

# ROLE OF ADENOSINE DEAMINASE ENZYME (ADA) IN DIFFERENTIATING TUBERCULOUS PLEURAL EFFUSION FROM MALIGNANT PLEURAL EFFUSION

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## ABSTRACT

**Objective:** To study the role of Adenosine deaminase enzyme (ADA) in differentiating tuberculous pleural effusion from malignant pleural effusion.

**Material and Methods:** It was a cross sectional descriptive study carried out in Pulmonology departments of Lady Reading Hospital, Khyber teaching Hospital Peshawar and department of Pathology, Khyber Medical College, Peshawar from April 2015 to Jan 2016. A total of 128 patients including 88 tuberculous and 40 malignant pleural effusion patients were selected through consecutive non-probability sampling technique.

**Results:** In our study, mean ADA levels in pleural fluid were significantly higher for tuberculous than for malignant pleural effusions ( $p < 0.001$ ). Pleural fluid ADA levels has sensitivity, specificity, PPV and NPV of 95.5%, 90%, 95.4%, and 50% respectively in differentiating tuberculous pleural effusions from malignant pleural effusion.

**Conclusion:** A pleural fluid ADA level of  $\geq 35$  U/L in lymphocyte predominant effusions makes mycobacterium tuberculosis most likely etiology. This test is not only very sensitive and specific but also is very cheap (cost effective), quick and easy to perform by routine colorimetric method.

**Key words:** Tuberculosis, diagnosis, Pleural effusion (P.E), malignant, Adenosine deaminase enzyme (ADA)

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## INTRODUCTION

Tuberculosis (TB) is caused by Mycobacterium Tuberculosis which was first isolated by Robert Koch in 1882<sup>(1)</sup>. TB is the second commonest cause of death among infectious diseases worldwide. One third of world population is suffering from TB infection<sup>(2)</sup>. According to WHO 2014 report, TB cases increased in 2013 as compared to 2012 which is attributed to increased drug resistance and poor compliance<sup>(3)</sup>. Situation in Pakistan is alarming as 0.298 million cases were reported in 2013 of which 12777 cases were multi drug resistant<sup>(4)</sup>. Mostly TB remains symptomless. About 10 % latent TB cases progress to full blown TB cases, if untreated, mortality rate rises to 50%<sup>(5)</sup>. TB has both pulmonary and extra pulmonary presentations. Tuberculous pleural effusion (TPE) is the second common extra pulmonary presentation of

TB. Sensitivity and specificity of conventional diagnostic tests for diagnosis of TPE are low and therefore new diagnostic biomarkers are under research<sup>(6)</sup>.

There are multiple causes of exudative pleural effusion with predominance of lymphocytes but TB and malignancy are most common (7). Sensitivity of diagnostic tests for TB are as follows; pleural fluid culture is 23%, pleural biopsy culture is 55% and histological diagnosis of pleural biopsy is 63%. Ziehl-Neelsen (ZN) staining of pleural fluid is although inexpensive and rapid but has very low sensitivity and produces negative results in confirmed diagnosed cases of tuberculous pleurisy. Diagnostic sensitivity of Adenosine deaminase enzyme (ADA) has been under research with varying sensitivity and specificity in

different parts of the world with excellent results in areas of the world where TB is endemic. ADA is naturally involved in metabolism of purine. Its concentration is more than 10 times in active T-lymphocytes than in Red blood cells. ADA levels are significantly raised in TPE above the level of 35 U/L. Main objective of the study is to determine the diagnostic accuracy of ADA in tuberculous pleural effusion in population living in the province of Khyber Pakhtunkhwa.

### METHODOLOGY

It was a descriptive cross sectional study conducted in the Pulmonology Department of Khyber Teaching Hospital (KTH), Lady Reading Hospital (LRH) Peshawar and Department of Pathology, Khyber Medical College, Peshawar from April, 2015 to January, 2016. A total of 128 patients were selected by non-probability consecutive sampling technique amongst the population from the rural, urban and semi-urban areas of Khyber Pakhtunkhwa. All the patients of pleural effusion which were either tuberculous or malignant were included in the study while the patients suffering from rheumatoid arthritis, lymphoma and emphysema were excluded from the study. Pleural fluid samples were analyzed for Adenosine deaminase enzyme (ADA) at Pathology Department, Khyber Medical College Peshawar

spectrophotometrically using Microlab-300 chemistry analyzer (By non-Guisti and Galanti method).

### RESULTS

A total of 128 patients were included in the study of which there were 80 male patients and 48 female patients (Figure 1). The male to female ratio is 1.67:1. Mean age of the patients was 51.73 ± 20.25 years. Of the total 128 patients, 88 patients were found out to be tuberculous in nature while 40 turned out to be malignant. As shown in Table 1, ADA levels were significantly higher in patients with tuberculous pleurisy (effusion) than in patients with malignant pleurisy ( $p < 0.001$ ).

By using a cut off of 35 U/L, for all effusion types ( $n = 128$ ), the sensitivity of pleural fluid ADA for detecting mycobacterium tuberculosis was 95.5% with a specificity of 90%, positive predictive value (PPV) 95.4% and negative predictive value (NPV) of 50% (Table 2). Area under the receiving operator curve (ROC) was 0.976 (ROC Curve) which suggest that the test is excellent in differentiating tuberculous pleural effusion from malignant pleural effusion (Figure 2). However it should be noted that in 4 malignant pleural effusions having ADA level above cut off value of 35 U/L, the maximum ADA level is 55 U/L which suggests that value above it almost exclude it from malignant and the cause is most probably tuberculous.

Figure 1: Showing Male to Female Ratio

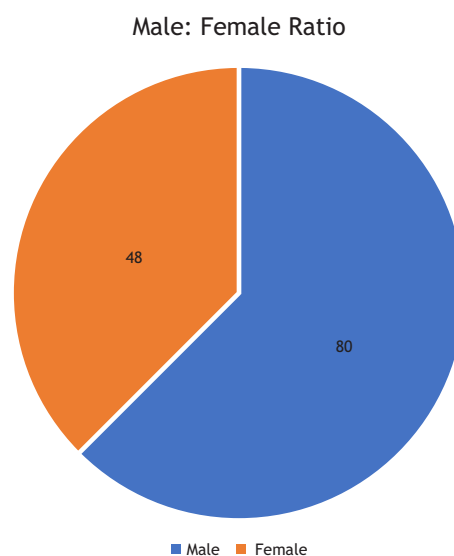
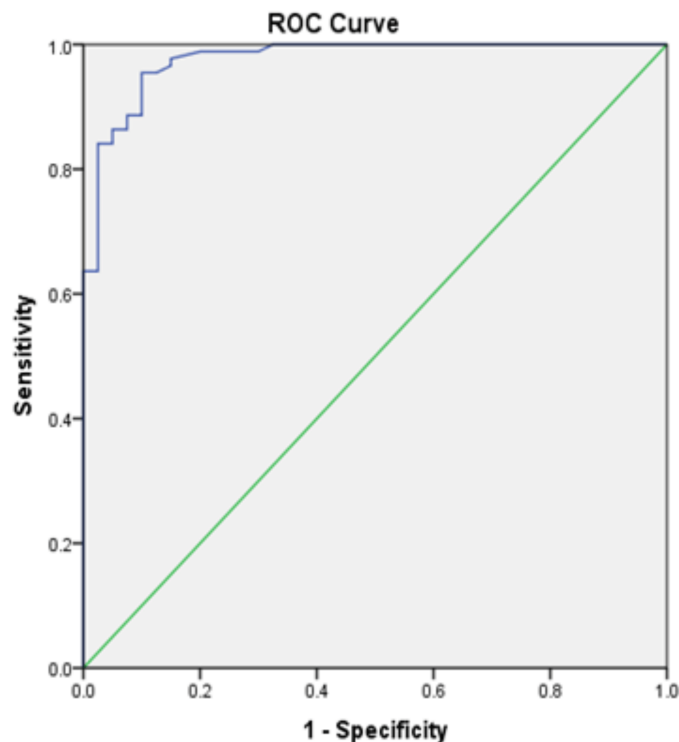


Figure 2: ROC Curve



Diagonal segments are produced by ties.

Table-1: ADA Count in Tuberculous and non-tuberculous Pleural effusion (P.E)

ADA U/L	Tuberculous P.E	Malignant P.E	Total
>35	84	04	88
<35	04	36	40
Total	88	40	128

Table-2: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ADA in diagnosis of tuberculous pleural effusion

Group	Cut off Value	Sensitivity	Specificity	PPV	NPV
Tuberculous	≥ 35	95.5%	90%	95.4%	50%
Malignant					

## DISCUSSION

Differentiating TPE from malignant pleural effusion is difficult because of low sensitivity and specificity of conventional diagnostic tests. Although definitive diagnostic tests for TPE are culture of pleural fluid and polymerase chain reaction (PCR), however test yield of these tests are low because pleural fluid culture apart from sensitivity of less than 40 %, also takes 42 days and so quick treatment cannot be started and

PCR has low sensitivity of 42.9 % because of the pauci bacillary nature of pleural fluid(11). In countries like Pakistan where the BCG is a part of immunization schedule, the Mantoux test has very low utility. Conventional open pleural biopsy has sensitivity of only 20 % to 51%. Therefore several biomarkers are understudy for diagnosis of tuberculous pleural effusion of which Adenosine deaminase (ADA) is the leading marker on the list. It is involved in purine metabolism and its level is increased during antigenic

and mitogenic lymphocytes responses. ADA is significantly raised in tuberculous pleural effusion. Bento et al. for the first time 50 years ago documented elevated ADA levels in tuberculous pleural effusion<sup>(5)</sup>. A meta-analysis was done by Liang in which he found highest values of sensitivity, specificity, positive predictive value and negative predictive value for ADA in diagnosis of tuberculous pleural effusion<sup>(15)</sup>. In some international studies sensitivity and specificity of ADA is observed for more than 90 % which highest among all the diagnostic tests for tuberculous pleural effusion<sup>(16)</sup>. In areas where tuberculosis is endemic, more than 50 % of pleural effusions are due to tuberculosis, while less than 50 % cases of pleural effusion are due to some other etiological factors<sup>(17)</sup>. In tuberculous pleural effusion lymphocyte count is high that's why the level of ADA in tuberculous pleural effusion is high as level of ADA rises in activated lymphocytes. In our study we divided pleural fluid in two groups; tuberculous and non-tuberculous. Cut off value for positive tuberculous pleural effusion was 35U/L. In our study, Pleural fluid ADA levels has sensitivity, specificity, PPV and NPV of 95.5%, 90%, 95.4%, and 50% respectively in differentiating tuberculous pleural effusions from malignant pleural effusions which correspond to 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<sup>(18)</sup>.

## CONCLUSION

Depending on our results we can conclude that ADA estimation in pleural fluid is very sensitive and specific and a level of >35 U/L in lymphocyte predominant pleural effusions makes TB the most likely etiology. It has also very low cost, can be performed in few minutes by routine colorimetric method. Therefore its estimation is recommended in all patients with exudative lymphocytes predominant pleural effusions.

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