INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

IJCMPR

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RESEARCH ARTICLE

USEFULNESS OF PLEURAL FLUID ADA LEVEL IN DIFFERENTIAL DIAGNOSIS OF EXUDATIVE PLEURAL EFFUSION – A PILOT STUDY

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ARTICLE INFO

Article History:

Received 16th May, 2016
Received in revised form 15th June, 2016 Accepted 28th July, 2016
Published online 27th August, 2016

Key words:

Pleural Fluid; Adenosine Deaminase; Exudative Pleural Effusion; Tuberculosis

ABSTRACT

Pleural effusion is an abnormal collection of fluid in pleural space resulting from excess fluid production. It is a frequent manifestation of serious thoracic disease whose specific diagnosis is a challenging task. Pleural effusion can be due to Infectious, malignant, parapneumonic disease and tubercular or other causes. Accurate diagnosis of etiology of pleural effusion (PE) by clinical or radiological methods is difficult due to overlapping symptoms and microbiological test are time consuming. Thus, diagnostic efficiency of Pleural fluid Adenosine Deaminase PF ADA was analysed in this study specifically for tubercular pleural effusion. TB is a global health problem and therefore requires a method which is of short duration with acceptable sensitivity and specificity to diagnose it. The study aims to assess utility of Pleural fluid ADA as a routine biochemical test for diagnosis of exudative pleural effusion arising due to tubercular and other causes.

Material and Method The study involved 187 adult patients diagnosed with exudative pleural effusion and classified into 5 groups as follows:1 Malignant pleural effusion (MPE), 2 Chronic non-specific inflammation (CNI), 3 Parapneumonic pleural effusion (PPE), 4.Tubercular pleural effusion (TBPE) and 5. Others. After complete clinical evaluation, routine Pleural fluid analysis and ADA was analysed. Reciever Operating Characterstics (ROC) analysis established the cutoffs of ADA for discriminating between groups.

Result and Discussion pleural fluid ADA was significantly higher in Tubercular effusion group (88.12± 35.9 U/L), followed by CNI group (73.41± 29.0 UL) than any others groups. Further, sensitivity and specificity of 94.1 and 69.4 % was obtained in Tubercular group at cut off 32.3 U/L. Though, at cut off 27.85 U/L PPE patients show 100% sensitivity but its specificity was much less i.e. 31.5%. The results show greater diagnostic accuracy of ADA in tuberculosis with acceptable Positive predictive value and Negative Predictive value. P F ADA is a simple, cost effective and useful in differential diagnosis of Tubercular pleural effusion where other cytological and biochemical test gives misleading results. This diagnostically effective in countries like India where large population lives in overcrowded and poorly ventilated areas and are thus highly prone to infectious diseases like Tuberculosis.

INTRODUCTION

Pleural Effusion (PE) is an abnormal accumulation of fluid in the pleural space lined by mesothelial cells resulting from excess fluid production or decreased absorption (1). Fluid accumulation can be due to increased permeability of pleural membrane, increased pulmonary capillary pressure, decreased negative intrapleural pressure, decreased oncotic pressure and obstruction of lymphatic flow (2). The etiological diagnosis of PE is often challenging because of spectrum of pulmonary disorders causing accumulation of fluid. While investigating the cause of PE, first step is to differentiate between exudative and transudative PE. According to a Meta analysis, exudative PE meet at least one of the following criteria i.e. PF protein >2.9g/dl, pleural fluid cholesterol > 45 mg/dl and lastly pleural fluid Lactate Dehydrogenase > 60% of upper limit for serum whereas transudative PE meet none (3,4). No further diagnostic investigation of fluid is required for transudative PE but exudative PE can be due to Tubercular or non bacterial infection, Parapneumonic, malignant or chronic non specific inflammation (CNI). Although it is easy to establish the presence of PE clinically and radiologically, it is often not always easy to determine its etiology. Diagnosis may not be ascertain for some patients despite performance of all diagnostic steps, such as imaging, cellular, microbiological and biochemical, closed and open pleural biopsy. Additionally, cytological examination of suspected malignant PE can result in false negative rates of upto 40 % (5). Thus, diagnostic

difficulties have led to search of novel biomarkers for exudative PE.

ADA is an enzyme that converts adenosine to inosine and deoxyadenosine to deoxyinosine and thus catalyze irreversible deamination. It is widely disrributed in human tissue especially in T- lymphocytes and therefore is a significant indicator of active cellular immunity and increases in disease where cell immunization is stimulated (6). Human ADA activity is found to be increased in various diseases like tuberculosis, HIV, thypoid, infectious mononucleosis and in certain malignancies (6). It acts in proliferation and differentiation of T-Lymphocytes and monocytes. Despite the fact that there are many causes of PE, it is estimated that 90% of all pleural effusion are the result of only 5 conditions, congestive heart failure (CHF), pneumonia, malignancy, viral infection and pulmonary embolism. An epidemiologic study from Czech Republic found that four leading cause of PE in prder of incidence are congestive heart failure, pneumonia, malignancy and pulmonary embo;ism with malignancy accounting for 24% of all pleural effusion (7). CHF cause almost all transudative PE whereas malignancy, pneumonia, pulmonary embolism and tuberculosis are the main cause of exudative PE. Bacterial pneumonia is associated with PE in 40% of cases (8).

Aim

The aim of the study is to evaluate the usefulness of PF ADA as a biomarker for differential diagnosis of various etiologies of exudative Pleural effusion including malignant, tubercular, chronic non specific inflammation and parapneumonic pulmonary disease. The study also focuses on the role of PF ADA in tuberculous pleurisy so that it can be used as a screening tool in routine diagnosis of PTB.

MATERIAL AND METHOD

This is a tertiary hospital based observational study conducted in the "Institute of Respiratory Disease, SMS Medical College", Jaipur during the year 2013-2014. 187 adult patients (both male and female) with exudative pleural effusion (both outdoor and indoor) who agreed to participate in the study were enrolled. After full explanation, written informed consent was obtained from all patients. Subject with transudative PE, those suffering from HIV, encephalopathy, renal disease, uncontrolled diabetes mellitus, cardiac disorder, major psychiatric illness, pregnant or lactating women were excluded from the study. Patients with hematological disease, respiratory failure and on treatment (including ATT) or any other therapy were also excluded from the study. All patients were classified into 5 etiologic classes on the basis of specific diagnostic criteria as follows.

Group1 (N=11): patients with malignant pleural effusion (MPE)

Group 2 (N=58) patients with chronic non-specific inflammation (CNI)

Group 3 (N=9) patients with parapneumonic pleural effusion (PPE)

Group 4 (N=102) patients with tubercular pleural effusion (TBPE)

Group 5 (N=7) patients with other causes of pleural effusion.
All subjects will be submitted for:

Thorough clinical history including smoking and occupational history, physical examination (fever, cough, hemoptysis, weight loss, appetite loss, night sweats, breathlessness etc) and signs such as cervical lymph node enlargement, clubbing, SVC obstruction were done.

Radiographical Investigation Chest X-ray (PA view) with side involved, amount of fluid, parenchymal involvement, cavitation and presence of any other abnormalities were recorded. CT of thorax & abdomen was done if necessary and ultrasonography of thorax and abdomen if obligatory.

Thoracocentesis and pleural fluid analysis PF was accumulated in detached vials for biochemical - Protein (Biuret method), glucose (GOD-POD method), Albumin (BCG-method), cytological-cell count, cell type and microbiological examination i.e. Gram staining and conventional Ziehl-Neelson's stain for acid fast bacilli. Culture of suspected tuberculous effusion was made by BACTEC rapid culture method for MTB.

Pleural Fluid ADA was analysed on Randox imola 3 Autoanalyzer by enzymatic method (kit Diazyne, Presicion). The end product quinonine dye is monitored in kinetic manner at 556 nm (10).

Routine laboratory Investigations Hemoglobin, Total Leucocyte count, Differential count (on Adonis Axiom-19 plus cell counter), ESR (by Westergren method), bleeding time (by Duke's method), clotting time (by Sabreze's capillary tube method).

- Tubercular pleurisy was diagnosed by tubercular skin test (Mantoux technique), lymphocyte count in pleural fluid, sputum/pleural fluid smear for AFB and pleural biopsy showing caseating granuloma
- Malignant effusion was confirmed by cytological examination of pleural fluid or by thoracoscopic pleural biopsy using rigid thoracoscope (KARL STORZ ENDOSKOPE TRICAM SL II 20223020). The procedure was carried out by method described by BOUTIN and coworkers. CT guided biopsy and Abram's needle pleural biopsy was also done for confirmation of MPE cases when required.
- Parapneumonic PE was diagnosed on basis of clinical, biochemical and radiological signs suspected acute inflammation, positive gram staining, positive bacterial culture or predominance of neutrophil cells in pleural fluid.

Data Analysis

Data collected was smudged in MS excel sheet 2007. Qualitative data were expressed as percentage (%) and proportions while quantitative data as Mean±S.D. P value less than 0.05 was considered to be statistically significant. Comparison among various groups was assessed by chi-square analysis, using ANOVA and Multiple Comparisons Tukey test. For the choice of optimal cut off, Reciever Operating Characterstics (ROC) curves were constructed and Youden Index calculated. Furthermore, accuracy of pf CRP in distinguishing between tubercular and non tubercular and between parapneumonic and non parapneumonic PE was established by calculating sensitivity, specificity, Negative predictive value (NPV) and Positive predictive value (PPV). The best cut off has the highest Youden Index. The commercial statistical software package used was SPSS 17.0 (SPSS, Inc, Chicago, IL, USA).

RESULTS AND DISCUSSION

Pleural effusion is often a clinical problem in medical practice, as its differential diagnosis includes a wide variety of local and systemic symptoms. Microbiological methods do provide some definitive results, however yield rate is not only low but also have a long turn around time that may result in delayed diagnosis. Diagnostic difficulties have led to the search of new novel biomarkers of Pleural Effusion. Various parameters have been used by many researchers for differential diagnosis of exudative PE. Table 1 shows complete gender distribution, demographic characteristics, physical and clinical symptoms of patients for all 5 groups. Incidence of malignant and tubercular effusion was predominantly common in Males (77.6% and 67 % respectively) than in females (22.4% and 33.3% respectively). Due to smoking and alcoholic habits being more common in males, incidence of malignancy is three times more prevalent in males (78%) than females (22%). Similarly, for PPE 67% cases were males while on other hand, for chronic non specific PE 72.7% cases were females. The observations were consistent to other reports where male female ratio in exudative pleural effusion was 4.45 showing a significant predominance of males in lung infection cases (9). The mean age of all patients was 44.12±16.5 years (table 1) and mean age of malignant group was significantly ($p<0.001$) highest among all i.e. 59.8± 11.86 years. Malignant mesothelioma usually presents in fifth to seventh decade of life and pleurisy develops only in later stages of disease. No significant difference was seen in BMI and other general symptoms of patients.

All etiological classes of PE are associated with cachexia, loss of appetite, generalized weakness, weight loss, respiratory insufficiency and fever. In the present study, chest pain was the most common complaint (80.74%) reported in study population followed by cough (77%), Loss of appetite (68.98%), fever (68.44%), and shortness of breath (60.8%) were seen in more than half of population. Weight loss (40.64%), expectoration (31.55%) and hemoptysis (10.69%) were less common symptoms. Majority of patients have more than one symptoms and none was without any chest symptoms. Other studies have shown comparable frequencies of these symptoms i.e. chest pain (86.8%), dyspnoea (81.6%), fever (68.4%) and loss of appetite (60.5%) while cough (44.7%) and weight loss (34.2%) were less commonly observed (10). Majority of patients had moderate amount of PE (59.9%) with right side predominance (54%). Presence of free fluid in pleural space (88.77%) was more common in contrast to loculated effusion (11.22%). Presence of loculi indicates an intense inflammatory response. Straw coloured Pleural fluid were found in 68.9% cases followed by hemorrhagic effusion in 27.27% patients in our study population. Among all hemorrhagic effusions, 77.59% cases belong to malignant group since malignancy is one of the most common causes of hemorrhagic pleural effusion.

Basic characterstics of pleural fluid samples are shown in Table 2 (figure 2). Cytological and biochemical analysis of pleural fluid constitutes an important part of differential diagnosis of exudative PE. An increased White cell count i.e. more than 7000/ul is commonly found in most infectious

Table 1 Distribution of General Demographic, Physical and Clinical Symptoms in Subjects

General characteristics of subject		CNI PE	Malignant PE	Parapneumonic PE	Tubercular PE	Others	Total	P value
No of patients (n)	N	11 (5.9)	58 (31)	9 (4.8)	102 (55)	7 (3.7)	187	-
	Males (N)	3 (27.3 %)	45 (77.6%)	6 {66.67 %}	68 {66.67 % }	6(85.7 %)	128 (68.4%)	0.017
	Female (N)	8 (72.7 %)	13 {22.4%}	3 {33.33 %}	34 { 33.33 %}	1 (14.3%)	59 (13.55%)	
Average age (in years) mean ± SD		39.9 ±15.3	59.81 ± 11.86	34.44 ± 11.4	36.19 ± 12.65	48.71±13.7	44.12±16.49	P< 0.001
Location	Urban (n=99)	4 (4.04 %)	34 {34.34%}	4 {4.04 }	55 { 55.56}	2 (2.02)	99 (52.94)	0.415
	Rural (n = 88)	7 (7.95%)	24 {27.27}	5 {5.68}	47 { 53.41 }	5 (5.68)	88 (47.05)	
BMI (kg/m^2) (mean ± SD)		19.96 ±4.32	20.47 ± 3.67	21.27 ± 2.43	20.00 ±3.17	19.86±0.8	20.20±3.31	0.771
Smoking Status	Ex Smoker	0 (0.00)	4 {6.90 }	1 {11.11}	1 (0.98)	0 (0.00)	6 (3.20)	0.000
	Non Smoker	8 (72.7)	10 {17.24 }	5 {55.56 }	51 (50.0)	3 (42.86)	77 (41.17)	
	Smoker	3 (27.3)	44 {75.86 }	3{33.33 }	50 (49.02)	4 (57.14)	104 (55.61)	
Alcoholic status	Ex alcoholic	0 (0.00)	2 {3.45 }	0{0.00 }	3 (2.94)	0 (0.00)	5 (2.67)	0.395
	Non Alcoholic	10 (90.91)	48 { 82.76 }	5{55.56 }	87 (85.29)	6 (85.71)	156 (83.42)	
	Alcoholic	1 (9.09)	8 {13.79 }	4 {44.44 }	12 (11.76)	1 (14.29)	26 (13.90)	
Side of effusion	Bilateral	0 (0.00)	3 {5.17 }	0 {0.00 }	1 (0.98)	0 (0.00)	4 (2.13)	0.609
	Left	6 (54.55)	24 {41.38 }	4 {44.44 }	44 (43.14)	5 (71.43)	83 (44.38)	
	Right	5 (45.45)	31 { 53.45 }	5 {55.56 }	57 (55.88)	2 (28.57)	100 (53.47)	
Amount of effusion	Massive	0 (0.00)	26 (44.83)	0(0.00)	3 (2.94)	0 (0.00)	29 (15.50)	P < 0.001
	Moderate	11 (100)	20 (34.38)	0 (0.00)	77 (75.49)	4(57.14)	112 (59.89)	
	Minimal	0 (0.00)	12 (20.69)	9 (100.0)	22 (21.57)	3 (42.86)	46 (24.59)	
Nature of Exudate	Free	9 (81.82)	58 (100.0)	9 (100.0)	84 (82.35)	6 (85.71)	166 (88.77)	P = 0.010
	Loculated	2 (18.18)	0 (0.00)	0 (0.00)	18 (17.63)	1 (14.29)	21 (11.22)	
Colour of exudate	Chocolate	0	0	0	0	1	1	P= 0.000
	Hemorrhagic	0	45	0	4	2	51	
	Milky	0	0	0	0	1	1	
	Straw	11	13	5	97	3	129	
	Turbid	0	0	4	1	0	5	
Respiratory symptoms	Cough	9	45	9	78	3	144	P= 0.113
	Expectoration	3	14	8	32	2	59	P= 0.004
	SOB	8	46	4	62	5	125	P=0.090
	Chest pain	6	46	8	85	6	151	P= 0.290
	Hemoptysis	0	11	1	8	0	20	P= 0.120
Non- Respiratory symptoms	Appetite loss	8	39	0	78	4	129	P= 0.001
	Weight loss	3	31	0	41	1	76	P=0.012
	Weakness	0	1	0	1	0	2	P= 0.900
	Fever	9	15	9	90	5	128	P=0.001

Exudates with majority of degenerate neutrophils in intense inflammatory conditions such as in Parapneumonic PE while lymphocytic predominance is commonly seen in Tubercular PE. Malignant PE is generally associated with moderate or slightly raised WBC count. Presences of Thrombocytes indicate acute haemorrhage or a contaimination during thoracocentesis.

Table 3 shows the mean value of PF ADA in all the groups. Difference was highly significant (p<0.001) with highest value in tubercular PE group (88.12± 35.90 U/L) followed by CNI group (73.41± 29.01 U/L). In parapneumonic (PPE) group, mean PF ADA was 36.22±5.65 U/L while in malignant and others group PF ADA levels were considerably low i.e 17.77±10.12 and 17.46±9.62 U/L respectively (Fig 1). When

Table 2 Basic Hematological and Biochemical Parameters in Pleural Effusion

Pleural fluid (pf) markers	CNI PE	Malignant PE	Parapneumonic PE	Tubercular PE	Others	P value	Significance
Pf Glucose (mg/dl)	76.8± 13.5	85.6 ±42.0	46.1 ± 5.3	69.8 ± 16.5	76.1 ± 19.7	<0.001	S
Pf Protein (g/dl)	5.5 ± 0.9	4.6 ± 1.1	4.0 ± 0.4	5.4 ± 1.1	4.59 ± 0.69	<0.001	S
Pf Albumin (g/dl)	3.1±1.1	3.0 ± 0.78	3.8 ± 1.3	3.06 ± 1.3	2.9 ± 1.51	<0.05	S
A/G Ratio	1.29 ± 0.76	1.87 ± 0.96	1.42 ± 0.71	1.27 ± 1.0	1.32 ± 0.9	<0.05	S
Pf TLC (per cubic mm)	7.6 ± 2.8	11.6 ± 2.71	30.2 ± 10.2	18.4 ± 3.2	10.87 ± 4.55	P= 0.70	NS
Pf Neutrophil count (%)	16.8 ± 11.0	18.7 ± 5.31	53.2 ± 35.2	20.6 ± 14.5	57.7 ± 29.6	<0.001	S
Pf Lymphocyte Count (%)	80.0 ± 8.1	64.7 ± 17.4	26.7 ± 15.0	79.3 ± 14.7	42.3 ± 29.6	<0.001	S
Pf Platelet Count (per ul)	2.2 ± 0.4	2.6 ± 1.4	3.3 ±1.2	2.4 ± 0.8	2.23 ± 0.79	P= 0.10	NS
Hemoglobin level (g/dl)	9.5 ± 1.7	10.7 ± 1.7	11.0 ± 1.2	10.9 ±1.5	10.9 ± 1.77	P= 0.10	NS

Values are in mean ± Standard Deviation: S is Significant and NS is non significant

In the present study, Total Leucocyte Count was highest in PPE group, followed by tubercular and malignant groups, but the difference in TLC was insignificant (p=0.70). Significant difference was observed (p<0.001) in neutrophil count (%) and lymphocyte (%) level among all groups. Neutrophil count was highest in others group (57.7±29.6) and PPE group (53.2±35.2) and lymphocyte count was highest in CNI (80.0±8.1), followed by TBPE (79.3±14.7) and malignant PE group (64.7±17.4) (Table2). Non-significant difference (p=0.10) was observed in platelet count and hemoglobin level among all classes of exudative PE (Table 2). Comparable results have been shown by other workers with significantly higher level of neutrophils (%) in parapneumonic PE patients than in other categories of PE and level of lymphocytes was highest in Tubercular PE patients than in other cases of Pleural effusion (11). Another study also found a higher leucocyte level in linfectious PE than Malignant PE. The level of neutrophils (%) in the PNPE group was significantly higher than those in other group and lymphocyte count higher in TBPE than other groups (12). A predominance of neutrophil in pleural fluid is a simple marker of parapneumonic PE. Immune stimulation causes recruitment of large number of Polymorphonuclear cells locally that are further proliferated under the effect of cytokines and other inflammatory markers. Pleural fluid glucose concentration was highest in MPE group (85.6±42.0), followed by CNI and others group (76.8±13.5 and 76.14±19.9) mg/dl respectively. Patients with PPE have lowest level of PF glucose i.e. 46.1±5.3 mg/dl. Glucose level < 60 mg/dl is often seen in complicated PPE. Various studies have shown low glucose level in infectious PE than non infectious or malignant condition (13). As regard to pleural fluid protein, significantly high level was obtained in CNI (5.5±0.9) and tubercular group (5.4±1.1) (g/dl). Mean PF Albumin level in CNI and PPE group were 3.1±1.1 g/dl and 3.8 ±1.3 g/dl respectively and were significantly higher than other classes (p<0.05). Exudative effusion mostly involves some types of inflammation which leads to increased leakage of fluid that has high protein concentration (14). Intense inflammation in CNI, PPE and TBPE groups accounts for increased level of PF protein and PF albumin in these patients than in malignant group due to intense inflammation that increases permeability of pleural membrane. Albumin being a low molecular weight protein enters from plasma to pleural space via inflamed pleura (Table 2).

Infectious and non infectious or malignant conditions are compared, ADA was higher in infectious (including TB, Parapneumonia and CNI) than in non infectious group (like malignancy). TB is a cell mediated immune response which shows a predominance of lymphocytes and ADA level higher than other forms of Pleural effusion. ADA is widely distributed in T- lymphocytes and thus tuberculosis is associated with high ADA activity. In another study, mean PF ADA in tuberculous Pleural effusion was 78.36 ± 19.5 that was significantly higher than that observed in Parapneumonic or malignant PE i.e 51.61 ± 43.1 UI/L and 19.73 ± 6.19 U/L respectively (15). MPE is a common condition seen in advanced stages of lung cancer with approx. 50% of cases developing PE at later stage of the disease (16).

Table 3 Mean±SD of ADA Level in Different Etiologies of Pleural Effusion

Diagnosis	No. of cases	Mean ADA IU/L	Std. Deviation	P value
Tubercular	102	88.12	35.90	
Malignant	58	17.77	10.12	
Chronic nonspecific pleuritis	11	73.41	29.01	
Parapneumonic	9	36.22	5.65	< 0.001
Others	7	17.46	9.62	
Total	187	60.29	43.32	Significant

ANOVA - Analysis of Variance ---F =145.14; p<0.001 S
Multiple Comparisons - Tukey Test ---

Increase PF ADA in malignant effusion may be associated with Parapneumonic effusion caused by lung cancer induced obstructive pneumonia or due to enhanced activity of lymphocytes during immune reaction against tumors. Patients with lung cancer or mesothelioma may be infected with TB concurrently. PF ADA is thus highly specific for tuberculous PE especially lymphocytic in origin. Various studies assess usefulness of ADA estimation in lymphocytic origin. For example, it was seen that ADA level <40.0 IU/L virtually excludes diagnosis in lymphocytic PE, whereas very high ADA are associated with lymphocytic and Tuberculosis PE (16). The above finding suggest TP either lymphocytic or non lymphocytic show significant rise in PF ADA, but cut off for non lymphocytic Effusion is less than lymphocytic showing much higher sensitivity and Specificity for diagnosis of lymphocytic TBPE. TB is the most common cause of Pleural effusion in developing countries like India. Definitive diagnosis of TB often difficult as in more than 50 % of cases, since pleura is the only site of infection. Tuberculin test in non

specific and finding can be negative, further when bacterial load is low, PF MTB culture is also not specific. PF ADA estimation is quick and non expensive (17). Other studies have established the role of pleural fluid ADA in diagnosing TB with effusion without performing invasive procedure like pleural biopsy. Their results were statistically significant between tuberculous and non tuberculous groups ($p< 0.001$) even when cut off was kept > 40.0IU/L (18). PF in TB is mostly associated with accumulation of mononuclear cell and T lymphocytes. Further, tissue injury and intense local inflammation and granuloma formation in PTB increases local synthesis of inflammatory markers and cytokines. These acts as proliferating agents which causes rapid multiplication of macrophages and T- lymphocytes. Cells that reproduce rapidly tend to have high ADA activity compared to cells that do not proliferate (19). This accumulation of activated cells in pleural space results in local intrapleural production of ADA in TBPE. This was observed and reconfirmed in our study. While PF ADA level higher than 70 U/L interpret in favour of Tuberculous pleurisy, values lower than 40 I/U suggest causes unrelated to TB. The enzyme may increase in lymphoma, emphysema or in malignancy. Number of other studies confirmed ADA level > 40 U/L highly suggestive of Tubercular etiology and obtained Sensitivity and specificity of 72% and 69% at this cut off (19). PF ADA level were established to be significantly higher in TBPE and PPE when compared with other PE cases (19).

thus is responsible for comparable value if PF ADA. The mean PF ADA in group-v others category could not be compared to rest of study groups, as it contains both infectious (amoebic liver abscess with hepatopleural fistula) and non infectious (pulmonary embolism, SLE etc.) diseases.

Diagnostic Efficiency of Pf Ada in Tubercular Pleural Effusion

The diagnostic efficiency of PF ADA with its sensitivity, specificity and AUC for differential diagnosis of different causes of PE is fully investigated in this study. Different cut off values of ADA ranging from 30-100 IU/L have been used in various studies resulting in different sensitivity and specificity. Positive predictive value (PPV) and Negative predictive value (NPV) of PF ADA have been studied in India and abroad. The observations have been tabulated in **table 5**. These discrepancies is the result of different method of ADA estimation, prevalence of TB in different areas as well as ethnic difference in the study population characteristics (21). In the present study ROC analysis of PF ADA for differential diagnosis of Tubercular effusion from non tuberculous effusions provided good sensitivity of 94.1 % and Specificity 69.4 % at cut off value 32.3 IU/L. At this cut off, AUC obtained after ROC analysis of PF ADA was 0.924 (at 95% CI with STD Error of 0.020) Fig 3 (Table 4). Present study showed that at PF ADA value 29.5 IU/L, there occurs increase in sensitivity to 95.1 % but at the cost of Specificity which fell

Table 4 Diagnostic Performance of Pleural Fluid ADA at Optimum Cut Off For Differential Diagnosis of Different Etiologies of PE At 95% CI

Diagnosis	Cut off level of PF ADA (IU/L)	Sensitivity	Specificity	PPV	NPV	AUC	Interval Lower bound-upper bound
Tubercular PE	≥ 32.32	94.1	69.4	82.46	89.04	0.924	0.885-0.963
Parapneumonic PE	≥ 27.85	100	31.46	6.87	100.0	0.681	0.561-0.800
CNI	≥36.45	90.9	42.0	-	-	0.623	0.512-0.733
Malignant PE	-	-	-	-	-	0.054	0.023-0.084

Table 5 Comparison of Studies at Different Places of PF ADA in Exudative Tubercular PE Cases.

S.No	Investigator	Country	Cut off (U/L)	Sensitivity (%)	Specificity (%)	PPV	NPV
1.	Sushmita *et. al.*	India (2010)	40	97	93	94	97
2.	Sharma *et. al*.	India (2010)	35	83.3	66.6	-	-
3.	Perlat *et.al*.	Albania(2011)	40	89	28.8	54.5	68.5
4.	Yoshiko O *et.al*.	Japan (2011)	36	85.5	86.5	69.7	93.6
5.	Ashmita A *et.al.*	South India (2014)	40	72.7	69.6	78.7	61
6.	Moung Chen *et.al*.	China (2014)	55	87.3	91.8	78.7	61.4
7.	Present study	India (2015)	32.2	94.1	69.4	82.5	89

PPV-Positive predictive value, NPV- Negative predictive value, SD- Standard deviation.

As in this study, previous reports have found increased PF ADA level in parapneumonic effusions wherein immune response mostly involes polymorphonuclear cells, macrophages and neutrophils rather than lymphocytes. ADA enzyme elevates in conditions like lymphoma, emphyma, Reumatoid arthritis or in malignancy. Approximately 20 % of patients with pneumonia develop Parapneumonic effusion, of whom 35 % have emphyema associated with high PF ADA (20). Since in our study, emphysema cases were excluded from PPE that were mostly neutrophilic effusions, PF ADA level was less than TBPE group. Neutrophilic effusion also causes high ADA level due to increased cellular activity. Mean PF ADA in CNI group was close to Tuberculous effusion group. These results are possibly due to misdiagnosis of early presentation of tubercular pleuritis. As all patients with CNI had same symptoms as TB with short duration of presenting complaints and approximately > 50% in this group were tuberculin positive and about 80% were relieved by Anti Tubercular Therapy, misdiagnosis might have occured and

To 67.1%. In another study that was conducted in Chinese population, ROC curve analysis of PF ADA provides an AUC of 0.941 (at 95% CI ; 0.899-0.983) at a cut off level 45 IU/L giving a sensitivity and Specificity of 93.8% and 82.5 % for diagnosis of Tuberculous Pleural effusion (21). The higher cut off in their study was due to lower proportion of Tuberculous pleurisy in Chinese than Indian or other races (36.9%, 80 % and 87.5% respectively). The Chinese appeared to have lower mean PF ADA compared to Indians and other races i.e. 52± 48, 94± 47 and 110 ± 48 IU/L respectively (21). This difference may be due to low socio economic status of majority of people in a developing country like India which are more prone to infectious disease like TB and develop pleurisy as a result of poor nutrition and improper health facilities. Some studies conducted abroad have shown PF ADA level 74.6 ± 7.9 U/L in Tuberculous pleurisy (TP) as against 21.9 ± 4.3 U/L in non tuberculous effusions. At a cut off level, 53.4 U/L, sensitivity and specificity was 91 % and 93 % respectively and suggested ADA as cheap, highly sensitive

and specific for diagnosis of TP (22) PE is widely associated with Tuberculosis specially in countries epidemic for it like India. Studies conducted in India have shown sensitivity of 83.3 % and Specificity 66.7 % have been obtained at a cut off value of PF ADA 35.0 IU/L with mean PF ADA level of 95.8IU/L. (23). There results were consistent to our observations. More of studies conducted in India showed that at a cut off level 40 IU/L gives a sensitivity and Specificity of 97.0% and 93.0 % respectively (24). Mean ADA for TBPE, MPE and PPE was 45.32± 18.21, 18 ± 12.78 and 27.45± 34.55 U/L respectively in a study that also showed 43 out of 49 patients with TBPE had ADA > 40.0 IU/L while only 3 out of 36 cases of MPE showed ADA > 40.0 IU/L. At this cut off PF ADA yielded 85.7% sensitivity, 80.8% specificity, 75 % PPV and NPP of 89 % for diagnosis of TB. Apart from TB, second most common cause of elevated ADA was parapneumonic PE (11-33 %) as similar increase found in our study. The later is usually neutrophilic unlike tuberculosis that is lymphocytic (25). When only tubercular and non tubercular respiratory diseases were compared, serum ADA increases significantly in Tuberculous pleurisy than other cases even when they are lymphocytic in origin (35.5± 6.9 and 16.20± 2.8 IU/L respectively). Further the high ADA activity in PF than serum in patients with TP indicates that cells in pleural space locally synthesize ADA (26). It is reasonable to suggest that high ADA in TP is a reflection of local activation of T-lymphocytes since ADA enzyme is typically of these cells and increases during Cell mediated immunity.

When AUC derived from ROC analysis for different parameters were studied individually for differential diagnosis of TBPE vs non TBPE, it was seen that ADA gave largest area under curve i.e. 0.940 at a cut off 42.2 IU/L. None of the other parameter like CRP, CEA, TNF-α, IL-6 and VEGF provided such large AUC, indicating that a high diagnostic efficiency in identification of Tuberculous pleurisy (27). Thus, ADA has emerged as a low cost and easy performance test that can be used as an effective screening tool for diagnosis of Tuberculous pleurisy.

In pathological condition, the clearance capacity of lungs is greatly decreased causing recirculation of activated lymphocytes. ADA activity increases during cellular activation to detoxify the toxic metabolities. This occurs in all disease characterized by enhanced CMI response as typhoid fever, bacterial pneumonia and tuberculosis etc.

Diagnostic Efficiency of Pf ADA in Parapneumonic Effusion

In the present study ROC analysis of PF ADA for differential diagnosis of Parapneumonic effusion from non parapneumonic effusion provided a sensitivity and Specificity of 100% and 31.46 % respectively at a cut off value 27.85 IU/L. The PPV was only 6.87% and NPV was 100 % with AUC of 0.681 (at 95 % CI with STD error 0.061) (Table 4). Various studies have shown comparable value of AUC for PF ADA in differential diagnosis of PPE vs non PPE, for example, at a cut off level 25 IU/L, researchers have shown AUC of 0.700 (0.550-0.840) with sensitivity and Specificity of 49 % and 66% respectively and a mean PF ADA 51.61 ± 43.1 IU/L, though significantly higher than malignant PE but less than tuberculous effusion (27). In their study, among all the parameters studied (ADA, CRP, CEA, TNF-α, IL-6 and VEGF), CRP provided the largest AUC (0.920) for discrimination of PPE from MPE and TBPE. Thus, it was found that PF ADA has limited significance in PPE but more diagnostic accuracy in TBPE.

Diagnostic Efficiency of Pf ADA in Effusion Arising From Chronic Non-Specific Inflammation (CNI)

The ROC curve of PF ADA for predicting CNI positivity was constructed. The AUC was found to be 0.623 (95 % CI with STD error of 0.056). The curve was not significantly different from 0.5 since valves > 0.05 i.e logistic regression not classify the group significantly better than by chance. At a cut off level 36.45 IU/L, sensitivity of 90.9% and Specificity of 42 % was obtained.

Diagnostic Efficiency of Pf ADA in Malignant Effusion

ROC analysis of pf ADA for differentiation of malignant from non-malignant effusion showed AUC=0.054 (**Table 4**) means that the test incorrectly classify all subjects with disease as negative and all subjects with non diseased as positive that is extremely unlikely to happen in clinical practice. The minimum AUC should be considered a chance level i.e AUC= 0.5.

CONCLUSION

The present study indicates high utility of pleural fluid ADA in differential diagnosis of exudative pleural effusion. ADA is simple, cost effective and diagnostically useful particularly in areas where TB is prevalent where other microbiological or biochemical tests fail to accurately determine the cause of exudation. At a very low cut off of 32.2 U/L, excellent sensitivity, NPV, good PPV and acceptable specificity is obtained. The study suggest level less than 30 U/L strongly predicts non tubercular origin of Pleural effusion that may be non bacterial or in few cases malignant. Very high ADA strongly suggest Tuberculous pleurisy, therefore can be usedas screening tool and for diagnosis of TP in countries with high prevalence of TP. Further its estimation serves as additive and supportive evidence for diagnosis of tuberculosis apart from routine standard methods. The authors declare that they have no competing interest.

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