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ORIGINAL ARTICLE

Validity of Adenosine Deaminase (ADA) Level in Pleural Fluid for the Diagnosis of Tuberculosis

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ABSTRACT

Introduction: The pleural fluid LDH/ADA ratio, which can be determined from routine biochemical analysis, is highly predictive of TPE at a cut-off level of 16.20. Measurement of this parameter may be helpful for clinicians in distinguishing between TPE and PPE. Despite wide variations in the reported sensitivity and specificity of pleural fluid ADA level, it can be used as a surrogate for pleural biopsy when the latter is not feasible. Adenosine deaminase (ADA) is the most cost-effective pleural fluid marker and is routinely used in high prevalence settings, whereas its value is questioned in areas with low prevalence. The lymphocyte proportion (LP) is known to increase the specificity of ADA for this diagnosis.

Objectives: To determine the diagnostic accuracy of ADA level in pleural fluid for the diagnosis of TB as compared to other conventional methods available like gene expert.

Materials & Methods: The design of this study was a cross sectional study design. This study was conducted in Medicine Unit III, S.P.H. Quetta and the duration of this study was from 23rd October 2019 to 22nd April 2020. A total of 423 patients with pulmonary TB between the ages of 25 years to 45 years, having developed pleural effusion were included. Patients with pleural effusion due to causes other than TB, having extra pulmonary TB and those with other respiratory infections were excluded. Tests done specifically for TB in our setup include sputum AFB, CXR, and genexpert. These patients' pleural fluid ADA were then sent.

Results: Adenosine deaminase (ADA) found that 217 were True Positive and 13 were False Positive. Among 193, adenosine deaminase (ADA) negative patients, 12 (False Negative) had TB on gene expert whereas 181 (True Negative) had no TB on gene expert (p=0.0001). Overall sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of ADA level in pleural fluid for the diagnosis of TB as compared to other conventional methods available like gene expert was 94.76%, 93.30%, 94.35%, 93.78% and 94.09% respectively.

Practical Implication: This study was conducted to determine the validity of ADA levels for the diagnosis of TB and the results of this study if favorable will decrease the need for other laboratory tests usually done which take longer time, thus helping to decrease the need for multiple tests, early availability of report and timely management of the disease. This will further help to reduce the contact rate of TB and the risk of developing MDR TB by starting early treatment.

Conclusion: This study concluded that diagnostic accuracy of ADA level in pleural fluid for the diagnosis of TB is quite high.

Keywords: Pulmonary Tuberculosis, Pleural Fluid, Adenosine Deaminase, Biochemical, Mycobacterium .

INTRODUCTION

Tuberculosis (TB) is the leading cause of morbidity and mortality worldwide, especially in underdeveloped and developing countries. Pulmonary Tuberculosis (pTB) is the most common infection caused by the Mycobacterium Tuberculosis as compared to other extra-pulmonary forms of TB. In developing countries pulmonary TB accounts for 30-80% of all pleural effusions encountered. Tb is a global health problem [1]. In 2014, an estimated 9.6 million people developed TB and 1.5 million died from the disease. Currently, 22 high burden countries account for over 80% of world's TB cases. In 2011, Pakistan ranked 5th among 22 high burden countries. Keeping in view these statistics there is need for early detection and treatment of TB in Pakistan to reduce disease burden. Pulmonary tuberculosis, caused by mycobacterium tuberculosis [2], when bacilli are inhaled through respiratory route, is classically associated with pulmonary diseases and complications. It typically involves upper lobes and superior segments of lower lobes. Cavity formation associated with tuberculosis is noted in 30-50 % of the cases. HRCT chest is most sensitive modality in diagnosis of such cavitary lesions [(3,4)].

There are a number of tests to help diagnose TB, however there is still need for a test that can be totally relied upon as a single test to diagnose TB in a short time. In Baluchistan, approximately 20,000 new cases emerge every year, and the conventional methods used for TB diagnosis here are sputum AFB which need three early morning samples for diagnosis, GeneXpert that is not routinely used, and culture for mycobacterium which is a much expensive and time-consuming test [(5)]. There is need for a test that is both reliable and takes lesser time. Studies done previously show that pleural fluid ADA can be used for the diagnosis of pTB. Demonstration of elevated pleural fluid ADA levels is useful in establishing the diagnosis of tuberculous effusions. An elevated pleural fluid ADA level predicts tuberculous pleuritis with a sensitivity of 90% to 100% [(6)].

The pleural fluid LDH/ADA ratio, which can be determined from routine biochemical analysis, is highly predictive of TPE at a cut-off level of 16.20. Measurement of this parameter may be helpful for clinicians in distinguishing between TPE and PPE. Despite wide variations in the reported sensitivity and specificity of pleural fluid ADA level, it can be used as a surrogate for pleural biopsy when the latter is not feasible [(7)]. Adenosine deaminase (ADA) is the most cost-effective pleural fluid marker and is routinely used in high prevalence settings, whereas its value is questioned in areas with low prevalence. The lymphocyte proportion (LP) is known to increase the specificity of ADA for this diagnosis [(8,9)]. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for ADA were 89%, 92.7%, 69.2% and 97.9%, respectively.

The disease burden of TB in Baluchistan is very high with 20,000 new cases emerging every year. The treatment and diagnosis of the disease is already a problem in the province due to lack of facilities and poverty [(10,5)]. The already available laboratory tests have a low sensitivity and specificity and usually need further tests. This study will be conducted to determine the validity of ADA levels for the diagnosis of TB and the results of this study if favorable will decrease the need for other laboratory tests usually done which take longer time, thus helping to decrease the need for multiple tests, early availability of report and timely management of the disease [(7)]. This will further help to reduce the contact rate of TB and the risk of developing MDR TB by starting early treatment.

Objectives: To determine the diagnostic accuracy of ADA level in pleural fluid for the diagnosis of TB as compared to other conventional methods available like gene expert.

MATERIALS & METHODS

The design of this study was a cross sectional study design. This study was conducted in Medicine Unit III, S.P.H. Quetta and the duration of this study was from 23rd October 2019 to 22nd April 2020. This study was conducted after getting the approval from the ethical review board.

Sample Size: Taking sensitivity of ADA test as 90% and specificity of 50%, with prevalence of TB in Pakistan being 0.27%, the margin of error 5% and level of significance taken as 95%, the calculated sample size was 423.

Sample Technique: Non-probability, Consecutive sampling.

Inclusion Criteria: All patients with pulmonary TB between the ages of 25 years to 45 years, having developed pleural effusion.

Exclusion Criteria: All patients with pleural effusion due to causes other than TB, having extra pulmonary TB and those with other respiratory infections. These patients were excluded to control the effect modifier and avoid bias in the study. Patients having only one or two symptoms or having a history of less than two weeks were not included in the study.

Data Collection Procedure: All the patients diagnosed with TB on history, examination, and sputum AFB, CXR, GeneXpert, coming to OPD and admitted in ward were included in the study. Patients were enquired about their symptoms, examined in detail for signs of TB, and their baseline labs were sent. Tests done specifically for TB in our setup include sputum AFB, CXR, and genexpert. These patients' pleural fluid ADA were then sent. The reports of the test when obtained were discussed with the senior and if recommended to be appropriate, were included in the study. During examination of female patients, female attendant was standing by the side. Due respect was given to all patients. Exclusion criteria was strictly followed to control bias in the study. Informed consent was taken from all patients and their information was kept confidential. The data obtained was saved in the proforma attached. The data was then subjected to statistical analysis.

Data Analysis Procedure: Statistical testing was done by SPSS for Windows version 19. Descriptive statistics such as frequency of ADA level being positive and negative for TB cases, frequency of positive pleural fluid culture which is the modality of choice to diagnose TB was also calculated. Mean age of the patient with SD and male and female patients' frequency were also calculated. Sensitivity, Specificity, NPV, PPV and diagnostic accuracy was calculated by their respective formulas given in operational definitions by 2×2 table.

Sensitivity: (TP/TP+FN) × 100.
Specificity: (TN/FP+TN) × 100.
Negative Predictive Value: (TN/FN+TN) × 100.
Positive Predictive Value: (TP/TP+FP) × 100.
Validity: {(TP+TN)/(TP+TN+FP+FN)} ×100

RESULTS

Age range in this study was from 25-45 years with mean age of 35.04 ± 4.50 years. Majority of the patients 251 (59.34%) were between 25-35 years of age as shown in Table I. Out of these 423 patients, 296 (69.98%) were males and 127 (30.02%) were females with ratio of 2.3:1 (Figure I). Adenosine deaminase (ADA) found that 217 were True Positive and 13 were False Positive. Among 193, adenosine deaminase (ADA) negative patients, 12 (False Negative) had TB on gene expert whereas 181 (True Negative) had no TB on gene expert (p=0.0001) as shown in Table II.

Overall sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of ADA level in pleural fluid for the diagnosis of TB as compared to other conventional methods available like gene expert was 94.76%, 93.30%, 94.35%, 93.78% and 94.09% respectively. Stratification of diagnostic accuracy with respect to age groups is shown in Table III and IV. Gender stratification is shown in Table V & VI.

Table-1: Distribution of Patients According to Age

Age (years)	No. of Patients	%Age
25-35	251	59.34
36-45	172	40.66
Total	423	100.0

Mean ± SD = 35.04 ± 4.50 years

Figure-1: Distribution of patients according to Gender (n=423).

Table-2: Diagnostic Accuracy of ADA Level in Pleural Fluid for The Diagnosis of TB As Compared To Other Conventional Methods Available Like Gene Expert.

	Positive Result on Gene Expert	Negative Result on Gene Expert	P-value
Positive result on ADA	217 (TP)*	13 (FP)***	0.0001
Negative result on ADA	12 (FN)**	181 (TN)****	

*-TP=True positive **-FP=False positive ***-FN=False negative ****-TN=True negative

Sensitivity: 94.76%
Specificity: 93.30%
Positive Predictive Value (PPV): 94.35%
Negative Predictive Value (NPV): 93.78%
Diagnostic Accuracy: 94.09%

Table 3: Stratification of Diagnostic Accuracy with Respect To Age 25-35 Years (n=251).

	Positive Result on Gene Expert	Negative Result on Gene Expert	P-Value
Positive result on ADA	107 (TP)	04 (FP)	0.001
Negative result on ADA	02 (FN)	138 (TN)	

Sensitivity: 98.17%
Specificity: 97.18%
Positive Predictive Value (PPV): 96.40%
Negative Predictive Value (NPV): 98.57%
Diagnostic Accuracy: 97.61%

Table 4: Stratification of Diagnostic Accuracy with Respect to Age 36-45 Years (n=172).

	Positive Result on Gene Expert	Negative Result on Gene Expert	P-Value
Positive result on ADA	110 (TP)	09 (FP)	0.001
Negative result on ADA	10 (FN)	43 (TN)	

Sensitivity: 91.67%
Specificity: 82.69%
Positive Predictive Value (PPV): 92.44%
Negative Predictive Value (NPV): 81.13%
Diagnostic Accuracy: 88.95%

Table 5: Stratification of Diagnostic Accuracy with Respect to Male Gender (n=296).

	Positive Result on Gene Expert	Negative Result on Gene Expert	P-Value
Positive result on ADA	171 (TP)	01 (FP)	0.001
Negative result on ADA	12 (FN)	112 (TN)	

Sensitivity: 93.44%
Specificity: 99.12%
Positive Predictive Value (PPV): 99.42%
Negative Predictive Value (NPV): 90.32%
Diagnostic Accuracy: 95.61%

Table 6: Stratification of Diagnostic Accuracy with Respect to Female Gender Years (n=127)

	Positive Result on Gene Expert	Negative Result on Gene Expert	P-Value
Positive result on ADA	46 (TP)	12 (FP)	0.001
Negative result on ADA	00 (FN)	69 (TN)	

Sensitivity: 100.0%
Specificity: 85.19%
Positive Predictive Value (PPV): 79.31%
Negative Predictive Value (NPV): 100.0%
Diagnostic Accuracy: 90.55%

DISCUSSION

Adenosine deaminase (ADA), an enzyme produced from lymphocytes and involved in purine metabolism, has been extensively studied as a biochemical marker in pleural fluid during investigation for TPE [(11)]. The test is simple, cheap, rapid, minimally invasive, and can be performed in most laboratories. Although pleural fluid ADA is not a perfect discriminator, its level is considerably elevated in patients with TPE. High ADA levels can sometimes be observed in pleural fluid from patients of empyema, malignancy, or rheumatoid pleurisy. Therefore, presence of raised pleural fluid ADA is considered a useful marker for diagnosis of TPE, especially in patients with exudative and lymphocytic pleural effusion in high TB burden settings [(12)]. These patients can empirically be started on anti-tuberculous therapy if no other investigation can provide a definite diagnosis. Similarly, low pleural fluid ADA may be useful in excluding TPE, especially in a patient with low pre-test probability. These patients usually require additional investigations to establish the etiology of pleural effusion. Previous meta-analyses have suggested good diagnostic performance for pleural fluid ADA [(13)].

I have conducted this study to determine the diagnostic accuracy of ADA level in pleural fluid for the diagnosis of TB as compared to other conventional methods available like gene expert. Adenosine deaminase (ADA) found that 217 were True Positive and 13 were False Positive. Among 193, adenosine deaminase (ADA) negative patients, 12 (False Negative) had TB on gene expert whereas 181 (True Negative) had no TB on gene expert (p=0.0001) [(14-16)]. Overall sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of ADA level in pleural fluid for the diagnosis of TB as compared to other conventional methods available like gene expert was 94.76%, 93.30%, 94.35%, 93.78% and 94.09% respectively [(17)]. In a study, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for ADA were 89%, 92.7%, 69.2% and 97.9%, respectively.

In a study conducted in Serbia in which the tuberculosis is moderately common, 54 TPE and 67 MPE cases were prospectively evaluated. On the TPE diagnosis, sensitivity was found as 89.2%, specificity 70.4%, PPV 84.4% and NPV 78.4% for the cutoff value of ADA 49 U/L [(18-20)]. All patients underwent pleuroscopy-guided pleural biopsy to confirm the diagnosis in the same study. Sensitivity of this process was calculated as 66.7%, specificity 100%, PPV 100% and NPV as 78.8%.101 In another retrospective study, Chen et al., evaluated 63 TPE and 147 nontuberculous pleurisy cases and reported higher diagnostic values like the sensitivity of 87.3%, specificity 91.8%, PPV 82.1% and NPV 94.4% for the cutoff value similar to ours (55.8 U/L) in the TPE diagnosis. They concluded total ADA value of the pleural fluid is an appropriate and fast diagnostic tool for the diagnosis of tuberculosis [(21)].

ADA levels are most useful when there is a moderate to high suspicion of TB in patients with negative pleural fluid or biopsy cultures, and non-diagnostic histology. There is a wide range of cut-off values used by authors but in the majority of studies the most accurate threshold was found to range between 40 and 60 U/L.103 In a study of 254 patients with pleural TB, 99.6% had ADA more than 47 U/L104 and in another group of 303 patients in a high TB prevalence population with exudative effusions [(22)], 58% had TB with a lymphocytic predominant effusion and ADA more than 50 U/L.105 The diagnostic usefulness of ADA depends not only on its sensitivity and specificity, but also on the local prevalence of TB. In populations with a high prevalence of TB and clinical suspicion of TB effusion, elevated ADA level might be considered as a confirmatory test justifying treatment initiation [(23,24)]. In contrast, in countries with a low prevalence of TB, the negative predictive value remains high even though the positive predictive value of pleural ADA declines [(25)]. This was illustrated by two studies done in populations with low TB prevalence and non-TB lymphocytic effusions, in which 97% and 98% had ADA levels less than 40 U/L. Therefore, a negative ADA test may justify abandoning further diagnostic procedures for TB and pursuing alternative diagnoses [(26)].

When interpreting ADA levels, the clinician must additionally be aware of situations which may increase the likelihood of both the false-negative and false-positive ADA results. In the early phase of the disease low levels of ADA in the pleural fluid may be found, giving rise to a false negative result. However, ADA levels will invariably be elevated if thoracentesis is repeated a few days later [(27)]. Additional care should also be taken when interpreting pleural ADA levels in elderly patients and/or current smokers, as ADA levels may be low in such TB patients. Conversely, raised ADA levels may be observed in a number of conditions potentially leading to a false positive diagnosis of TB. These include rheumatoid effusion, empyema due to other bacteria, mesothelioma, lung cancer, parapneumonic effusion, and haematological malignancies [(28)].

The diagnostic accuracy of ADA can be improved by measuring different ADA isoenzymes. ADA-2 is increased in TB effusions, while ADA-1 is increased in other bacterial empyema's, and distinguishing between these two principal isoenzymes can increase the specificity of ADA for diagnosing TB [(29)]. Use of the ADA-2 isoenzyme measurement increased the specificity for TB from 91% to 96% and 92.1% to 98.6% , in two different studies [(30)]. Although it has been suggested that ADA might be a less sensitive marker of TB in immunocompromised patients, there is currently little evidence to support this view. Baba et al demonstrated that ADA is a reliable marker of pleural TB in HIV-positive patients, even for those with low CD4 counts, while Chung et al confirmed that ADA is an accurate marker in renal transplant recipients.

CONCLUSION

This study concluded diagnostic accuracy of ADA level in pleural fluid for the diagnosis of TB is quite high and has not only dramatically improved our ability of accurate diagnosis of TB but also improved patient care by timely and proper treatment, which consequently reduces patients' morbidity and mortality. So, we recommend that ADA level in pleural fluid should be used routinely as a prime test for diagnosis of TB in order to reduce the morbidity and mortality.

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