Original Article

Diagnostic Accuracy of Adenosine deaminase enzyme (ADA) in the diagnosis of tuberculous pleural effusion

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[2,3] *Conception of study*
[1,4] *Experimentation/Study conduction*
[2,3,4] *Analysis/Interpretation/Discussion*
[1,5] *Manuscript Writing*
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[1,4,6] *Facilitation and Material analysis*

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Article Processing
Received: 06/01/2020
Accepted: 20/11/2020

Cite this Article: *Rasheed, H., Khan, E.H., Shafi, M., Rafiq, A., Ali, A., Shiaib, S.L. Diagnostic Accuracy of Adenosine deaminase enzyme (ADA) in the diagnosis of tuberculous pleural effusion. Journal of Rawalpindi Medical College. 30 Dec. 2020; 24(4): 311-315.*
DOI: https://doi.org/10.37939/jrmc.v24i3.1318

Conflict of Interest: *Nil*
Funding Source: *Nil*

Access Online:

Abstract

Objective: To study the diagnostic accuracy of Adenosine deaminase enzyme (ADA) in the diagnosis of tuberculous pleural effusion (TPE).

Material and Methods: It was a cross-sectional descriptive study conducted in the Pulmonology departments of Lady Reading and Khyber Teaching Hospital Peshawar and department of Pathology, Khyber Medical College, Peshawar from April 2015 to Jan 2016. A total of 210 tuberculous and non-tuberculous pleural effusion patients were selected through consecutive non-probability sampling techniques. After physical and systemic examination, 3cc of pleural fluid was taken. ADA was estimated by Non-Guisti and Galanti method through the simple colorimetric method. All the data was entered in a specially designed proforma and SPSS v16 was used for statistical analysis.

Results: Out of 210 tuberculous and non-tuberculous pleural effusions, the commonest cause of pleural effusion was tuberculosis followed by malignancy. In our study, Pleural fluid ADA levels have sensitivity, specificity, positive predictive value(PPV), and negative predictive value (NPV) of 95.5%, 92.3%, 92.4%, and 96% respectively in differentiating tuberculous pleural effusions from non-tuberculous lymphocytes predominant pleural effusions.

Conclusion: Tuberculosis is the commonest infectious disease worldwide. A pleural fluid ADA level of ≥ 35 U/L in lymphocyte-predominant effusions makes mycobacterium tuberculosis most likely etiology. This test is not only very sensitive and specific but also it is very cheap, quick, and easy to perform by routine colorimetric method.

Keywords: Tuberculosis (TB), Tuberculous Pleural effusion (TPE), Adenosine deaminase enzyme (ADA).

Introduction

Robert Koch in 1882 discovered Mycobacterium Tuberculosis as a causative agent of Tuberculosis (TB).[1] The second commonest infectious etiology of death throughout the world is TB with 1/3rd of the population on the planet has been suffering from TB.[2] World Health Organization (WHO) report' 2020 suggests the emergence of more TB cases due to high drug resistance and poor patient's compliance.[3] Statistics in Pakistan is further alarming as 0.298 million patients were diagnosed in 2013 out of which 12777 patients were labeled as multi-drug-resistant.[4] TB remains asymptomatic in the majority of patients. Ten percent of latent TB patients progress to symptomatic TB and if left untreated, the mortality rate may rise to 50%.[5] TB has extra-pulmonary as well as pulmonary presentations. Tuberculous pleural effusion (TPE) ranks 2nd most common extra-pulmonary presentation of TB.[6] Conventional tests for diagnosis of TPE have low specificity and Sensitivity and as a consequence new diagnostic parameters are under research.[7] There are many causes of lymphocytes predominant pleural effusion but TB and carcinoma are more common.[8] Conventional diagnostic tests for diagnosis of TB have the following sensitivity; culture of pleural fluid 23%, the culture of pleural biopsy 55%, and pleural biopsy histopathologic diagnosis 63%. Pleural fluid Ziehl Neelson (ZN) staining, is rapid, very inexpensive but has very low sensitivity and produces usually negative results even in diagnosed cases of tuberculous pleural effusion.[9] TPE mostly occurs due to Mycobacterial antigens and only rarely by tuberculous bacilli as a whole. Due to pauci-bacillary nature of this effusion, its AFB culture, Polymerase chain reaction (PCR), and ZN staining are less sensitive.[10]

The diagnostic role of Adenosine deaminase enzyme (ADA) in TPE is under research with varying specificity and sensitivity in different regions of the world having excellent results in TB endemic areas.[11] ADA is an enzyme that has a natural role in purine metabolism. Its concentration is 10 times more in-activated T-lymphocytes as compared to Red blood cells.[12] Elevated ADA levels ≥35/40 U/L have been found in TPE.[13] Atalay in 2005 found low ADA level (< 30 U/L) in transudative pleural effusions and thus low ADA level can also be used in labelling pleural effusion as transudative and vice versa.[14]

The main objective of the present study is to know about the diagnostic accuracy of Adenosine deaminase (ADA) in TPE in the Khyber Pakhtunkhwa population of Pakistan.

Materials and Methods

It was a cross-sectional study of the descriptive type conducted in the Pulmonology ward Khyber Teaching Hospital Peshawar, Lady Reading Hospital Peshawar, and Pathology Department Khyber Medical College Peshawar, Khyber Pakhtunkhwa from April 2015 to Jan 2016. A total of 210 patients of pleural effusions including tuberculous and non-tuberculous were selected by non-probability consecutive sampling technique amongst the urban, rural, and semi-urban population of Khyber Pakhtunkhwa, Pakistan. Consent was taken from all the patients. After detailed general and systemic examination, 3cc pleural fluid was taken which was stored at -20 degrees after centrifugation. All the patients with lymphocytes predominant exudative pleural effusions with biopsy-proven tuberculosis, malignancy, and idiopathic (biopsy inconclusive), as well as transudative pleural effusions due to chronic renal failure, cirrhosis, and congestive heart failure, were included along with Neutrophilic predominant empyema and pneumonia. Patients suffering from Rheumatoid Arthritis and Emphysema were excluded.

Pleural fluid samples were measured for Adenosine deaminase enzyme (ADA) by non-Guisti and Galanti method spectrophotometrically through Microlab-300 chemistry analyzer. We selected the cut off value of 35 U/L in differentiating tuberculous pleural effusion from non-tuberculous pleural effusion. All the data was entered in a specifically designed proforma and SPSS version 16 was used for statistical analysis. We calculated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) by estimating ADA level in both biopsy-proven tuberculous pleural effusion and non-tuberculous pleural effusion. We also determined the significance of ADA level via Receiver Operating Curve (ROC-curve).

Results

Out of the total of 210 pleural effusion patients, 128 were males and 82 were females. Their mean age was 49.28 years. There were multiple causes of pleural effusion, with Tuberculosis accounting for 42% and Malignancy 19% as shown in Table 1.

Table 1: Causes of Pleural Effusion

Cause	Number (Percentage)
Tuberculosis	88 (41.91 %)
Malignancy	40 (19.04 %)
Idiopathic	22 (10.48 %)
Lymphoma	02 (0.95 %)
Congestive cardiac failure	09 (4.29 %)
Chronic renal failure	10 (4.76 %)
Multiple Myeloma	01 (0.48%)
Empyema	12 (5.71%)
Pneumonia	18 (8.57%)
Cirrhosis	08 (3.81%)
Total	210 (100 %)

Table 2: ADA Counts in Tuberculous and Non-tuberculous Pleural Effusion

ADA Count (U/L)	Tuberculous PE	Non-Tuberculous PE	Total
> 35	84	25	109
< 35	04	97	101
Total	88	122	210

By using a cut off of 35 U/L, for all effusion types (n=210), the sensitivity of pleural fluid ADA for detecting mycobacterium tuberculosis was 95.5% with a specificity of 79.5%, positive predictive value (PPV) 77.1%, and negative predictive value (NPV) of 96%. The area under the receiving operator curve (Figure 1) was 0.846. When using the same cut-off in the non-tuberculous group excluding empyema and para-pneumonic effusions i.e. by excluding neutrophilic predominant pleural effusions which are easily diagnosed by naked eye appearance or routine fluid examination, (n=62), specificity increases to 92.3% from 79.5%, PPV increases to 92.4% from 77.1% without changing the sensitivity and NPV. The area under the roc curve increases from 0.846 to 0.966 (Figure 2) taking the test from very good to excellent category. NPV falls to 98.9%. Sensitivity remains the same and specificity improves to 98.9%.

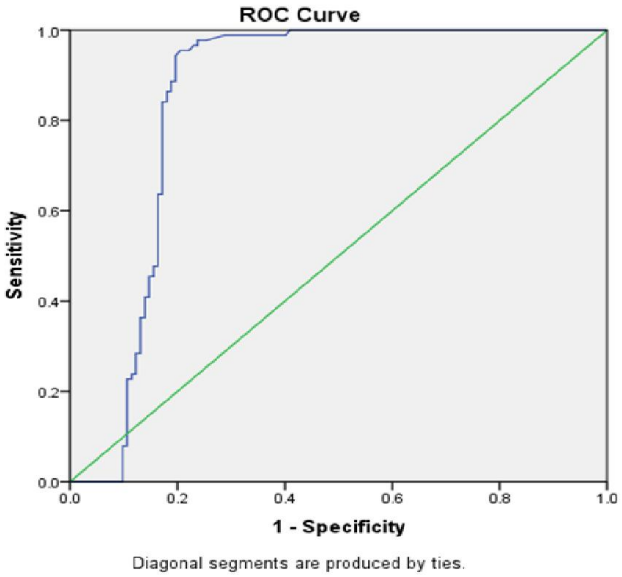

Figure 1: ROC CURVE showing sensitivity and specificity of ADA in the diagnosis of Tuberculous Pleural Effusion

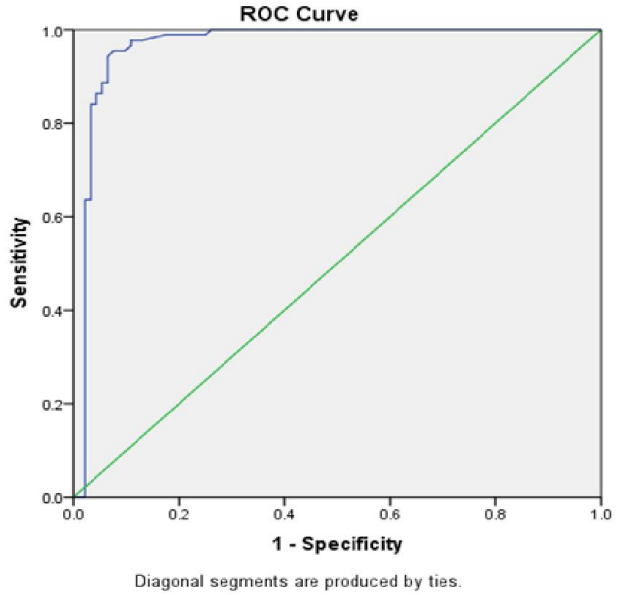

Figure 2: ROC CURVE showing sensitivity and specificity of ADA in the diagnosis of TPE after excluding Neutrophilic Predominant Non-tuberculous pleural effusion

Table 3: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ADA in the diagnosis of tuberculous pleural effusion

Group	Cut off value	Sensitivity	Specificity	PPV	NPV
Tuberculous Non-tuberculous	≥35	95.5%	79.5%	77.1%	96%
Tuberculous *Non-tuberculous**	**≥35**	**95.5%**	**92.4%**	**92.3%**	**95.5%**
Tuberculous Non-tuberculous	≥40	88.6%	80.3%	76.5%	90.7%
Tuberculous Non-tuberculous*	≥40	88.6%	93.5%	92.9%	89.6%

**Non-tuberculous group excluding Empyema and Parapneumonic effusion*

Discussion

Conventional diagnostic markers/tests for the diagnosis of TPE have low sensitivity and specificity. Although definitive diagnostic tests are the culture of pleural fluid and PCR for detection of Mycobacterial Tuberculosis, these are less sensitive due to pauci-bacillary nature of tuberculous pleural fluid with the culture of pleural fluid has a sensitivity of just 40% and that of PCR 42.9%. Also, culture apart from low sensitivity takes a maximum of 42 days, and thus early and quick treatment cannot be initiated.[15] In Pakistan, BCG being part of immunization schedule, Mantoux test has low utility.[5] Open pleural biopsy has a sensitivity of just 20 to 51%.[16] Therefore many biomarkers are under research for diagnosis of tuberculous pleural effusion of which Adenosine deaminase (ADA) is one of them. It is naturally involved in the metabolism of purine and its level is raised due to increased lymphocyte activity.[17] ADA is raised in tuberculous pleural effusion. Bento et al 50 years ago for the first time documented elevated ADA levels in tuberculous pleural effusion.[18] Liang did a meta-analysis in which he concluded high specificity, sensitivity, positive predictive value, and negative predictive value for ADA in labelling a pleural effusion as tuberculous.[13] In some international studies ADA has more than 90% sensitivity and specificity which is highest among all the current diagnostic tests for the diagnosis of tuberculous pleural effusion.[19] In tuberculosis endemic areas, 50% or more of pleural effusions are because of Mycobacterial Tuberculosis.[20] In tuberculous pleural effusion, high activated lymphocyte count is responsible for high ADA level. In this study, pleural fluid has been divided into two groups; tuberculous & non-tuberculous. Cut off value for positive tuberculous pleural effusion was taken as ≥35 U/L. In our study, Pleural fluid ADA levels have sensitivity, specificity, PPV, and NPV of 95.5%, 92.3%, 92.4%, and 96% respectively in differentiating tuberculous pleural effusions from non-tuberculous lymphocytes predominant pleural effusions which correspond to the recent international meta-analysis of sixty-three studies having 2798 patients of TPE and 5298 patients of non-tuberculous pleural effusions with ADA sensitivity and specificity of 92% and 90% respectively.

Study Limitations

We did not receive patients of Pleural Effusions due to Systemic Lupus Erythematosis (SLE) and Rheumatoid Arthritis which have been reported with ADA level > 35 U/L in some studies. Therefore it is suggested to be cautious in the interpretation of high ADA levels in these patients.

Conclusion

In our study, ADA ≥ 35 U/L in Pleural fluid has a sensitivity of 95.5%, the specificity of 92.3%, PPV of 92.4%, and NPV of 96% in differentiating tuberculous pleural effusions from non-tuberculous lymphocytes predominant exudative pleural effusions. Therefore ADA level of ≥ 35 U/L in lymphocyte-predominant pleural effusions makes TB the most likely cause. ADA estimation in pleural fluid is thus very sensitive and specific particularly in areas of the world where tuberculosis is endemic. It has a very low cost, can be estimated in a few minutes by routine colorimetric method. Therefore its estimation is recommended in all patients with exudative lymphocytes predominant pleural effusions.

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