Diagnostic accuracy of high pleural fluid Adenosine De Aminase levels in exudative pleural effusion in patients keeping pleural biopsy as gold standard

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Date Received: Aug 15, 2018
Date Revised: Oct 26, 2018
Date Accepted: Nov 25, 2018

Author Contributions

SSK RU conceived idea, MZ SSK RM SA drafted the study, RU SN MAK collected data, MAK RU RM did satisfied analysis & inter-pretation of data, SN MAK HBK AG critical reviewed manuscript, All approved final version to be published

Declaration of conflicting interests

The Authors declares that there is no conflict of interest.

Abstract

Background: The diagnosis of tuberculous pleural effusion (TPE) is difficult even today because of low sensitivity and specificity of conventional diagnostic investigation. The diagnostic accuracy of adenosine deaminase (ADA) in TPE varies in different parts of the world. The rationale of this study is to find out the diagnostic accuracy of ADA in differentiation of TPE from non-TPE.

Materials and methods: This cross sectional study was conducted between May 2015 and February 2016 in Pulmonology Departments Khyber Teaching Hospital (KTH) Peshawar. Samples were collected using non-probability consecutive sampling technique. Patients of both gender with Exudative Pleural effusion and aged 18-60 years were included in the study. Patients with transudative pleural effusion, patients not willing for pleural biopsy and those with inconclusive biopsy report were excluded. Pleural biopsy was done through abrahm's needle for histopathology and fluid was taken for cytology, gram staining and ADA level. ADA 40U/L has been taken diagnostic for TPE. Statistical analysis was done using SPSS- 16.

Results: Out of 100 patients, 61 were male and 39 female. 42 were tuberculous and 58 non-tuberculous pleural effusion. Among non-tuberculous pleural effusion group, 19 were malignant, 11 idiopathic, 23 para-pneumonic and 5 empyema. The sensitivity of pleural fluid ADA for detecting TPE was 95.2%, specificity of 79.3%, positive predictive value (PPV) 77% and negative predictive value (NPV) of 96%.

Conclusion: In this study, Pleural fluid ADA level has improved sensitivity, specificity, PPV and NPV in differentiating tuberculous pleural effusions from non tuberculous pleural effusions.

Keywords: Adenosine Deaminase; Tuberculous Pleural effusion; Diagnostic accuracy

This article may be cited as: Khattak SS, Khan AM, Zaib M, Ullah R, Muhammad R, Ashraf S, Naz S, Khan MA, Khan HB, Gul A Zaman M. Diagnostic accuracy of high pleural fluid Adenosine De Aminase levels in exudative pleural effusion in patients keeping pleural biopsy as gold standard. Pak J Chest Med 2018; 24 (4):203-207.

Introduction

Mycobacterium Tuberculosis, which belongs to the family of Mycobacteriaceae, is the etiologic agent of tuberculosis in almost all the cases. It was first isolated in 1882 by Robert Koch.[1] Tuberculosis is such a deadliest communicable disease that it ranks second worldwide regarding mortality rate among the infectious diseases.[2] One third population of the world is infected with

Mycobacterium Tuberculosis. In 2013, 9 million humans suffered and 1.5 million died from tuberculosis. Tuberculosis usually occurs in lungs but extra-pulmonary types of tuberculosis are also common. Common extra-pulmonary sites of tuberculosis include pleura, peritoneum, meninges and synovial membranes. Extra-Pulmonary TB diagnosis is usually difficult because the sensitivity and specificity of conventional diagnostic procedures are low.[3] There are several causes of pleural effusion. Exudative effusions with predominant neutrophils are be-cause of acute processes like pneumonia whereas exudative pleural effusions with predominant lymphocytes have many causes but tuberculosis and malignancy are more common particularly in areas of the world where tuberculosis is endemic.[4] Tuberculous pleural effusion is because of delayed hypersensitivity response in pleural space due to mycobacterial antigens resulting in lymphocytes activation and differentiation into lymphokines leading into macrophages activation for increased bactericidal re-sponse.[5] Two thirds of tuberculous pleural effusion patients are men, usually occur in 15-44 years and has more chances in patients having human immunodeficiency virus. Symptoms vary being more acute in younger age and chronic in old age group. The effusion is mostly a unilateral exudate, mostly right sided, and one third cases have negative Tuberculin Test. Drug Treatment is similar to any TB case. The corticosteroids have no role and chest drainage may improve symptoms quickly in massive effusions.[6]

Definitive diagnosis of Tuberculous Pleural Effusion (TPE) can be achieved only through pleural fluid culture and polymerase chain reaction but unfortunately the positive rates are low as in many cases Tuberculous Pleural effusion is paucibacillary in nature.[7] Also due to prolonged culture period (up to 6 weeks), decisions are necessary before the arrival of culture results. The sensitivity of pleural fluid culture is 23%, pleural biopsy culture is 55% and histological diagnosis of pleural biopsy is 63%. Zeal Neelson (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.[8, 9] The Pleural Effusion is most likely Tuberculous to several researchers if the level of ADA is above 40 U/L.[10] The elevated ADA (>40 U/L) activity in tuberculous pleurisy has been globally documented in several studies with varying levels of sensitivity and specificity in different parts of world except in Peshawar and Khyber Pakhtoonkhwa (KPK). Keeping in view of the above this study has been conducted to document diagnostic accuracy of ADA in KPK, Peshawar.

Methodology

This cross sectional study was conducted between May 2015 and February 2016 in Pulmonology Departments Khyber Teaching Hospital Peshawar Khyber Pakhtunkhwa, Pakistan. After approval from hospital ethical committee a total of 100 patients of pleural effusions including both tuberculous and non tuberculous were selected by non-probability consecutive sampling technique amongst the population from the rural, urban and semi-urban places of Khyber Pakhtunkhwa. Patients with Exudative Pleural effusion from both gender and aged between 18 and 60 were included in the study. Those patient with transudative pleural effusion, patients with exudative pleural effusion but not willing for pleural biopsy and patients with inconclusive biopsy report were excluded from the study. After explaining the whole protocol, benefits verses risks to the patient and informed written consent were obtained. Pleural tap done under local anesthesia in the 8th or 9th intercostal space. The analytical procedures were done at Pathology Department, Khyber Medical College. Gross examination of fluid including turbidity, volume and color was noted. Microscopic examination includes TLC (Total Leucocytes count), DLC (differential leukocyte count), Gram Staining and ZN Staining, Bio-chemical examination of fluid include total proteins, pH, and LDH.

ADA was analyzed by non Guisti and Galanti methodology. Turbid effusions and hemorrhagic fluids were properly centrifuged and supernatant was used for ADA estimation. Manual Chemistry Analyzer Microlab 300 (Germany) was used for ADA estimation. ADA 40U/L has been taken as cut-off for tuberculous pleural effusion.

Results

Among 100 cases of pleural effusion (PE), 61 were male and 39 females (Table 1). Mean age was 49.28 with Std. Deviation of 19.61(Table 2). 42 were tuberculous and 58 were non-tuberculous pleural effusion (Table 3). Only those tuberculous pleural effusion cases which were biopsy proven had been taken and included in tuberculous group. The remaining 58 cases were in non tuberculous group. Using cut off value of >40, 40 out of 42 tuberculous effusion patients fell into the high pleural fluid ADA group. Regarding non tuberculous group only 12 out of 58 fell into high pleural fluid ADA group.

Of these 58 non tuberculous groups, 19 were malignant PE, 11 were idiopathic, 23 were para-pneumonic effusion and 5 were empyema (Table 4). All the empyema cases (n=05) had high ADA level. 05 out of 23 para-pneumonic effusions and 2 out of 19

malignant pleural effusions also fell into high ADA group. The highest ADA levels were seen in a Non-Hodgkin's lymphoma case measuring 427 U/L followed by 05 cases of empyema with mean ADA level of 261.5 U/L and then followed by 42 cases of tuberculosis with mean ADA level of 72.2.There were 2 cases of biopsy proven TPE which fell into the low pleural fluid ADA group i.e. below 40 U/L. The mean pleural fluid ADA value was 72.2 in tuberculous pleural effusions with standard deviation (SD) of 35.6 and 50.6 in non tuberculous group with standard deviation of 91.1 (Table 5). Sensitivity, Specificity, Negative predictive value and Positive predictive value given in Table 6.

Table 1. Age distribution of patients

Total no. of cases	**100**
Missing	0
Mean Age	49.28
Std. Deviation of Age	19.612
Minimum Age	18
Maximum Age	60

Table 2. Gender distribution of study cases

Gender	**Frequency**	**Percent**
Male	61	61%
Female	39	39%
Total	100	100%

Table 3. Tuberculous and non tuberculous pleural effusions with Pleural Fluid ADA level

	High ADA (?40)	**Low ADA(? 39.9)**	**Total count**
Biopsy proven TPE	40	02	42
Biopsy proven Non-TPE	12	46	58
Total count	52	48	100

ADA= adenosine deaminase
TPE= tuberculous pleural effusion

Table 4. Causes of non tuberculous pleural effusions (N= 58)

Diagnosis	**Number**
Malignant	19
Idiopathic	11
Para-pneumonic	23
Empyema	05

Table 5. Mean and Standard Deviation of ADA in the tuberculous and Non-tuberculous pleural effusions

	Outcome	**No. of cases**	**Mean**	**Std. Deviation**
Pleural Fluid ADA level	Non-tuberculous	58	50.70	91.1
	Tuberculous	42	72.23	35.6

Table 6. Sensitivity, Specificity, Negative predictive value and Positive predictive value

Sensitivity	95.2%
Specificity	79.3%
Negative Predictive Value	96%
Positive Predictive Value	77%

Discussion

The diagnosis of tuberculous pleural effusion is a challenging job even today due to low sensitivity and specificity of conventional diagnostic investigations. Definitive diagnosis of Tuberculous Pleural Effusion (TPE) can be achieved only through pleural fluid culture and polymerase chain reaction but the positive rates are low as in many cases TPE is pauci-bacillary in nature. The sensitivity of pleural fluid culture is only 23%, pleural biopsy culture 55% and histological diagnosis of pleural biopsy is up to 63%. Zeihl Neelson staining of pleural fluid is although inexpensive and rapid but is very much less sensitive and produces negative results in confirmed diagnosed cases of tuberculous pleurisy. In countries like Pakistan where the BCG is a part of immunization schedule, the Mantoux test has very low utility.8-10 The Xpert(R) MTB/RIF test (PCR) on pleural fluid has very low sensitivity of 15 % due to pauci bacillary nature of pleural fluid.

To aid in diagnosis of TPE, several biomarkers are under research. One of them is Adenosine deaminase (ADA).It is the enzyme which is involved in metabolism of purine. It converts adenosine to inosine and deoxyadenosine to deoxyinosine and liberates ammonia. Its concentration is more than ten times in active T-lymphocytes than in erythrocytes. The ADA level is increased during antigenic and mitogenic lymphocytes responses.11

Piras et al. 50 years ago were the first who documented elevated ADA levels in tuberculous pleural effusion. Since then, a lot of authors have endorsed this finding and hence recommending the implementation of high ADA level in pleural fluid as sensitive, simple, rapid and cost-effective diagnostic parameter for diagnosing TPE.10,12 We demonstrated that pleural fluid ADA ≥ 40 IU/L detects TPE with excellent sensitivity and specificity. 40 out of 42 biopsy proven TPE cases have ADA level ≥ 40 U/L. Mehta et al., 2014 concluded that Pleural fluid ADA >40U/l has 85.8% sensitivity, 81% specificity , 76% positive predictive value and 90% negative predictive value.10,13 Our results match with that of Devkota et al., 2012 concluded in Nepal that if 42.19 U/L cut-off value is considered for diagnosis of TPE, sensitivity was 90.8% and specificity 82.8%11,14 In this study all the empyema thorax patients (n=5) and 1/3 of para-pneumonic effusions (n=23) had ADA level above the cut off level set for diagnosis of TPE. Similar findings were reported by Bielsa et al., 2013 who concluded that most TPE patients have more than 50% lymphocytes in pleural fluid but in 10% cases neutrophils are predominant especially in early TPE patients.12,15 In this study one case of Non-Hodgkin's lymphoma had extremely high ADA levels of 427. Therefore very high ADA level i.e. above 250U/L should arouse the suspicion of lymphoma than TPE. The highest ADA level in TPE in our study was 192.9 i.e. not a single TPE case out of 42 crossed the 200 U/L mark. Also TPE is much more common than pleural effusion due to lymphoma. Similar findings were also reported by Porcel et al., 2013 who found that nearly half of pleural effusions due to lymphomas have extremely high ADA levels usually > 250 U/L. Therefore when such high ADA levels are found in pleural fluid, always suspect empyema or lymphoma rather than tuberculosis16 In our study 2 out of 19 malignant pleural effusions fell into high ADA group but none of these 2 had ADA level more than 55U/L suggesting that in 10% malignant pleural effusion ADA may be ≥ 40 U/L but ≤ 55 U/L. Therefore ADA level ≥ 55U/L excludes the possibility of malignant pleural effusion in almost all cases. Similar findings were evaluated by Ogata et al and Yildiz PB et al.[16,17]

Conclusion

In this study, Pleural fluid ADA levels has sensitivity, specificity, PPV and NPV of 95.2%, 79.3%, 77%, and 96% respectively in differentiating tuberculous pleural effusions from non tuberculous pleural effusions. A pleural fluid ADA level of ≥ 40 U/L in lymphocyte predominant effusions makes mycobacterium tuberculosis the 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 chemistry analyzer. Therefore it is recommended to be routinely used in evaluating patients with exudative lymphocytes predominant pleural effusions.

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