A study on adenosine deaminase activity in pleural effusion of tuberculous and non tuberculous origin

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Abstract

Introduction: Accumulation of fluid in the pleural space that exceeds the physiological amount is defined as pleural effusion. Pleural effusion can be either transudative or exudative. The exudative pleural effusion is predominantly observed in tuberculosis. Adenosine deaminase (ADA) level remains high in disorders where cellular immunity is stimulated. Tuberculosis is one such disease.

Materials and Methods: The cases were classified as two groups. 30 patients of tubercular pleural effusion were enrolled in Group I and 30 patients of non-tubercular pleural effusion were Group II. In non-tubercular pleural effusion, 6 cases were malignant effusions, 12 cases were parapneumonic effusions and 12 cases were transudative effusions, which include 4 cases of congestive cardiac failure, 4 cases of nephrotic syndrome and 4cases of hepatic cirrhosis. The following biochemical parameters in pleural fluid were estimated. They are Glucose, Protein and Adenosine deaminase.

Results: In this study mean pleural fluid glucose level was significantly higher in transudative when compared to exudative pleural effusions. In exudative pleural effusions, mean pleural fluid protein level was increased significantly as compared to transudative pleural effusions. The activity of ADA in tubercular effusion was significantly higher when compared to non tubercular pleural effusions of different etiology such as malignant parapneumonic and transudative.

Conclusion: The assessment of ADA act as better marker for identifying tuberculosis than the traditional markers.

Keywords: Pleural effusion, Exudative, Transudative, Tuberculosis, Adenosine deaminase.

Introduction

Accumulation of fluid in the pleural space that exceeds the physiological amount is defined as pleural effusion. Effusion develops when there is an alteration in hydrostatic and osmotic forces that affects the formation of pleural fluid, when lymphatic drainage is impaired, or when mesothelial or capillary endothelial permeability is increased.[1]

Pleural effusion can be either transudative or exudative.[2] Transudative pleural effusions are usually bilateral due to systemic conditions results in elevated capillary hydrostatic pressure or declined pleural oncotic pressure. The exudates are more often unilateral, associated with localized disorders that increase vascular permeability or interfere with lymphatic resorption.[3,4] The transudative pleural effusions are due to congestive cardiac failure, nephrotic syndrome and left ventricular failure. The transudative pleural effusion occurs because the increased amount of fluid in the lung interstitial space exists in part across the visceral pleura. This overwhelms the capacity of the lymphatics in the parietal pleura to remove the fluid.[5] The exudative pleural effusions are due to tuberculosis, malignancy and pneumonia. Parapneumonic effusions are associated with bacterial pneumonia, lung abscess or bronchiectasis.[6]

Tuberculosis is the prime cause of exudative pleural effusion in our country and this is due totuberculosis protein hypersensitivity in the pleural space. The exudate pleural fluid predominantly contains small lymphocytes.[7] Adenosine deaminase catalyzes the conversion of adenosine to inosine.[8] Its main biological activity is detected in T-lymphocytes and its level remains high in disorders where cellular immunity is stimulated. Tuberculosis is one such disease.[9,10]

Materials and Methods

The study was undertaken at Maharajah's Institute Medical Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, during the period December 2016 to March 2018. Patients attended at MIMS OPD of Chest and TB department and admitted in the Medical / Surgical wards, during the study period were chosen for the study. The study subject comprised of 60 cases of pleural effusions of different etiology in the age group between 25 to 65 years of both the sexes. The diagnosis is based on detailed history, clinical examination and chest X-ray. The Institutional Ethical Committee (Ref. No. MIMS/IEC/Lr. No. 027/2016) approval was also taken.

Inclusion Criteria: Patients with tuberculous pleural effusion, malignant effusion, parapneumonic effusion, congestive cardiac failure, nephrotic syndrome and hepatic cirrhosis were included.

Exclusion Criteria: Patients with HIV, Viral hepatitis and age greater than 65 years were excluded

The cases were classified as two groups.

30 patients of tubercular pleural effusion and 30 patients of non-tubercular pleural effusion patients were considered as Group I and Group II. The study and objectives were explained to them and their consent in written form was taken.

Sample Collection: On confirmation of pleural effusion by X-ray and clinical examination, pleural fluid was aspirated from the posterior axillary line. 5ml of 2% lignocaine was infiltrated and Pleural fluid was aspirated from the site.

The following biochemical parameters in pleural fluid were estimated by different methods. They are Glucose by Glucose oxidase Peroxidase method,[11] Protein by Biuret method[12] and Adenosine deaminase by Giusti and Galanti method of enzymatic analysis.[13]

Statistical Analysis

Data was expressed as Mean and Standard deviation (mean ±SD). Statistical significance between control and patient groups, the Z test was performed using SPSS software 16.0.The statistical significance was determined at 5% ($p < 0.05$) level.

Results and Discussion

Depending on the patient history, clinical examination, Chest X-ray and other investigations, 60 cases of pleural effusions were diagnosed and categorized into two groups. Group I (n= 30) tuberculous pleural effusion and Group II (n= 30) non-tubercular pleural effusion patients, which included 6 cases of malignant effusions, 12 cases of parapneumonic effusions and 12 cases of transudative effusions. The transudative effusions included 4 cases of congestive cardiac failure, 4 cases of nephrotic syndrome and 4cases of hepatic cirrhosis. (Table 1).

Glucose levels in Pleural Fluid: In comparison to pleural glucose values in tubercular with non tubercular of different etiology, it was observed that pleural fluid glucose in transudative effusions of congestive cardiac failure, nephrotic syndrome and hepatic cirrhosis (100.58 mg/dl ±6.24) had statistically significant higher values ($p<0.001$) than the exudative effusions of tuberculous, malignant and parapneumonic origin (65.12 mg/dl ±11.04). (Table 2 and Table 4). Parapneumonic effusions and tubercular effusions had low pleural fluid glucose because of utilization of glucose by the bacteria for the growth. Whereas in malignant pleural effusions, the growing malignant mass consumes ever increasing amounts of glucose at the expense of the host's energy reserve. Most cancer cells have enzyme systems for both oxidative metabolism and anerobic glycogenic pathway utilizing majority of glucose produced by gluconeogenesis liberating lactic acid that often must be converted in to glucose before being utilized. In cancer there is an increase in energy expenditure and an inability to adjust the metabolic rate consequent to malnutrition.[14] Growth continues throughout the entire day in malignant cells rather than follows the diurnal pattern of the metabolic activity of normal tissues. All these causes are responsible for low glucose level in malignant pleural effusions when compared to transudates.[15]

Protein Levels in Pleural Fluid: In comparing with the protein levels in tubercular and non tubercular pleural effusions, the pleural fluid protein in transudative effusions (2.05 g/dl ±0.36) showed statistically significant lower values ($p<0.001$) than the exudative effusion (tuberculous, malignant and parapneumonic) (5.27 g/dl ±0.61) and it is in agreement with the Light's criteria (Table 3 and Table 4). The pleural fluid protein level helps in classifying transudates and exudates but it does not provide diagnostic information regarding the etiology of pleural effusion. In our present study, the pleural fluid protein in transudative effusions had statistically significant lower values than the exudative (tuberculous, malignant and parapneumonic) effusion It is in agreement with Light's criteria to differentiate transudative and exudative effusions. The pleural fluid protein level helps in classifying transudates and exudates but it does not provide diagnostic information regarding the etiology of pleural effusion.[16]

ADA Activity in Tubercular and Non Tubercular Pleural Effusion: The ADA activity in tubercular and non-tubercular pleural effusion was compared and it was observed that pleural fluid ADA in tubercular effusions (85.76 U/L ±15.62) had statistically higher values ($p<0.001$) than the non-tubercular effusions of malignant, parapneumonic and transudative origin (23.3 U/L ±7.55). (Table 5 and Table 6).

Tuberculosis is one of the dreadful disease and the severity of the disease can be judged by the fact that it affects all ages, irrespective of the sex. No other disease has so much socio economic health significance as tuberculosis in India and other tropical countries. In tuberculosis, the presence of antigens of mycobacteria in the pleural space causes delayed hypersensitivity which results in pleural effusion.[17] In turn these antigens can enter the pleural space due to rupture of a sub pleural focus.[18] The delayed hypersensitivity will cause the differentiation of lymphocytes and releases the lymphokines that activate the macrophages to increase mycobactericidal effect.[19] The biochemical markers like adenosine deaminase, γ-interferon, and lysosome got utmost clinical importance in the early diagnosis of pleural tuberculosis.[20] ADA is found in majority of cells, however its role mostly concerned to the proliferation and differentiation of T-lymphocytes. The enzyme ADA predominantly a T lymphocyte enzyme and its plasma activity is high in disease where cellular immunity is stimulated such as tuberculosis. In tubercular pleural effusion, the isoform ADA_2 is predominant that accounts for 88% and ADA_1 accounts for 70% of total activity of ADA.[21] Monocytes or macrophages infected by mycobacterium are thought to be main source of ADA_2 in pleural fluid. ADA_2

produced by macrophages in response to pathogen invasion leads to activation of immune cells at the site of inflammation.[22] The non tuberculous lymphocytic pleural effusion includes malignant effusion, parapneumonic effusions, miscellaneous exudative effusions and transudative effusions. A high level of ADA is observed in parapneumonic effusions and emphysemas. The ADA level in non tuberculousis does not exceed the 40 IU/L, which is considered as a cut-off for tuberculous effusions.[23]

Table 1: Distribution of cases

Groups	Type of pleural effusion	Number of cases	Percentage
I	Tuberculous	30	50%
II	Non tuberculous	30	50%
Group II cases were subdivided into three subgroups.			
II-A	Malignant	06	10%
II-B	Parapneumonic	12	20%
II-C	Transudative	12	20%

The above table shows distribution of 12 transudative cases which include 4 cases of congestive cardiac failure, 4 cases of nephrotic syndrome and 4 cases of hepatic cirrhosis.

Table 2: Glucose levels in pleural fluid

Groups	Type of pleural effusion	Pleural fluid Glucose range (mg/dL)	Mean ± SD (mg/dL)
I	Tuberculous	50-90	66.9±10.58
Group II cases were Non tuberculous subdivided into three subgroups.			
II-A	Malignant	65-87	76.17±8.11
II-B	Parapneumonic	49-61	55.17±3.67
II-C	Transudative	91-110	100.58±6.24

The above table shows the glucose levels of pleural fluids in different groups of pleural effusion.

Table 3: Protein levels in pleural fluid

Groups	Type of pleural effusion	Pleural fluid Protein range (g/dL)	Mean ± SD (g/dL)
I	Tuberculous	4.1-6.6	5.36±0.73
Group II cases were Non tuberculous subdivided into three subgroups.			
II-A	Malignant	4.9-5.4	5.01±0.26
II-B	Parapneumonic	4.7-5.7	5.17±0.33
II-C	Transudative	1.5-2.6	2.05±0.36

The above table shows the protein levels of pleural fluids in different groups of pleural effusion.

Table 4: Pleural fluid glucose and protein levels in transudative and exudative pleural effusion

Biochemical Parameters	Transudates Mean ± SD	Exudates Mean ± SD	Z value	p value
Pleural fluid Glucose (mg/dl)	100.58±6.24	65.12±11.04	14.73	<0.001
Pleural fluid Protein (g/dl)	2.05±0.36	5.27±0.61	23.58	<0.001

The above table shows the glucose and protein levels of pleural fluids in different groups of pleural effusion.

Table 5: Adenosine deaminase (ADA) in pleural fluid

Groups	Type of pleural effusion	Pleural fluid ADA range (U/L)	Mean ± SD (U/L)
I	Tuberculous	54-117	85.77±15.6
Group II cases were Non tuberculous subdivided into three subgroups.			
II-A	Malignant	24-36	31.83±5.11
II-B	Parapneumonic	12-33	25.08±5.92
II-C	Transudative	11-25	17.25±4.13

The above table shows the adenosine deaminase levels of different pleural effusion groups.

Table 6: Adenosine deaminase activity in tubercular and non tubercular pleural effusion

Type of pleural effusion	ADA range (U/L)	Mean ± SD (U/L)
Tubercular	54-117	85.77±15.6
Non tubercular	11-37	23.3±7.55

Z value: 19.7 p value<0.001

The above table shows the adenosine deaminase levels in the pleural effusion of tubercular and non tubercular patients.

Conclusion

In comparison to non tubercular pleural effusion, the tubercular pleural effusion showed a marked and significant elevation of pleural fluid ADA. The association of pleural effusion tuberculosis and the rise of ADA can be considered as early and sensitive marker for the early diagnosis and to prevent further disease severity.

References

1. Zocchi L. Physiology and pathophysiology of pleural fluid turnover. *European Respiratory Journal.* 2002;20(6):1545-58.
2. Padilla NI. Pleural effusion: criteria for distinguishing between transudates and exudates. *An Internal Med.* 1996;13(9):460-8
3. Pappenheimer JR, Renkin EM, Borrero LM. Filtration, diffusion and molecular sieving through peripheral capillary membranes: a contribution to the pore theory of capillary permeability. *American Journal of Physiology-Legacy Content.* 1951;167(1):13-46.
4. Bodega F, Zocchi L, Agostoni E. Macromolecule transfer through mesothelium and connective tissue. *Journal of Applied Physiology*. 2000;89(6):2165-73.
5. Gazquez I, Porcel JM, Vives M, De Vera MV, Rubio M, Rivas MC. Comparative analysis of Light's criteria and other biochemical parameters for distinguishing transudates from exudates. *Respiratory Medicine.* 1998;92(5):762-5.
6. Light RW, Macgregor MI, Luchsinger PC, Ball WC. Pleural effusions: the diagnostic separation of transudates and exudates. *Annals of Internal Medicine.* 1972;77(4):507-13.
7. Morisson P, Neves DD. Evaluation of adenosine deaminase in the diagnosis of pleural tuberculosis: a Brazilian meta-analysis. *Journal Brasileiro de Pneumologia.* 2008;34(4):217-24.
8. Weihofen WA, Liu J, Reutter W, Saenger W, Fan H. Crystal structure of CD26/DPPIV in complex with adenosine deaminase reveals a highly amphiphilic interface. *Journal of Biological Chemistry*. 2004;279(41):43330-43335.
9. Gakis C. Adenosine deaminase (ADA) isoenzymes ADA1 and ADA2: diagnostic and biological role. *European Respiratory Journal.* 1996;9:632-633.
10. Ungerer JP, Oosthuizen HM, Bissbort SH, Vermaak WJ. Serum adenosine deaminase: isoenzymes and diagnostic application. *Clinical Chemistry.* 1992;38(7):1322-6.
11. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. *Annals of Internal Medicine.* 1969;6:24–27.
12. Friedman RB. Anderson RE and Hirshberg SB: Effects of diseases in clinical laboratory tests. Clincal. *Chemistry.* 1980;26:209-11.
13. Giusti G, Galanti B. Adenosine deaminase. In: Bergmayer HU. Methods of Enzymatic analysis. 2nd edition. New York: 1092-1099.
14. Fadaka A, Ajiboye B, Ojo O, Adewale O, Olayide I, Emuowhochere R. Biology of glucose metabolization in cancer cells. *Journal of Oncological Sciences.* 2017;3(2):45-51.
15. Sahn SA, Heffner JE. Pleural fluid analysis. Textbook of pleural diseases. 2008;2:209-26.
16. Light RW. Pleural effusion. *New England Journal of Medicine.* 2002;346(25):1971-7.
17. Leibowitz S, Kennedy L, Lessof MH. The tuberculin reaction in the pleural cavity and its suppression by antilymphocyte serum. *British journal of experimental pathology.* 1973;54(2):152.
18. Stead WW. Operative and pathologic findings in twenty-four patients with syndrome of idiopathic pleurisy with effusion, presumably tuberculosis. *Am Rev Tuberc*. 1955;71:473-502.
19. Yamamoto S, Dunn CJ, Willoughby DA. Studies on delayed hypersensitivity pleural exudates in guinea-pigs. II. The interrelationship of monocytic and lymphocytic cells with respect to migration activity. *Immunology.* 1976;30(4):513-19.
20. Gilhotra R, Sehgal S, Jindal SK. Pleural biopsy and adenosine deaminase enzyme activity in effusions of different aetiologies. *Lung India.* 1989;7(3):122-24.
21. Ungerer JP, Oosthuizen HM, Retief JH, Bissbort SH. Significance of adenosine deaminase activity and its isoenzymes in tuberculous effusions. *Chest.* 1994;106(1):33-7.
22. Chen ML, Yu WC, Lam CW, Au KM, Kong FY, Chan AY. Diagnostic value of pleural fluid adenosine deaminase activity in tuberculous pleurisy. *Clinica Chimica Acta.* 2004;341(1-2):101-7.
23. Lee YG, Rogers JT, Rodriguez RM, Miller KD, Light RW. Adenosine deaminase levels in nontuberculous lymphocytic pleural effusions. *Chest.* 2001;120(2):356-61.

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