural effusion having no echoes inside (ii) complex septated: complex effusions containing septations inside, (iii) complex non-septated: complex effusions without any internal septations, and (iv) homogenous echogenic: pleural effusions with homogenous hyper-echoic pattern.[14] Ultrasound-guided pleural aspiration was done. Pleural fluid samples were examined for biochemistry, cytology, Gram staining, ZN-staining, pyogenic culture and ADA measurement. Sputum AFB and cytology were done. Sonographic septations were identified. TUS-guided pleural biopsy was done. Response to anti-TB treatment was noted. Exudative effusions were separated from transudative by using Light' Criteria.[15] Neutrophilic exudates were isolated by fluid differentiated counts. Pyogenic effusions were diagnosed by typical history, leukocytosis, Gram staining, culture sensitivity and homogenous echogenic appearance on ultrasound. The lymphocytic exudates included tuberculosis and malignant etiologies. Malignancy was diagnosed by finding malignant cells in PF, sputum and biopsy reports. The diagnosis of tuberculosis was made by pyrexia of unknown origin, history of contact with a TB patient, suggestive physical examination and supported by either isolation of AFB or caseating granuloma or response to anti-TB treatment. The diagnostic yield was obtained by pleural fluid ADA levels at cut-off value 40 IU/L and was compared with the final clinical diagnosis. The sonographic septations were identified in ADA-false negative (FN) cases and were added to the ADA based diagnostic yield.

The diagnostic yield was calculated once again. Sensitivity, Specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy were calculated by using standard formulae and compared with that of ADA based diagnostic yield. Conclusions were drawn by statistical analysis using SPSS version 21.

RESULTS

Total 415 patients were enrolled with age range was 14-64 years. Their mean age was 30.9 ± 17.38 years. The mean age for non-malignant effusions was 22 years while mean age for malignant effusions was 39 years. Out of 415 patients, 282 (67.95%) were males while 133 (32.04%) were females. Male to female ratio was 2.12:1. Two hundred and thirty-six (56.86%) patients had right-sided and 142 (34.21%) had left-sided pleural effusion, while bilateral PE was observed in another 37 (8.91%) patients. Total 381 (91.8%) PF were straw-colored, 25(6.02%) blood-stained and 9 (2.17%) were watery in appearance. Clot formation was noted in 321 (77.34%) cases. Thirty-seven (8.91%) patients were reported with diabetes mellitus and 17 (4.09%) were drug users. History of contact with the TB patients was available in 79 (19%) patients. Transudative PE was reported in 35 (8.43%) patients while 380 (91.56%) had exudative PE. Out of these 380 cases, neutrophilic exudates were observed in 26 (6.84%) while lymphocytic exudate was reported in 354 (93.15%) patients. The clinical features of the study population are summarized in Table 1. Basic hematological and pleural fluid findings in 380 exudative pleural effusions are depicted in Table 2.

LFTs, RFTs, HBs Ag, Anti-HCV and HIV results were non-conclusive in all patients with exudative effusions. Final diagnosis was made on the basis of clinico-pathological findings and response to treatment. Patients were followed for six months. Various etiological groups identified in 380 exudative pleural effusions included: (i) pyogenic PE in 26 (6.84%), (ii) tuberculous PE in 323 (85%) and (iii) malignant PE in 31 (8.15%). Total 323 patients were diagnosed as having pleural tuberculosis on clinical grounds. Two hundred and ninety-five patients were diagnosed as TB with ADA level ≥40 IU/L (true-positive cases) while 28 cases of TB showed ADA level <40 IU/L (false negative cases). In 57 patients, TB was ruled-out by ADA, but 5 cases of non-TB patients had ADA level >40 U/L (false positive test). The ADA level for TB PE ranged 32—328 IU/L with mean 79.9 IU/L, while the range for non-TB group was between 9—42 IU/L with mean

Table 1. Clinical features of the study participants

Clinical features	Frequency (*n*)	Percentage
Chest pain	339	81.68
Cough	325	78.31
Fever	312	75.18
Dyspnoea	280	67.46
Weight loss	271	65.30
Loss of Appetite	263	63.37

Table 2. Pleural fluid and hematological features in lymphocytic exudates (n = 380)

Characteristics	Exudative pleural effusions		
	Tuberculous	Malignant	Pyogenic
Total exudates (*n*)	323	31	26
Clot formation (%)	98	0	7.6
Protein (g/dL) [mean]	5.7	4.4	6.2
Fluid cytology (%)	Lymphocyte = 98.93	Lymphocyte = 78	Poly = 87
Positive growth culture (%)	0	0	65.38
ZN staining (%)	0	0	0
Malignant cells (%)	0	58.06	0
ADA concentration (IU/L) [mean]	73.48	14.3	18.9
ESR (mm/1st hour) [mean]	40.97	38	67
TLC (No. of cells/mm^3) [mean]	8,755	10,220	14,950
Hemoglobin (g/dL) [mean]	10.86	11.4	8.2
Serum protein (gm/dL) [mean]	7.21	7.9	7.4

ZN: Ziehl-Neelsen; ADA: adenosine deaminase ESR: erythrocyte sedimentation rate; TLC: total leukocyte count

value 18 IU/L. Diagnostic yield of tuberculous pleural effusion (TBPE) is shown in Table 3. Diagnostic Accuracy in ADA based efficacy was found as 91.31%. Regarding transthoracic ultrasonographic (TUS) findings, out of total 323 TBPE, 258 (79.87%) showed complex septated effusion, 43 (13.31%) complex non-septated and 22 6.81%) were anechoic in character.

Fifty seven of 323 cases (17.6%) belonged to non-TB group; 14 (24.56%) showing homogenous echogenic pattern, 7 (12.28%) complex septated, 25 (43.85%)complex non-septated and 11 (19.29%) anechoic pattern. A complex septated pattern was found in 265 of 380 (69.73%) cases. Total 258 of 265 (97.35%) cases had tuberculous etiology while 7 cases belonged to non-TB group. Only 22 (6.81%) cases showed anechoic pattern in tuberculous pleural effusions while 43 (13.31%) cases with complex non-septated pleural effusions were also encountered with TBPE. Total 295 patients were diagnosed as tuberculosis by PF-ADA level (TP). Twenty-eight cases of tuberculous pleural effusions were not identified by ADA (false negative). Nineteen of these 28 (67.85%) displayed sonographic septations. These 19 cases were added to the diagnostic yield of ADA and diagnostic yield was recalculated. The corrected yield being: true positive cases (TP) - 314 (295 +19), False Negative cases (FN) - 9, True negative cases (TN) - 52, False positive cases (FP) - 5. The statistical analysis results are shown in Table 3.

DISCUSSION

In this study, patients aged 20 to 30 years constituted 72.88% of the study population and the mean age was 30.94 years which is similar to other reports who have quoted mean age of around 31.5 years or more.[14,15] TB PE in elderly were encountered in previously treated patients, due to re-activation and re-infection as shown by other authors as well.[16] The comparison between the mean ages of non-malignant (24 years) and malignant effusions (44 years) was statistically significant ($p<0.05$). Other authors reported similar findings in their studies.[17-18] Male to female ratio (2.12:1) showed that pleural effusion is more common in males than in females and is consistent with other reports.[17-18] On clinical grounds, 323 (85%) cases of TB PE, 31 (8.15%) cases with malignant pleural effusions and 26 (6.84%) cases with pyogenic PE were diagnosed while TB was the most common etiology. This is supported by other researchers as well.[19] Twenty-six (6.84%) cases of pyogenic PE were isolated. These included 11-cases of para-pneumonic effusions and 15-cases of empyema. Only two cases of empyema were found in younger age group while 13 cases of empyema were diagnosed in elderly, out of which 07 cases were identified in diabetics and 06 cases of empyema were found in drug abusers which is because DM and alcohol abuse alters immune regulation, leading to immunodeficiency and increased susceptibility to bacterial pneumonia, tuberculosis, and other infectious diseases.[20] Total 354 out of 380 (93.15%) cases with lymphocytic-exudates included tuberculous and malignant etiologies with almost similar features. The discrimination between these two entities is really a challenge, because tissue diagnosis is required for neoplastic etiology.[21] It is a risky procedure, not readily available and suitable for serious patients, the diagnostic yield is not more than 80%.[22,23] Because Pakistan ranks 5th among the high burden countries, with an annual incidence of 497/100,000 and pleural effusion is the most common type of extra-pulmonary tuberculosis. Similarly malignant pleural effusions are increasing because of smoking and environmental pollution. Differentiation

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Table 3. Diagnostic accuracy of combined sonographic findings and pleural fluid adenosine deaminase levels for tuberculous pleural effusions

Statistics	Type of diagnostic test	
	PF ADA levels	PF ADA levels + septations
True positive	295	314
False positive	5	5
False negative	28	9
True negative	52	52
PPV (%)	98.33	98.43
NPV (%)	65	85.25
Sensitivity (%)	91.33	97.21
Specificity (%)	91.22	91.22
Accuracy (%)	91.32	96.32

PF: pleural fluid; ADA: adenosine deaminase; PPV: positive-predictive value; NPV: negative-predictive value

between the two entities is essential, as both exist as lymphocytic exudates. The diagnostic yield for tuberculosis in this study by PF ADA level with cut off value 40 IU/L obtained a sensitivity of 91.3%, specificity 91.2%, PPV 98.3%, NPV 65% and diagnostic accuracy 91.3% for TB diagnosis. Many current researchers have reported that pleural fluid ADA level has got a high discriminative value between tuberculous and malignant PE in high prevalence populations.[24-28] In this study, 295 cases of TB were found with PF ADA level ≥40 IU/L (true-positive cases) while 28 cases of tuberculosis showed PF ADA level <40 IU/L (false negative cases). The ADA level for TB PE ranged 32—328 IU/L with mean 79.88 IU/L, while the range for non-TB group was between 09—42 IU/L with mean value 18.0 IU/L. Only 5/380 non-TB cases showed ADA level >40 IU/L. The cases of TB showing PF ADA level <40 IU/L were in the range of 32-39. These lower marginal levels were found in tuberculous PE in elderly age group, diabetes, gross pleural thickening containing micro-loculations, minimal pleural effusions and those who were previously treated symptomatically with quinolones. The current literature states that in early disease, ADA levels are low while higher level of ADA are demonstrated in the same patient when estimated later, with established disease. Similarly, the ADA level is reduced in response to anti-TB treatment. This may be the explanation of lower marginal ADA levels in quinolone treated patients in this study, because quinolones are shown to have anti-tuberculous activity. A negative correlation was found between PF ADA level and the age of the patient. Higher ADA level values were observed in younger patient as compared to the elderly patients, since slightly lower marginal levels (<40 IU /L) of ADA were observed in old age.[29] This may reflect a need to set different cut-off values for younger and elderly groups.

Twenty-eight false negative cases were found in this study which were not diagnosed by ADA, 19 of these showed complex septated pattern. These 19 cases which were not picked by ADA were readily diagnosed by sonographic septations. When these 19 cases were added to the ADA positive cases (295), the combined diagnostic yield was further improved (Table 3). Several authors have reported that sonographic septations in exudative lymphocytic pleural effusions is a useful diagnostic predictor for tuberculosis in high prevalence area. Martinez and group reported that the winding bands were seen frequently in patient with TPE.[30] Current study has the limitation that only patients with tuberculous, pyogenic and malignant pleural effusions were included in the differential diagnosis. Pyogenic effusions are easily segregated but actual problem is encountered in differentiating TB from malignancy. As the pleural space was evacuated at the time of diagnostic aspiration, repeated estimation of ADA in patients with low ADA level TB PE was difficult or not possible. Similarly, prognostic value of ADA levels could not be studied for the same reasons.

CONCLUSION

ADA estimation and chest sonography are readily available, cost effective, easy to perform, safer than biopsy and give excellent diagnostic yield. It is concluded that diagnostic yield is tremendously increased after combining sonographic septations with pleural fluid ADA level in high prevalence populations.

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