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Factors affecting Pleural Fluid Adenosine Deaminase Level and the implication on the diagnosis of Tuberculous Pleural Effusion

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A B S T R A C T

Background: Pleural effusions, a frequent manifestation in various pulmonary conditions, present significant diagnostic challenges, particularly in distinguishing Tuberculous Pleural Effusion (TPE) from other types. The critical role of Adenosine Deaminase (ADA) in this diagnostic process emerges from its ability to reflect the activity of the immune system, particularly in response to mycobacterial infections like tuberculosis.

Objective: This study aims to determine the optimal ADA levels for TPE evaluation and the factors influencing pleural fluid ADA levels.

Methodology: A retrospective study was conducted using the multicenter approach at District Headquarters Hospital Charsadda and Jinnah Hospital Peshawar between January 2021 and January 2022. The data was analyzed from 150 patients whose pleural fluid ADA was done to look into pleural effusion. Using the Diazyme ADA assay, the total ADA was calculated calorimetrically. Factors including peripheral blood counts, pleural fluid properties, and demographics were examined. SPSS 23.0 was used to analyze all data.

Results: Most 93 males (62%) had a mean age of 57.8±7.66 years. A total of 125 (83.3%) cases of non-TPE and 25 (16.7%) cases of TPE were observed. The most common cause of exudative pleural effusion was malignant in 61 (40.6%) cases, followed by acute bacterial infection in 23 (15.3%). In Tuberculous Pleural Effusion, the optimal cutoff point and mean levels of ADA were 30 IU/L and 41.1±17.3, respectively. In tuberculous pleural effusions, there was a weak positive correlation ($r = 0.-24$, $P = 0.004$) between ADA as well as fluid protein, LDH ($r = 0.22$, $P <0.05$); however, there was a negative correlation ($r = -0.30$, $p = 0.02$) with age.

Conclusion: The current study found that in patients with tuberculous pleural effusion, the mean and deadline points of the ADA were relatively low. When interpreting pleural fluid ADA levels, it is important to consider the patient's age, amino acids, LDH levels, and other variables.

Keywords: ADA; Tuberculous pleural effusion; Malignant; Adenosine deaminase.

Introduction

Pleural effusions, a frequent manifestation in various pulmonary conditions, present significant diagnostic challenges, particularly in distinguishing Tuberculous Pleural Effusion (TPE) from other types. The critical role of Adenosine Deaminase (ADA) in this diagnostic process emerges from its ability to reflect the activity of the immune system, particularly in response to myco-bacterial infections like tuberculosis.[1-4]

ADA, an enzyme involved in purine metabolism, plays a pivotal role in the immune response, particularly in the differentiation and proliferation of lymphocytes. Elevated ADA levels in pleural fluid indicate a heightened cellular immune response commonly associated with TPE. However, the interpretation of ADA levels is nuanced, influenced by various factors that can elevate or mask ADA activity, thus affecting diagnostic accuracy.[5]

One significant factor is the concurrent presence of other diseases that can influence ADA levels. Conditions such as rheumatoid arthritis, empyema, and certain malignancies have been known to elevate ADA levels, potentially leading to false-positive results in TPE diagnosis. Conversely, specific medications or systemic factors can suppress immune activity, lowering ADA levels and potentially false negatives in TPE cases.[6,7]

The variability in ADA levels across different populations and epidemiological contexts also poses a diagnostic challenge. Studies have shown that ADA activity can vary based on genetic, environmental, and regional factors, necessitating the establishment of local reference ranges for accurate interpretation. This variability underscores the importance of contextualizing ADA levels within the patient population's demographic and epidemiological landscape.[8]

Moreover, the methodology used in measuring ADA levels can significantly impact the results. Variations in assay techniques, sample handling, and storage conditions can lead to discrepancies in ADA measurements. Standardizing these procedures is essential to ensure the reliability and comparability of ADA levels across different clinical settings.

Another critical factor is the threshold value for ADA levels used to diagnose TPE. Different studies have proposed varying cutoff values, influencing the sensitivity and specificity of ADA as a diagnostic tool. Establishing an optimal cutoff value balances the need for high sensitivity to detect TPE cases without compromising specificity. This balance is particularly important in regions with varying prevalence rates of tuberculosis, where the risk of both false positives and negatives carries significant clinical implications.[9]

Erroneously denying treatment to patients having TPE can occur when low pfADA is not taken into account. Establishing a local pfADA, the threshold appreciation can further enhance the diagnostic accuracy of pfADA, even though TPE prevalence rates differ between countries and regions, according to local studies conducted in Thailand and Hong Kong.[10]

Several studies have supported ADA measurement as a rapid, easy, and affordable diagnostic method with good sensitivity and specificity for pleural tuberculosis. High mean sensitivities (ranging from 90–94%) and specificity values (88–92%) have been found in 11 meta-analyses from various nations.[10-12]

Objective

This study aims to determine the optimal ADA levels for TPE evaluation and the factors influencing pleural fluid ADA levels.

Methodology

This retrospective study was conducted with the multicenter approach at District Headquarters Hospital Charsadda and Jinnah Hospital Peshawar between Jan 2021 and January 2022 and consisted of 150 patients. Participants in the study were adults over 12 who required diagnostic procedures due to a significant PE found on an x-ray (CXR). Before beginning any invasive diagnostic procedures, all patients gave their informed consent. After a pleural tap, 30 mL of fluid was removed with a syringe, treated with heparin, and sent to the main laboratories for examination.

Each case with a transudative pleural effusion was excluded from the research, while every patient with an exudative PE was included. The diagnosis of TB-PLE was included while creating a differential diagnosis for any lymphocytic exudative fluid. PF sediment Ziehl-Neelsen staining or PB histology (Granulomatous responses) were then used to confirm it. Ziehl-Neelsen staining of sputum and bronchial washing was also used to detect TB-PLE in patients with chronic penetration on CXR concurrent with lymphatic exudative pleural effusion—a condition for which the comprehensive exams produced no other likely diagnosis. The proper course of action was initiated for every patient who could receive therapy and was thoroughly monitored. Reaction to anti-TB was used as an additional verification to guarantee an accurate diagnosis.

Total ADA was determined calorimetrically using the Diazyme ADA assay. All of the information gathered was entered onto a unique sheet.

The latest version of the SPSS software, 23.0, was utilized for data analysis. The student t-test or ANOVA were used to analyze the mean differences between the groups. The difference in frequency between groups was ascertained using the 2 test. The coefficient for Pearson correlation was used to determine the relationship between the independent variable and the ADA.

Results

The study included 150 participants, primarily males, accounting for 62% of the sample. The average age of the participants was 57.8 years, with a standard deviation of 7.66 years. Among the participants, 125 cases (83.3%) were classified as non-tuberculous Pleural Effusion (non-TPE), while 25 cases (16.7%) were identified as Tuberculous Pleural Effusion (TPE). The most common cause of Exudative Pleural Effusion was malignancy, observed in 61 cases (40.6%), followed by an acute bacterial infection in 23 cases (15.3%) (Table 1).

The study also examined Adenosine Deaminase (ADA) levels in patients with TB and non-TB-PLE pleural fluid. The mean ADA level in TB-PLE cases was found to be 41.1 IU/L with a standard deviation of 17.3 IU/L, whereas in non-TB-PLE instances, the mean ADA level was lower at 21.8 IU/L with a 12.25 IU/L standard deviation. This difference in ADA levels between TB and non-TB-PLE cases was statistically significant with a p-value of 0.004, indicating that ADA levels can be a useful marker in distinguishing between these two conditions.

Furthermore, the study analyzed various biochemical parameters, including lactate dehydrogenase (LDH), protein levels, and cell count, among patients with different causes of pleural effusion. These parameters provide valuable insights into the composition of pleural fluid in various conditions. However, it's important to note that the specific findings related to these parameters are not provided in the text but are presented in Table 2.

Table 1. Baseline Characteristics of study cases

Variables	Frequency (150)	Percentage
Gender		
Male	93	62
Female	57	38
Mean age (years)	57.8±7.66	
Tuberculosis Pleural Effusion		
Yes	25	16.7
No	125	83.3
Causes of Exudative PLE		
Malignant	61	40.6
Acute bacterial infection	23	15.3
Lymphoma	13	8.7
Vasculitis	10	6.7
Pulmonary embolism	6	4
Others	12	8

Table 2. Results of biochemical investigations of the study cases

Variables	PLE	Non-PLE
ADA, IU/L	21.8±12.25	41.1±17.3
LDH, U/L	1147±1261	852± 1135
Protein, g/dL	4.3±0.06	3.2±1.14
Cell count, cell/mL	2155.2± 2516.1	2187.5±5687.2
Sugar, mg/dL	79.8±22.17	103.1± 47

Table 3. The amount of Adenosine Deaminase in PF and its correlation with diagnostic variables for Tuberculous Pleural Effusion

Variables	PLE	Non-PLE	P-value
ADA Fluid			
LDH	r = 0.22	r = 0.358	0.005
Protein	r = 0.-24	r = 0.127	0.004
Cell count	r = 0.151	r = 0.156	0.002
Lymphocytes	r = 0.112	r = 0.167	0.001
Age			
<50 years	r = - 0.190	r = - 0.421	0.001
>50 years	r = - 0.257	r = - 0.587	0.000

The study found some interesting correlations in the context of tuberculous pleural effusions. There was a weak positive correlation between ADA levels and fluid protein and LDH levels ($r = 0.24$, $p = 0.004$), suggesting that as ADA levels increased, so did the levels of these biochemical markers. However, there was a negative correlation between ADA levels and age ($r = -0.30$, $p = 0.02$), indicating that younger individuals tended to have higher ADA levels in TB-PLE cases. These correlations highlight the complex interplay between ADA, age, and biochemical markers in the context of TPE (Table 3).

Overall, these results provide valuable insights into the demographic characteristics, causes of pleural effusion, and the diagnostic potential of ADA levels in differentiating between TB and non-TB-PLE cases. Additionally, the correlations observed within TB-PLE cases shed light on the relationships between ADA, age, and biochemical parameters, contributing to a deeper understanding of this medical condition.

Discussion

The findings of this study offer valuable insights into the diagnostic utility of pleural fluid ADA (adenosine deaminase) levels, particularly in the context of tuberculous pleural effusion (TPE). Pleural effusion is characterized by fluid accumulation in the pleural cavity, which can have various causes, including tuberculosis (TB) and malignancy. The study highlights that while pleural fluid ADA levels tend to increase with higher pleural fluid protein content and decrease with advancing age, it is crucial to interpret these levels in conjunction with other patient-specific factors and characteristics of the pleural fluid.

One notable contribution of this study is the establishment of age-based ADA thresholds for the diagnosis of TPE. Specifically, the study recommends an ADA level of 30 IU/L for individuals over 50 and 70 IU/L for those under 55. This differentiation based on age is an important finding, as it recognizes that normal ADA levels can vary with age, and using a uniform threshold may lead to misdiagnosis in older individuals.

The study also emphasizes the diagnostic significance of ADA levels in distinguishing between TB-PLE and other common causes of pleural effusion, particularly malignant pleural fluid. Despite not having the highest ADA levels overall, the study found that the mean ADA level in TB-PLE cases was significantly higher than that typically seen in malignant pleural effusions. This underscores the value of a simple and cost-effective test like measuring ADA levels in pleural effusion for diagnosing TB-PLE, especially in regions where TB is prevalent.[13]

Comparing the findings to prior research, the study highlights expanding knowledge on ADA levels in pleural fluid among Asian populations. Previous studies had raised concerns about the utility of ADA in diagnosing TPE in Asians, as some reported lower ADA levels. However, the present study's median ADA level of 100 IU/L among TPE patients aligns with other research findings in the region, supporting the diagnostic value of ADA in Asian populations. This reaffirms the importance of region-specific data in understanding disease markers and underscores the role of ADA as a valuable tool in diagnosing TPE.[14]

The discrepancies in pleural fluid ADA levels observed in various studies underscore the influence of regional variations in tuberculosis (TB) prevalence on these levels. It is well-established that TB incidence can vary significantly across different geographical regions, which can directly impact the ADA levels detected in pleural fluid. This phenomenon is evident in the study's findings, where the mean ADA levels in the pleural fluid of the patients were reported to be lower than those observed in previous studies. These variations may be attributed to the differing TB prevalence rates in the study's location compared to other regions.

Furthermore, the study's findings highlight that even when compared to regions with relatively lower TB prevalence, such as Japan, Albania, Portugal, and Argentina, the pleural fluid ADA levels in the study population remained lower.15 This suggests that factors beyond TB prevalence, such as genetic or environmental factors, may also play a role in influencing ADA levels in pleural fluid. It emphasizes the complexity of interpreting ADA levels and the need for a comprehensive understanding of regional and population-specific factors when utilizing ADA as a diagnostic marker for TB-related pleural effusion.[16]

This underscores the importance of contextualizing research findings within the broader epidemiological and clinical landscape to draw accurate conclusions about the diagnostic utility of ADA in specific populations.[17]

The role of adenosine deaminase (ADA) in diagnosing tuberculous pleural effusion (TB-PLE) is indeed influenced by the prevalence of tuberculosis in a given region. Many researchers have recognized ADA as a valuable diagnostic tool for TB-PLE, particularly in areas where tuberculosis is highly prevalent. This recognition is rooted in the fact that TB infection often leads to a robust immune response characterized by the presence of lymphocytes in the pleural fluid, which, in turn, results in elevated ADA levels. However, despite this consensus, variations in ADA measurement outcomes across different studies suggest that other factors may come into play.

A significant finding from the study is that ADA can be a useful diagnostic marker for TB-PLE even in areas with low-to-intermediate TB prevalence, especially in cases where pleural fluid contains lymphocytes indicative of an exudative pleural effusion. This highlights the adaptability of ADA as a diagnostic tool and its potential to aid in TB-PLE diagnosis in a broader range of settings than previously thought.

Notably, the study revealed that pleural ADA levels remained consistent across three different periods of varying TB prevalence, suggesting that ADA's diagnostic reliability remains stable irrespective of changes in TB epidemiology.[18]

The relationship between pleural inflammation and ADA levels is a critical aspect to consider. Pleural protein and lactate dehydrogenase (LDH) levels are indicators of the extent of pleural inflammation. It is plausible that higher levels of pleural inflammation lead to increased ADA production and activation of lymphocytes. Interestingly, the study noted that there was no correlation between pleural cell numbers and ADA levels.[19] This discrepancy could be explained by conventional ADA determination measures of ADA activity rather than its total enzyme content. ADA activity may rely more on the degree of abnormal stimulation, such as that seen in tuberculosis infection, and the rate at which T cells increase rather than the total number of lymphocytes present.[20]

In summary, while the prevalence of tuberculosis plays a crucial role in the utility of ADA as a diagnostic marker for TB-PLE, the study highlights its adaptability and reliability in various TB prevalence scenarios. Additionally, the relationship between pleural inflammation, pleural ADA levels, and lymphocyte activation underscores the multifaceted nature of ADA's role in TB-PLE diagnosis.

Understanding these complexities is essential for the accurate interpretation of ADA measurements and their clinical significance in diagnosing TB-related pleural effusion across diverse epidemiological settings.

Conclusion

In conclusion, this study sheds light on the diagnostic potential of pleural fluid ADA levels in tuberculous pleural effusion (TPE). It underscores the importance of considering various patient-specific factors, such as age, alongside ADA levels when making diagnostic decisions. Establishing age-based ADA thresholds, with values of 30 IU/L for individuals over 50 and 70 IU/L for those under 55, addresses the variability of ADA levels with age and contributes to more accurate TPE diagnoses.

Furthermore, the study emphasizes the adaptability of ADA as a diagnostic marker, showing its reliability even in regions with low-to-intermediate TB prevalence. Regional variations in TB incidence influence ADA levels in pleural fluid. Still, this study's findings suggest that factors beyond TB prevalence, such as genetic and environmental influences, also play a role. As a result, the diagnostic utility of ADA in TPE extends beyond high TB prevalence areas, making it a valuable tool for clinicians worldwide. The study's insights into the relationship between pleural inflammation, ADA levels, and lymphocyte activation add depth to our understanding of ADA's multifaceted role in TPE diagnosis. Overall, this research contributes to improving the accuracy of TPE diagnosis and highlights the importance of contextualizing diagnostic markers within the specific epidemiological and clinical landscape of the population being studied.

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