Original Article

DOI: 10.7860/NJLM/2020/44941:2427

Role of Pleural Fluid D-Dimer as a Novel Marker in the Diagnosis of Pleural Effusion

Biochemistry Section

MIHIR MEHTA[1], SIMBITA MARWAH[2]

ABSTRACT

Introduction: Tuberculous Pleural Effusion (TPE) is associated with enhanced fibrinolytic activity which may lead to high levels of D-dimer.

Aim: The present study aimed to investigate whether pleural fluid D-dimer plays a diagnostic role for TPE.

Materials and Methods: It is a cross-sectional study comprising of 101 patients diagnosed with pleural effusion that were divided into TPE (41 patients) and Non TPE (60 patients). Pleural D-dimer levels were measured by latex agglutination assay. The capacity of pleural D-dimer to differentiate TPE from non TPE was assessed with Receiver Operating Characteristic (ROC) curve analyses.

Results: Subjects with TPE showed a marked elevation of pleural D-dimer than those with Non TPE (Mean: 1690.5 mg/L FEU vs 305 mg/L Fibrinogen Equivalent Unit (FEU); p<0.0001). The area under curve when pleural D-dimer was used to differentiate TPE from non TPE was 0.962 (95% confidence interval: 0.92 to 0.99). With a cut-off value of >501 mg/L FEU, the sensitivity and specificity were 90.24% and 86.67%, respectively. Pleural fluid D-dimer levels were higher in TPE as compared to Non TPE.

Conclusion: D-dimer might be useful as a novel marker for the diagnosis of TPE.

Keywords: Fibrinolytic activity, Tuberculosis, Sensitivity, Specificity

INTRODUCTION

Tuberculosis (TB) still remains a major global health problem as approximately 10.4 million people has fallen ill with TB in 2016. TB is the ninth leading cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS. In 2016, there were an estimated 1.3 million TB deaths among HIV-negative people (down from 1.7 million in 2000) and an additional 3,74,000 deaths among HIV-positive people [1]. TPE is one of the most common extrapulmonary manifestations of TB, which can lead to TB morbidity [2]. The diagnosis of TPE is made by detecting acid fast bacilli from the pleural effusion, sputum, or pleural tissue [3]. However, due to the paucity of the acid fast bacilli in pleural effusion, the diagnostic tools, such as acid fast bacilli test, cytological examination of fluid for inflammatory cells, microbiological examination of pleural tissue and molecular tests like the Xpert MTB/RIF assay show poor sensitivities [4,5]. Lactate dehydrogenase (LDH) is an enzyme found in almost all body tissues. It plays an important role in cellular respiration, the process by which glucose is converted into usable energy for the cells. Normally, the blood levels of LDH are low but when tissues are damaged by injury or disease, they release more LDH into the blood. The pleural fluid LDH level is elevated in approximately 75% of cases of pleural effusion, with levels commonly exceeding 500 IU/L [6]. Adenosine deaminase (ADA) is an enzyme produced by cells throughout the body and is associated with the activation of lymphocytes. Conditions such as TB may cause increased amounts of ADA to be produced in the areas where the bacteria are present and thus the amount of ADA present in pleural fluid is useful to diagnose a TB pleural effusion. However, pleural fluid LDH and ADA levels are commonly used to distinguish between TPE and non TPE, this can be challenging as the LDH level may vary from normal to severely increased in parapneumonic pleural effusion and a significantly elevated ADA is frequently measured in both conditions [7]. The D-dimer is a product of fibrin degradation that is formed by the sequential action of enzymes of coagulation cascade [8]. Coagulation cascade plays an important role in pleural diseases and several studies have reported that TPE is associated with enhanced local fibrinolytic activity [9,10]. However, in India there is no data available suggesting the precise role of D-dimer in TPE. The aim of the present study is to investigate the role of D-dimer in the diagnosis of TPE.

MATERIALS AND METHODS

A cross-sectional study was conducted over a period of one year during 2017-18 in Dhiraj hospital, a tertiary care hospital situated in Vadodara district of Guajrat. The study consisted of 101 patients in the age groups 30-80 years, divided into case group (41 patients with TPE) and control group (60 patients with non TPE) admitted in Emergency Department and in Intensive Care Unit. In the case group, 41 individuals diagnosed with TPE were enrolled after getting approval from Institutional Ethical Committee (Approval no. SVIEC/ON/Medi/RP/17019). Selection was made based on positive results of cytological examination, clinical features of TB infection associated with a sustained positive response to anti TB therapy. In the control group, 60 patients of Non TPE such as patients with Malignant Pleural Effusion (MPE) (n=32), Parapneumonic pleural effusion (n=15), pleural effusion caused by heart failure (n=7) or liver cirrhosis (n=6) were enrolled. The diagnosis of MPE was made when malignant cells were found on cytological examination and or on closed pleural biopsy, or on lung tissue biopsy. While effusion caused by heart failure or liver cirrhosis were diagnosed by clinical findings or abnormal cardiac markers or liver function tests. However, due to the higher D-dimer level in certain cases, such as patients who had an operation, pulmonary embolism, experienced trauma, kidney diseases, Disseminated Intravascular Coagulation (DIC), and females who were pregnant, were excluded from the study [11].

After taking informed consent from the patients, the pleural fluid samples were collected in Ethylene Diamine Tetra Acetic acid (EDTA) and plain vacutainer by using aseptic precautions and centrifuged for 10 minutes. D-dimer testing was done by latex agglutination kit for the detection of circulating derivatives of cross-linked fibrin degradation products in human plasma on fully automated analyser ERBA EM-200®. Proteins were estimated by colorimetric endpoint Biuret method whereas LDH levels were estimated by using DGKC method which is based on the use of pyruvate substrate as per

Henry RJ et al., [12]. ADA levels were estimated by colorimetric Non Giusti and Galanti Method [13]. Proteins, LDH and ADA were also analysed from pleural fluid samples on ERBA EM-200® by using ERBA reagent system packs of those parameters. Normal range of proteins was 0-3.0 g/dL, LDH 5-275 IU/L, ADA was 3-30 IU/L and D-dimer was 100-500 mg/L FEU as given in the kit literature. Fluid samples were diluted 100 times before estimation as fluid D-dimer level is higher than in blood.

STATISTICAL ANALYSIS

Statistical analysis was performed using the commercially available statistical software SPSS 14.0 version, MedCalc version 12.5 and Microsoft excel. The p-value of less than 0.05 was considered statistically significant. ROC curve analysis and calculation of the Area Under the Curve (AUC) was done for all parameters included in the study population. The optimum cut-off was used to dichotomously classify the positive or negative D-dimer levels, and it was also used for calculating the diagnostic sensitivity and specificity.

RESULTS

The present study comprised of 101 individuals between the age of 30-80 years (41 cases and 60 controls) with a mean age of 56.69±13.46. Among these 101 individuals, 51 were males and 50 were females [Table/Fig-1]. Among 41 patients from case group mean age was 56.59±13.32 years and among them 20 individuals were males. The 60 patients from control group had a mean age of 55.75±13.35 years and among them 30 were males. The unpaired t-test showed t=0.455, degree of freedom=99 and p-0.650. This showed that there was no significant difference between these age groups.

Age in years	Gender		Total (%)
	Female (%)	Male (%)	
31-40	13 (12.9)	12 (11.9)	25 (24.8)
41-50	20 (19.8)	23 (22.8)	43 (42.6)
51-60	11 (10.9)	12 (11.9)	23 (22.8)
61-70	4 (4.0)	3 (3.0)	7 (6.9)
71-80	2 (2.0)	1 (1.0)	3 (3.0)
TOTAL (%)	50 (49.5)	51 (50.5)	101 (100)

[Table/Fig-1]: Demographic data of all individuals included in study groups (n=101). TPE: Tuberculous pleural effusion; PPE: Parapneumonic pleural effusion; MPE: Malignant pleural effusion; HFPE: Pleural effusion due to heart failure; HPE: Hepatogenous pleural effusion

Biochemical analysis of pleural fluid showed increase in proteins, LDH, ADA and D-dimer levels in TPE group. To find out whether there was any correlation of fluid D-dimer levels in between case and control group, unpaired t-test was performed and p-value was derived. The value p<0.001 suggested significant difference in values of fluid D-dimer between these two group [Table/Fig-2].

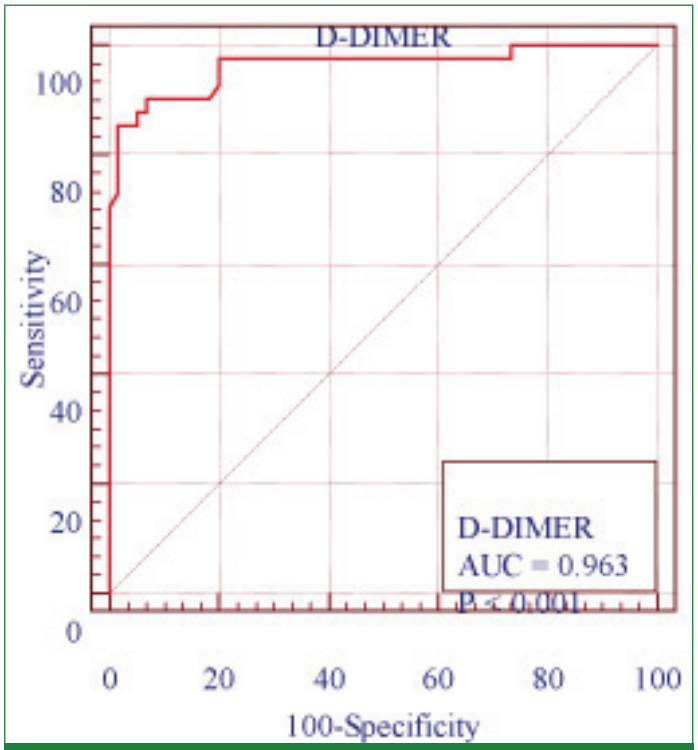
Pleural fluid	Case mean (95% CI)	Control mean (95% CI)	p-value
Proteins (G/Dl)	6.50 (6.19-6.82)	3.66 (3.37-3.97)	<0.0001
LDH (IU/L)	592.03 (540.2-648.83)	450.14 (405.05-500.25)	0.0004
ADA (IU/L)	43.56 (40.84-46.46)	18.60 (17.52-19.76)	<0.0001
D-DIMER (mg/L FEU)	1690.5 (1314-2175)	305 (267-348)	<0.0001

[Table/Fig-2]: Comparison of biochemical parameters in case and control groups. *95% CI- 95% Confidence interval

The ROC curve was constructed to test the performance of fluid D-dimer to differentiate TPE from non TPE. Sensitivity, specificity, Negative Predictive Values (NPV) and Positive Predictive Values (PPV) were calculated from ROC curves. The area under the ROC curve (AUC) for fluid D-dimer at the optimum cut-off value >501 mg/L FEU was 0.963 (0.92–0.99; p<0.001). On considering the cut-off point as >501 mg/L FEU for fluid D-dimer, the sensitivity was 90.24%, specificity was 86.67%, PPV was 81.86%, NPV was 93.0%, Positive Likelihood Ratio (PLR) was 6.77 and Negative Likelihood Ratio (NLR) was 0.11 [Table/Fig-3,4].

Fluid D-dimer	Cut-off value >501 mg/L FEU in ROC (95% CI)
Sensitivity	90.24% (76.9-97.3)
Specificity	86.67% (75.4-94.1)
PPV	81.86% (70.1-89.7)
NPV	93.0% (83.9-97.1)
PLR	6.77
NLR	0.11
AUC	0.963 (0.92-0.99)

[Table/Fig-3]: Sensitivity, specificity, Positive Predictive Valve (PPV), Negative Predictive Value (NPV), Positive Likelihood Ratio (PLR), Negative Likelihood Ratio (NLR) and Area Under the Curve (AUC) of fluid D-dimer.



[Table/Fig-4]: ROC curve of fluid D-dimer in the diagnosis of TPE.

DISCUSSION

Currently most of the laboratories use LDH and ADA for the diagnosis and differentiation of TPE and non TPE in biochemical examination. This can be challenging as LDH can increase in TPE, PPE, and MPE, and the level is likely to range greatly from normal to very high, due to its low sensitivity which limits the use of LDH for identifying PPE in a patient [14,15], In many cases, an ADA level ≥40 U/L in an exudate has been the most widely accepted indicator for the diagnosis of TPE but ADA levels are also higher in case of PPE and so it is very difficult to differentiate TPE from PPE by using ADA levels [7]. Although conventional methods like histological examination and mycobacterial culture of closed pleural biopsied tissue can be considered gold standard due to high sensitivity, they may not be widely used in hospitals as they are not well tolerated and can increase morbidity as well [16]. In such scenario, there is need of a novel biomarker which can be used in diagnosis and differentiation of TPE and non TPE along with the other diagnostic modalities so that the diagnosis can be done early. Characteristically, an exudative effusion increases the cellularity, higher protein levels and various inflammatory biomarkers. The coagulation system is fundamental for the maintenance of homeostasis and should be considered due to its close relationship to the inflammatory process. When blood enters the pleural space, the coagulation system comes into action when there is severe inflammatory response of pleura as the presence of TPE may injure pleura and induce coagulation activation; which leads to enhanced fibrinolytic activity due to the large amounts of plasminogen and plasminogen activators present in the pleural space and results in a

high pleural D-dimer level [17]. The plasminogen activators convert the plasminogen into active plasmin, which, in turn, enzymatically breaks down fibrin. The D-dimer is the primary degradation product of cross-linked fibrin which serves as a marker of coagulation with fibrinolysis [17,18].

In the present study, when the fluid D-dimer levels were compared between case and control groups it was found that mean in case group was 1690.5 mg/L FEU with 95% Confidence interval (95% CI) between 1314 mg/L FEU and 2175mg/L FEU and that in control group was 305 mg/L FEU with 95% CI between 267 mg/L FEU and 348 mg/L FEU. The levels of fluid D-dimer were significantly higher in TPE group as compared to non TPE group ($p<0.0001$).

Shen Y et al., conducted a similar study in which 87 patients with pleural effusion were included (32 TPE cases and 55 non TPE controls) [19]. Pleural D-dimer level was markedly increased in TPE patients than those with other aetiologies (1082.66±453.83 vs. 319.98±266.78 mg/L FEU, $p<0.05$). The AUC was 0.928 with a cut-off value of 622.5 mg/L FEU (95% CI: 0.878-0.979) and the sensitivity and specificity were 84.38% and 85.45%, respectively. Whereas in the present study, the area under the ROC curve (AUC) for fluid D-dimer at the optimum cut-off value >501 mg/L FEU was 0.963 (95% CI 0.92-0.99; $p<0.001$) and the sensitivity and specificity were 90.24 and 86.67% respectively which were higher than their study. Lu YD et al., conducted a study of D-dimer in pleural fluid of different effusion in 45 patients [20]. The levels of D-dimer in both tuberculous and empyema pleural effusion were significantly higher than in MPE ($p<0.01$; $p<0.05$). D -dimer was positively correlated with LDH in pleural fluid ($r=4168$, $p<0.01$). No similar study has been done in India showing the role of fluid D-dimer in the diagnosis and differentiation of TPE and non TPE.

Emami Ardestani M et al., in their study reported the comparison of D-dimer levels in each group between MPE vs. non MPE (NMPE) [21]. The mean pleural and serum D-dimer levels were 3472±1312 ng/dL and 3259±1220 ng/dl in patients with MPE, and 3425±32.5 ng/dL and 2425±1311 ng/dL in patients with NMPE, respectively. The serum D-dimer levels were not statistically different between two groups; while the pleural D-dimer levels were higher in MPE group in comparison with NMPE patients ($p<0.05$). However, in the present study, the levels of fluid D-dimer were significantly higher in TPE group as compared to non TPE group ($p<0.0001$).

Limitation(s)

This study has been conducted with a small sample size. It is still advisable to conduct similar studies with more participants, there by confirming the role of D-dimer as a novel marker in the diagnosis and differentiation of TPE and non TPE in a large scale population.

CONCLUSION(S)

From this study it can be concluded that the levels of fluid D-dimer are higher in TPE as compared to non TPE and hence it can be used as a novel marker in the diagnosis and differentiation of TPE and non TPE.

REFERENCES

[1] World Health Organization. Global tuberculosis report 2017. WHO (World Health Organization) 2017.
[2] Porcel JM. Tuberculous pleural effusion. Lung 2009;187:263-70.
[3] Zhai K, Lu Y, Shi HZ. Tuberculous pleural effusion. J Thorac Dis. 2016;8:E486 -94.
[4] Light RW. Update on tuberculous pleural effusion. Respirology. 2010;15: 451-58.
[5] Porcel JM, Palma R, Valdés L, Bielsa S, San-José E, Esquerda A. Xpert® MTB/RIF in pleural fluid for the diagnosis of tuberculosis. Int J Tuberc Lung Dis. 2013;17(9):1217-19.
[6] Vorster MJ, Allwood BW, Diacon AH, Koegelenberg CF. Tuberculous pleural effusion: advances and controversies. J Thorac Dis. 2015;7(6):981-91. doi:10.3978/j.issn.2072-1439.2015.02.18.
[7] Wang J, Liu J, Xie X, Shen P, He J, Zeng Y. The pleural fluid lactate dehydrogenase/adenosine deaminase ratio differentiates between tuberculous and parapneumonic pleural effusion. BMC Pulm Med. 2017;17(1):168.
[8] Schouten HJ, Geersing GJ, Koek HL, Zuithoff NP, Janssen KJ, Douma RA, et al. Diagnostic accuracy of conventional or age adjusted D-dimer cut-off values in older patients with suspected venous thromboembolism: systematic review and meta-analysis. BMJ. 2013;346:f2492.
[9] Vaz MA, Vargas FS, Marinho FC, D'amico EA, Rocha TR, Teixeira LR. Does the evaluation of coagulation factors contribute to etiological diagnosis of pleural effusion? Clinics (Sao Paulo). 2009;64:891-95.
[10] Lu XG, Mao JS, Tong JF, Zhu L, Liu J, Gong XB, et al. Fibrinolytic characteristics and their significance in malignant, tuberculous and cirrhotic pleural and ascitic fluids. Int J Lab Hematol. 2007;29:132-38.
[11] Kelly J, Hunt BJ. A clinical probability assessment and D-dimer measurement should be the initial step in the investigation of suspected venous thromboembolism. Chest. 2003;124:1116- 19.
[12] Henry RJ, Chiamori N, Golub OJ, Berkman S. Revised spectrophotometric methods for the determination of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, and lactic acid dehydrogenase. Am J Clin Pathol. 1960;34:381-98.
[13] Methods of Enzymatic Analysis; Giusti, G.; Galanti, B.; Pp. 1092-1099.
[14] Lee J, Yoo SS, Lee SY, Cha SI, Park JY, Kim CH. Pleural fluid adenosine deaminase/serum C-reactive protein ratio for the differentiation of tuberculous and parapneumonic effusion with neutrophilic predominance and high adenosine deaminase levels. Infection. 2017;45(1):59-65.
[15] Davies CW, Gleeson FV, Davies RJ. Pleural Diseases Group, Standards of Care Committee, British Thoracic Society. BTS guidelines for the management of pleural infection. Thorax. 2003;58 Suppl 2:ii18-28.
[16] Mcgrath EE, Anderson PB. Diagnostic tests for tuberculous pleural effusion. Eur J Clin Microbiol Infect Dis. 2010; 29:1187-93.
[17] Ribelles JMQ, Tenias JM, Grau E, Borras JMQ, Climent JR, Gomez E, et al. Plasma D-dimer levels correlate with outcomes in patients with community acquired pneumonia. Chest. 2004;124:1087-92.
[18] Dikensoy O, Stathopoulos GT, Zhu Z, Light RW. D-dimer levels in pleural effusion. Respir Med. 2006;100(8):1337-41.
[19] Shen Y, Yang T, Jia L, Wang T, Chen L, Wan C, et al. A potential role for D-dimer in the diagnosis of tuberculous pleural effusion. Eur Rev Med Pharmacol Sci. 2013;17: 201-05.
[20] Lu YD, Chen P, Xu M. Detection of D-dimer in pleural effusion. Hunan Yi Ke Da Xue Xue Bao. 2000;28:71-72.
[21] Emami Ardestani M, Modaemzadeh M, Mohammadi AR. Comparative analysis of mean pleural fluid D-dimer level in malignant and non-malignant pleural effusion patients. Tanaffos. 2019;18(1):47-51.

PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Biochemistry, Parul Institute of Medical Sciences and Research, Vadodara, Gujarat, India.
2. Associate Professor, Department of Biochemistry, Parul Institute of Medical Sciences and Research, Vadodara, Gujarat, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Simbita Marwah,
Department of Biochemistry, Parul Institute of Medical Science & Research,
Limda, Vadodara, Vadodara, Gujarat, India
E-mail: mail2sims@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: May 07, 2020
- Manual Googling: Jun 20, 2020
- iThenticate Software: Sep 26, 2020 (07%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **May 06, 2020**
Date of Peer Review: **Jun 02, 2020**
Date of Acceptance: **Jun 21, 2020**
Date of Publishing: **Oct 01, 2020**