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Original Research Article

DIAGNOSTIC UTILITY OF ADA, MALIGNANT CYTOLOGY, AND CBNAAT IN EXUDATIVE PLEURAL EFFUSIONS: A PROSPECTIVE OBSERVATIONAL STUDY IN AN INDIAN TERTIARY CARE HOSPITAL

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Abstract

Background: Pleural effusion is an excessive collection of fluid in the pleural space. Exudative pleural effusions are often caused by tuberculosis, malignancy, and parapneumonic conditions in India. This study evaluates the diagnostic utility of adenosine deaminase (ADA), malignant cytology, and Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) in exudative pleural effusions. **Materials and Methods:** This prospective observational study was conducted at a tertiary care hospital from November 2019 to January 2021. One hundred adult patients with exudative pleural effusions were enrolled. Clinical features, blood investigations, imaging studies, and pleural fluid analyses were performed, including ADA levels, CBNAAT, and cytology with the cell block method. **Result:** The study population included 59 males and 41 females, with a mean age of 44.82 ± 16.69 years. The most common cause of pleural effusion was tuberculosis (52%), followed by parapneumonic (20%) and malignant effusions (13%). ADA levels demonstrated a sensitivity of 93.48% and a specificity of 83.33% for tubercular effusions. Malignant cytology had a diagnostic yield of 72.72%. The sensitivity and specificity of pleural fluid CBNAAT were 19.57% and 85.19%, respectively. Lymphocyte predominance was noted in tubercular (94.23%) and malignant effusions (84.61%), while neutrophil predominance was observed in parapneumonic effusions (80%). **Conclusion:** Non-invasive tests such as ADA, CBNAAT, and pleural fluid cytology, combined with biochemical and cellular analysis, can effectively diagnose the etiology of exudative pleural effusions. These findings support their use as first-line diagnostic tools, potentially reducing the need for invasive procedures like pleural biopsy.

INTRODUCTION

Pleural effusion, defined as an excessive collection of fluid in the pleural space, poses a significant diagnostic challenge in clinical practice. This condition can be broadly classified into transudative and exudative pleural effusions based on Light's criteria, which utilize the pleural fluid to serum protein ratio and lactate dehydrogenase (LDH) levels.[1,2] In India, tuberculosis remains the most common cause of exudative pleural effusions, followed by malignancies, parapneumonic effusions, and other etiologies such as viral infections and pancreatitis.[3]

The diagnostic approach to pleural effusions involves a combination of clinical assessment, imaging, and laboratory analysis of pleural fluid. Traditional methods like pleural biopsy, although definitive, are invasive and associated with complications.[4] Hence, there is a growing interest in non-invasive diagnostic tools such as adenosine deaminase (ADA) levels, cytology, and Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) for Mycobacterium tuberculosis.[5,6]

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ADA is an enzyme involved in purine metabolism, and its levels are significantly elevated in tuberculous pleural effusions due to the high cellular activity of T lymphocytes.[7] Multiple studies have demonstrated the utility of ADA as a marker for tuberculosis, with a cut-off value typically around 40 IU/L offering high sensitivity and specificity.[8,9] However, ADA levels can also be elevated in other conditions, including malignancies and rheumatoid effusions, which necessitates the use of complementary diagnostic tests.[10]

Malignant pleural effusions often present with non-specific symptoms such as dyspnea, cough, and chest pain. Cytological examination of pleural fluid, particularly when combined with cell block technique, enhances the diagnostic yield for malignancies.[11,12] Studies have reported a cytological diagnostic yield ranging from 50% to over 70% when utilizing cell block methods, underscoring its importance as a first-line diagnostic tool in suspected malignant effusions.[13,14]

CBNAAT, a rapid molecular test for detecting Mycobacterium tuberculosis and rifampicin resistance, has revolutionized the diagnosis of tuberculosis in pulmonary and extra-pulmonary samples. Despite its high specificity, the sensitivity of CBNAAT in pleural fluid samples is relatively low, highlighting the need for a multimodal diagnostic approach.[15,16] Studies indicate a sensitivity range of approximately 13-20% for pleural fluid CBNAAT, with specificity reaching up to 100%.[17,18]

This study aims to evaluate the diagnostic utility of ADA, malignant cytology, and CBNAAT in patients with exudative pleural effusions, providing insights into their effectiveness as non-invasive diagnostic tools in a tertiary care hospital in India.

MATERIALS AND METHODS

This prospective observational study was conducted in the Department of Pulmonary Medicine at a tertiary care hospital from November 2019 to January 2021. The aim was to evaluate the diagnostic utility of adenosine deaminase (ADA), malignant cytology, and Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) in patients with exudative pleural effusions. The study population consisted of 100 adult patients presenting with clinical and radiological features of exudative pleural effusions. Inclusion criteria required patients to have exudative pleural effusion based on Light's criteria. Exclusion criteria included patients with transudative pleural effusions, empyema, hemothorax, chylothorax, post-traumatic effusions, clinical features of cardiac, renal, or liver failure, severe terminal illnesses, and those already on anti-tubercular therapy (ATT) or chemotherapy, as well as those on long-term steroids or immunosuppressants.

Informed consent was obtained from all participants, and the study protocol was approved by the Institutional Ethics Committee. Detailed histories and physical examinations were conducted for all patients, noting age, demographic features, clinical features, and associated comorbidities and risk factors. Routine blood investigations, imaging studies, and pleural fluid analyses were performed.

Blood investigations included complete blood picture (CBP), random blood sugar (RBS), liver function tests (LFT), renal function tests (RFT), viral markers (HBsAg, HIV, HCV), serum LDH, serum proteins, serum cholesterol, and serum amylase and lipase as needed. D-dimer tests were performed when required. Additionally, sputum gram stain, culture, and CBNAAT were conducted, alongside rheumatoid factor (RA), antinuclear antibody (ANA), and other markers for connective tissue diseases (CTD) if clinically indicated.

Imaging studies consisted of chest X-ray, ultrasound (US) of the chest and abdomen, computed tomography (CT) of the chest, and CT pulmonary angiography (CTPA) if needed. Endoscopic retrograde cholangiopancreatography (ERCP) and magnetic resonance cholangiopancreatography (MRCP) were performed when necessary.

Pleural fluid was analyzed for total and differential cell count, glucose, protein, LDH, gram stain, and culture. ADA and CBNAAT tests were performed on the pleural fluid, along with cholesterol, triglycerides, and chylomicrons if required. Amylase and lipase tests were conducted if indicated. Malignant cytology was performed on 3-5 samples using the cell block method, and pleural fluid hematocrit was measured in hemorrhagic effusions.

All patient-related parameters were recorded and transferred to Microsoft Excel for statistical analysis. Demographic data were expressed as mean ± standard deviation (SD). Comparisons between groups were performed using the Chi-Square test, with statistical significance set at $p < 0.05$.

The primary outcome measure was the diagnostic utility of ADA, malignant cytology, and CBNAAT in determining the etiology of exudative pleural effusions. Secondary outcome measures included the prevalence of various etiologies of exudative pleural effusions and the correlation of clinical features with different etiologies.

RESULTS

The study included 100 patients with exudative pleural effusions, comprising 59 males and 41 females, with a mean age of 44.82 ± 16.69 years. The etiological distribution revealed that tuberculosis was the most common cause, accounting for 52% of the cases. Other causes included parapneumonic effusions (20%), malignancies (13%), pancreatic effusions (9%), pulmonary embolism (2%), and undiagnosed causes (4%).

Clinical features varied with the etiology. Patients with tubercular effusions commonly presented with cough (76.92%) and fever (61.53%), while those with malignant effusions predominantly experienced

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dyspnea (69.23%). Parapneumonic effusions were frequently associated with cough (90%) and fever (80%). Other symptoms observed across different etiologies included chest pain, hemoptysis, loss of appetite, and weight loss.

Pleural fluid analysis showed that tubercular effusions were predominantly lymphocytic (94.23%), similar to malignant effusions (84.61%). In contrast, parapneumonic effusions were mainly neutrophilic (80%). The mean pleural fluid to serum protein ratio was 0.742, with a mean pleural fluid glucose value of 71.75 ± 2.7 mg/dL. The ADA levels were significantly higher in tubercular effusions (mean 59.63 ± 2.64 IU/L) compared to malignant (30.15 ± 5.49 IU/L) and parapneumonic effusions (20.35 ± 1.34 IU/L).

The diagnostic utility of pleural fluid CBNAAT was evaluated, showing a sensitivity of 19.57% and a specificity of 85.19%. Malignant cytology using the cell block method had a yield of 72.72%. Chest X-ray findings revealed that the majority of effusions were left-sided (53.85%).

Risk factors identified in the study population included diabetes (55%), alcoholism (38%), smoking (49%), and immunocompromised status (10%). The study demonstrated that simple, non-invasive tests like ADA, CBNAAT, and pleural fluid cytology, along with biochemical and cellular analysis, could determine the etiology in 94% of patients without the need for more invasive procedures like pleural biopsy.

Table 1: Age and Gender Distribution.

Sl. No	Age group	Male	Female
1	11-20	1	6
2	21-30	10	5
3	31-40	11	7
4	41-50	14	11
5	51-60	14	5
6	61-70	4	3
7	71-80	5	1
8	Above 80	0	3

Table 2: Etiology of Pleural Effusions

Sl.no	Etiology	Frequency
1	Tubercular effusion	52
2	Parapneumonic effusion	20
3	Malignant effusion	13
4	Pancreatic effusion	9
5	Pulmonary effusion	2
6	Undiagnosed	4

Table 3: Clinical Features

Sl.No	Symptom	Malignant	Tubercular	Pancreatic	Pulmonary embolism	Parapneumonic	Undiagnosed
1	Cough	5	40	6	1	18	3
2	Shortness of breath	9	38	6	1	6	1
3	Fever	3	32	4	0	16	2
4	Chest pain	7	28	2	2	12	1
5	Weight loss	5	24	4	0	2	1
6	Loss of appetite	2	40	4	0	6	1
7	Hemoptysis	4	4	1	1	4	1

Table 4: Differential Cell Count

Sl.No	Etiology	Lymphocyte predominant	Neutrophil predominant
1	Malignant	11	2
2	Tubercular	49	3
3	Pancreatic	2	7
4	Pulmonary embolism	0	2
5	Parapneumonic	3	17
6	Undiagnosed	4	0

Table 5: ADA Levels in Different Etiologies

Sl. No	ADA value (IU/L)	Malignant	Tubercular	Pancreatic	Pulmonary embolism	Parapneumonic	Undiagnosed
1	0-30	10	6	6	2	16	2
2	31-40	0	3	2	0	4	1
3	41-70	3	27	1	0	0	1
4	>70	0	16	0	2	0	0

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DISCUSSION

This study aimed to evaluate the diagnostic utility of ADA, malignant cytology, and CBNAAT in patients with exudative pleural effusions. Our findings indicate that tuberculosis is the most prevalent cause of exudative pleural effusions, followed by parapneumonic and malignant effusions. These results align with other studies conducted in similar settings, confirming tuberculosis as a major etiology in India.[1,2]

The mean age of patients in our study was 44.82 ± 16.69 years, with a male predominance (59%). This male preponderance is consistent with other studies, such as the one conducted by Laxmareddy et al., which reported a male to female ratio of 1.7:1 in exudative pleural effusions.[3] The prevalence of tuberculosis-related pleural effusions (52%) in our study corroborates with findings by Reddy et al., who reported a 38% incidence of tubercular effusions in their cohort.[19]

Clinical features varied according to the etiology of the effusions. Tubercular effusions were most commonly associated with cough and fever, similar to findings by Mehta et al., who reported these as predominant symptoms in tuberculous pleural effusions.[9] Malignant effusions, on the other hand, predominantly presented with dyspnea, which is consistent with the observations of Goyal et al.[20]

Our study revealed that lymphocyte predominance in pleural fluid was characteristic of tubercular (94.23%) and malignant effusions (84.61%), whereas neutrophil predominance was noted in parapneumonic effusions (80%). These findings are in line with the study by Jimenez et al., which emphasized the diagnostic value of differential cell count in pleural effusions.[7]

The diagnostic utility of ADA in our study showed a sensitivity of 93.48% and a specificity of 83.33% for tubercular effusions, with a mean ADA level of 59.63 ± 2.64 IU/L. These results are comparable to those reported by Duggal et al., who found a sensitivity of 91% and specificity of 100% for ADA at a cutoff value of 40 IU/L.[21] The role of ADA as a reliable marker for tuberculous pleural effusions has been consistently supported by various studies, including those by Mehta et al. and Asim et al.[9,22]

Malignant cytology using the cell block method had a diagnostic yield of 72.72% in our study. This is higher than the yield reported by Marrium Asim et al. (58%) and comparable to the findings of Theerada et al., who reported a yield of approximately 57% using a combination of conventional cytology and cell block methods.[12] The high yield in our study underscores the importance of utilizing cell block technique in enhancing the diagnostic accuracy of pleural fluid cytology.

CBNAAT showed a sensitivity of 19.57% and a specificity of 85.19% in detecting Mycobacterium tuberculosis in pleural fluid. This low sensitivity is consistent with the findings of Duggal et al., who reported a sensitivity of 13.4% for pleural fluid CBNAAT.[21] The specificity, however, was comparable to that reported in other studies, highlighting the test's utility as a confirmatory diagnostic tool despite its limited sensitivity.[23]

Limitations and Future Perspectives

This study has several limitations. Firstly, the sample size of 100 patients, while adequate for preliminary analysis, may not be sufficient to generalize the findings to broader populations. Additionally, the exclusion of patients with transudative pleural effusions, empyema, hemothorax, and those on long-term immunosuppressive therapy might have introduced selection bias. The study did not include advanced molecular diagnostic techniques beyond CBNAAT, such as next-generation sequencing, which could provide deeper insights into the etiology of pleural effusions. Furthermore, the lack of viral testing, particularly in the context of emerging respiratory pathogens, limits the comprehensiveness of the etiological assessment. Future research should focus on larger, multicentric studies to validate these findings across diverse populations. Incorporating advanced molecular diagnostics and comprehensive viral panels could enhance the diagnostic accuracy and provide a more detailed understanding of pleural effusion etiologies. Additionally, longitudinal studies examining the long-term outcomes of patients based on initial diagnostic strategies would be beneficial in refining clinical guidelines for the management of pleural effusions.

CONCLUSION

In conclusion, our study demonstrates that simple, non-invasive tests such as ADA, malignant cytology, and CBNAAT, along with biochemical and cellular analysis of pleural fluid, can effectively determine the etiology of exudative pleural effusions in the majority of cases. These findings support the use of these diagnostic tools as first-line investigations in the evaluation of pleural effusions, potentially reducing the need for more invasive procedures like pleural biopsy.

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