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PLEURAL FLUID MDA AND SERUM-EFFUSION ALBUMIN GRADIENT IN PLEURAL EFFUSION

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ABSTRACT

Pleural fluid malondialdehyde (PMDA) and serum effusion albumin gradient(SEAG) were estimated in 60 patients of pleural effusion of diverse etiologies. The results were compared with Light's criteria to distinguish between transudates and exudates. The mean PMDA level was 0.68±0.24nmol/ml and 1.17±0.25nmol/ml in transudates and exudates respectively showing a statistically significant ($p<0.05$) rise in exudates in comparison to transudates. SEAG registered a significant fall in exudates ($P<0.001$) when compared with transudates. PMDA revealed a positive correlation with pleural protein($r=+0.30$) and a significant negative association with SEAG ($r= -0.33$).Sensitivity and specificity of PMDA were better than the parameters of Light's criteria.Whereas SEAG documented approximately equal sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) with Light's criteria. Therefore PMDA and SEAG can be taken together in addition to Light's criteria to strengthen the discrimination between transudates and exudates in borderline cases of pleural effusion.

KEY WORDS

Pleural Fluid , Exudates , Transudates

INTRODUCTION

Pleural effusion is a common clinical disorder and is either a manifestation or a complication of one or other respiratory or non-respiratory diseases (1). It heralds a serious prognosis if not diagnosed or treated properly. Approximately one million patients develop pleural effusion each year. Frequently the cause of pleural effusion is obvious. But sometimes the cause is elusive and presents a difficult diagnostic challenge.(2)

The use of Light's criteria to separate transudates from exudates has been generally admitted as the first step in the study of a pleural effusion of unknown cause. However, concerns about the usefulness of this approach are growing. Biochemical parameters like pleural fluid cholesterol level (3,4), pleural fluid bilirubin level (5), serum-effusion albumin gradient (7), serum effusion protein gradient (8), have been analyzed.

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None has been found to be 100% sensitive and specific. The recent doubts are about their actual utility and better importance than the initial diagnosis based on clinical judgement. The most widely accepted is Light's criteria i.e. (a) Pleural protein to serum protein ratio greater than 0.5 (b) Pleural fluid to serum LDH ratio greater than 0.6 and(c) Pleural fluid LDH >200, denoting an exudate (9).This has become a standard method for the separation because of their high sensitivity in identifying exudates. The main disadvantage appears to be the misclassification of transudates as exudates.

Later, for evaluation of pleural effusion Light recommended that a decision be made on the serum effusion albumin gradient (SEAG), when the patient's pleural fluid meets the exudative criteria, but the patient appears to have a transudative effusion. SEAG is thought to directly reflect the colloid osmotic pressure.The major advantage of SEAG was the reduction in the number of patients with transudates receiving concurrent diuretic therapy being misclassified as exudates(10).

Recently oxidative status of pleural fluid has been analyzed by taking pleural fluid malondialdehyde level (PMDA), as well as different antioxidant enzymes to differentiate transudates from exudates (11) as the lung represents unique tissue in

both its exposure to higher oxygen tension and high concentration of antioxidants. The imbalance between oxidants and antioxidants, referred as oxidative stress has been associated with various respiratory disorders. Various markers of oxidative stress, including hydrogen peroxide and 8-isoprostane,have been reported to be increased in the lung, which have been determined in various biological samples, as in blood, bronchoalveolar lavage fluid and exhaled breath condensate. These samples express either local or systemic levels of oxidative stress.The pleural cavity is a closed space that is segregated from rest of the respiratory system,but interacts with lungs in different disease processes. However the local production of free radicals and the role of oxidative stress in the pathogenesis of pleural effusion have not been extensively studied. Some workers have observed raised PMDA concentration in exudative cases(12).

With the above facts the present study was designed to evaluate SEAG as well as PMDA level as parameters to differentiate transudates from exudates and their relative usefulness in pleural effusion cases as compared to criteria of Light et al(9).

MATERIALS AND METHODS

The present study was conducted in the Department of Biochemistry in collaboration with Department of Pulmonary Medicine of S.C.B. Medical College; Cuttack. Sixty patients of diverse etiology were taken into consideration. The effusion secondary to diseases that directly involve pleural surface were considered exudates and the rest were transudates. Out of these 60 cases of pleural effusion 42 were males and 18 were females with in age group of 31 to 50 years.

Group-1 (Transudates) : This group comprised of 20 patients of pleural effusion due to severe anaemia, congestive cardiac failure, Nephrotic Syndrome and hypoproteinemia.
Group-2 (Exudates) : This group comprised of 40 pleural effusion patients of tuberculosis, malignancy and pneumonia.

The cases in which either no cause was definitely diagnosed or more than one cause was present, as well as pseudochylous and chyliform effusions were excluded from the study. After a detailed history and thorough clinical examination routine laboratory investigations were done in each case. The clinical presumption of the nature of the effusion (transudate or exudates) was based on all available information obtained just before performing thoracocentesis. Investigations including thoracocentesis, pleural biopsy and pleural fluid analysis for total protein, LDH, albumin and MDA were also performed. Investigations of blood included estimation of serum total protein, serum albumin, serum LDH and serum MDA.The characterization of effusions as exudates was further validated using the criteria of Light et al. Other investigations like chest X-Ray, ultrasonography, were carried out as per requirement

Total proteins were estimated by Biuret method (13) and serum albumin was measured by BCG method (14). LDH was estimated by modified IFCC method (15) in which rate of oxidation of NADH to NAD was measured as a decrease in absorbance that was proportional to the LDH activity in the sample. All these above estimations were carried out using Technicon RA-1000 auto analyzer. MDA estimation was performed as thiobarbituric acid reactive substances at 532 nm (16). All these parameters were statistically analyzed using student's 't' test. Pearsons correlation co-efficient was used to establish the level of any correlation amongst them.

RESULTS

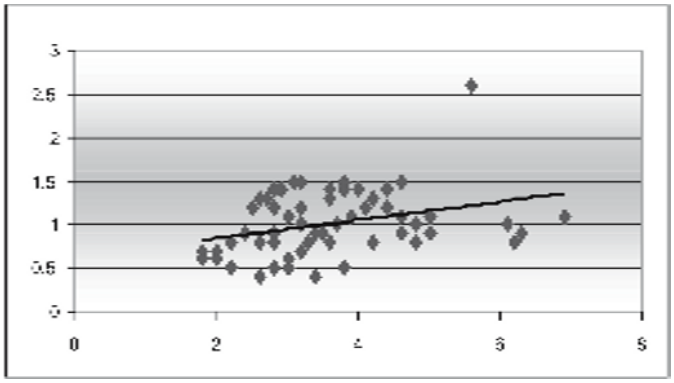
Radiological view of pleural effusion revealed the extent of involvement to be moderate in 73.33% of cases. 20 were of transudative pleural effusion and 40 had exudative effusion. The biochemical parameters (Table 1) by Light's criteria revealed a marked rise in pleural protein in exudates($p<0.001$) as compared with transudates. Pleural fluid to serum protein ratio was observed to be significantly($p<0.001$) increased in exudates in relation to transudates. Similarly pleural LDH documented a significant elevation in exudates($p<0.001$).The ratio of mean of pleural fluid LDH to serum LDH was observed to be >0.6 in exudates and <0.6 in transudates. SEAG registered a significant fall in exudates($p<0.001$)in relation to transudates. PMDA was also observed to be significantly increased in these exudate cases ($p<0.001$). Pearson correlation analysis in the whole study population revealed a positive correlation of PMDA with pleural protein with r-value of+0.30(Figure 1) whereas PMDA registered a significant

Table 1 : Biochemical Parameters in Pleural Effusion

Parameters	Transudate (n=20)	Exudate (n=40)
Pl. Protein(gm%)	2.65 ± 0.54	4.08 ± 1.05 *
Pl. LDH (U/L)	97.08 ± 30.01	1200.05 ± 347.13*
P/S. Protein	0.44 ± 0.19	0.86 ± 0.41**
P/S. LDH	0.53 ± 0.35	1.61 ± 0.68*
Pleural MDA (nmol/ml)	0.68 ± 0.24	1.17 ± 0.25*
SEAG (gm/dl)	1.82 ± 0.48	1.05 ± 0.33*

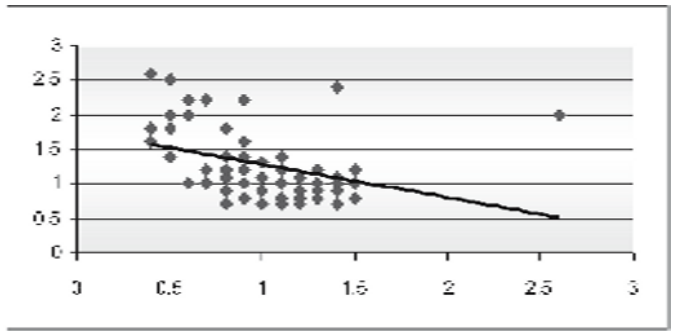
* $p < 0.001$, highly significant; ** $p < 0.01$, significant

Figure 1 : Correlation between PMDA & Pleural Protein



Pleural Protein (gm%)

Figure 2 : Correlation between PMDA & SEAG



SEAG (gm%)

negative association with SEAG having r- value of-0.33 (Figure 2).

Table 2 demonstrated the number and percentage of cases misclassified by each parameter studied. Taking the cut off value of pleural MDA as < 0.7nmol/ml, the number of cases misclassified was only one in case of transudates as well as exudates and the percentage was lower as compared to Light's criteria. Where as SEAG revealed more or less same number of misclassified cases as compared to Light's criteria. Sensitivity and specificity of PMDA was more in comparison to Light's criteria with more or less equal positive and negative predictive value(Table 3). SEAG documented approximately equal sensitivity, specificity, PPV and NPV with Light's criteria.

DISCUSSION

In abnormal states, pleural fluid can be accumulated for a number of reasons. Generally it is due to increased fluid formation or decreased fluid absorption. It is generally accepted that an effusion due to pleural disease more closely resembles plasma (exudates) while that occurring in the presence of a normal pleural membrane is due to hemodynamic aberrations or oncotic changes and is an ultrafiltrate of plasma (transudate). Transudate effusion resulting from different causes occurs in association with an intact microvasculature, thus maintaining the gradient between serum and pleural fluid protein(1).

Etiologies for the production of exudates involve some type of inflammation that results in a compromised pulmonary or pleural microvasculature which in turn leads to increased fluid leaking, a higher protein concentration and a decrease in albumin gradient(2). Both albumin and globulin fraction in pleural fluid are believed to originate from serum via diffusion. However some proteins like LDH come from within pleural space, i.e from pleural fluid leukocytes. Therefore SEAG should be taken as an effective means of discriminating exudates from transudates as this method only relies on measurement of effusion and serum albumin concentration and the result should be treated cautiously when the patient is suspected of having hypoalbuminemia(7). Taking a cut off value of 1.2gm/dl , one study revealed all the transudates and 95% of all exudates were classified correctly. However other authors were not fortunate enough to attain such good results(8,9).

Table 2 : Number & Percentage of Misclassification of Exudates and Transudates with each Parameter

Parameters	Transudate (n=20)	Percentage (%)	Exudate (n=40)	Percentage (%)
Pl. Protein(gm%)	3	15	5	12
P/S Protein	3	15	3	8
Pl. LDH (U/L)	3	15	0	0
P/S. LDH	5	25	1	2.5
Pl. MDA (nmol/ml)	1	5	1	2.5
SEAG (gm/dl)	3	15	3	8

Table 3 : Sensitivity, Specificity, Positive and Negative Predictive Values for Parametes Studied

Parameters	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Pl. Protein(gm%)	92	77	87	85
P/S Protein	92	85	92	85
Pl. LDH (U/L)	93	100	100	85
P/S. LDH	88	93	97	75
Pl. MDA (nmol/ml)	97	95	97	95
SEAG (gm/dl)	92	85	92	85

Recently usefulness of MDA has been studied to differentiate transudates from exudates (11). This lipid peroxidation product MDA accumulates in pleural fluid from two sources. The first possible source is plasma proteins, plasma MDA being bound to plasma proteins enter into pleural space due to increased leakage of plasma proteins to pleural space through inflammed pleura. Local production of MDA by inflammatory cells in pleural space in conditions like tuberculosis, malignancy, forms the second source of raised MDA in pleural fluid(12).

In the present study exudates with higher levels of PMDA than transudates, represented local oxidative burst in the former, the origin of which being related to the nature of each disease entity involving pleural cavity. On the contrary, transudative effusion is not related to local pleural pathology and results from an imbalance between hydrostatic and oncotic pressure. Therefore it does not lead to the formation of reactive oxygen species.

PMDA registered a positive correlation with pleural protein pointing towards increased leakage of plasma proteins responsible for raised pleural MDA value. The negative association between pleural MDA and SEAG strengthens this fact. SEAG revealed a sensitivity and specificity of 92% and 85% respectively with a PPV of 92% and NPV of 85% respectively, misclassifying cases of transudative and exudative effusions similar to Light's criteria. PMDA with a cut off value of 0.7nmol/ml documented a sensitivity of 97% and specificity of 95% with PPV and NPV of 97% and 95% respectively, misclassifying only one case in transudates as well as in exudates.

Thus, in the group of well characterized pleural effusion the measurement of oxidative stress (PMDA) proved to be a better marker for the differentiation of exudates and transudates, as this method provided a high sensitivity, specificity and accuracy for the characterization of effusion as an exudates when compared with Light's criteria. This again may explain the different pathophysiology behind the production of exudates and transudates. However, to overcome the limitation of misclassification by using criteria of Light et al, SEAG along with PMDA can be practised in addition to Light's criteria for better differentiation of exudates and transudates in clinical practice.

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