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Study on Oxidative Metabolic Changes to Differentiate Exudative from Transudative Pleural Effusions

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ABSTRACT

The purpose of this present study was to differentiate transudates and exudates in pleural effusions. Oxidative stress has been associated with various respiratory disorders. Ninety patients of pleural effusions of diverse etiologies were participated in this study. Subjects underwent diagnostic thoracentesis and standard biochemical parameters (total protein, lactate dehydrogenase, glucose, MDA levels) were measured in pleural fluid and serum. MDA, total protein, lactate dehydrogenase (LDH), glucose levels in plural fluid were higher in exudates compared to transudates ($p<0.001$). Total protein pleural fluid/ total protein serum ratio, LDH pleural fluid/LDH serum ratio and MDA pleural fluid/MDA serum ratio were raised in exudates compared to transudates ($p<0.001$). The present study showed that oxidative stress was more in exudates compared to transudates, probably due to the production of reactive oxygen species and it may serve as a marker for differentiation between transudates and exudates in clinical practice.

Key Words: Exudates, Melondialdehyde, Oxidative Stress, Pleural Effusion, Transudates

INTRODUCTION

The imbalance between oxidants and anti-oxidants is referred to as oxidative stress and has been associated with various respiratory disorders. Increased oxidative stress participates in the pathogenesis of both airways and parenchymal lung diseases. Asthma, COPD, and bronchiectasis have been associated with inflammation and increased levels of oxidative stress (Caramori and Papi, 2004; Horvath et al., 1998; Montuschi et al., 2000).The lung represents unique tissue in both its exposure to higher oxygen tensions and its high concentration of antioxidants (Kinnula and Crapo, 2003). Inflammatory cells generate free radicals in patients with interstitial lung diseases such as pulmonary fibrosis and sarcoidosis (Kuwano et al., 2003; Psathakis et al., 2004). Furthermore; free radicals are closely associated with diseases such as cystic fibrosis, primary pulmonary hypertension and bronchopulmonary dysplasia (Archer and Rich, 2000; Jobe and Bancalari, 2001; Paredi et al., 2000). The pleural cavity is a closed space that is segregated from the rest of the respiratory system but interacts with the lung in different disease processes. However, the local production of free radicals and the role of oxidative stress in the pathogenesis of pleural effusions have not been extensively studied. A primary diagnostic step is the Classification of pleural effusion as either an exudates or transudate.Transudative pleural effusions are of non-inflammatory and exudative effusions are of inflammatory in etiology. Pleural effusions are often a diagnostic dilemma for the physician as the differential diagnosis is wide. The first step in the evaluation of pleural effusions is the distinction between exudates and transudates. The criteria described by Light et al., 1972 have become a standard method for this separation because of their high sensitivity in identifying exudates. More recent studies have proposed other methods for the differentiation of exudates and transudates (Meise et al., 1990; Roth et al., 1990; Valdes et al., 1991). The main disadvantage appears to be the misclassification of transudates as exudates (Chakko, 1990). Mycobacteria can induce reactive oxygen species (ROS) production by activating phagocytes and although these are an important part of the host defence against mycobacteria, enhanced ROS generation may promote tissue injury and inflammation (Beers and Sizer, 1952). This may further contribute to immunosuppression (Grimble, 1994; Jack et al., 1994; Nathan et al., 1979), particularly in those with impaired antioxidant capacity, such as HIV infected patients (Aukrust and Muller, 1999; Favier et al., 1994; Muller et al., 2000). Moreover, the malnutrition which is commonly present in patients with tuberculosis can add to the impaired antioxidant capacity in these patients. Lipid peroxidation, a general mechanism of tissue damage

by free radicals is known to be responsible for cell damage and may induce many pathological events. During pulmonary inflammation, increased amounts of Reactive Oxygen Species (ROS) and Reactive Nitrogen Intermediates (RNI) are produced as a consequence of phagocytic respiratory burst (Kwiatkowska et al., 1999). The objective of the present study was to investigate the levels of lipid peroxidation products (Malondialdehyde, MDA), total protein, lactate dehydrogenase (LDH) and glucose levels in pleural fluid and serum samples of pulmonary tuberculosis patients.

EXPERIMENTAL

Subjects

This study was conducted on patients who were hospitalized in the Govt. Chest diseases and Tuberculosis Hospital, Warangal. During this study 90 consecutive patients who had undergone diagnostic thoracentesis for pleural effusions were studied. The study protocol was approved by the local ethics committee and all subjects gave their written informed consent. These patients were divided into two groups:

Group I (Transudates): This group comprised of 18 patients of pleural effusion due to Congestive heart failure (CHF), nephrotic syndrome and hypoproteinemia.

Group II (Exudates): This group comprised of 72 patients due to pleural effusion: tuberculosis (30 cases), malignancy (20 cases) and synpneumonia (22 cases).

Sample Collection and Analysis

Initial evaluation of a patient with effusion consists of history, physical examination, laboratory investigations and roentgen graphic studies. Prior to aspiration analgesic or sedative (I.V. midazolam) was administered if patient shows excessive anxiety. 1% xylocaine was infiltrated intradermally or subcutaneously and into muscles and parietal pleura and continued till the fluid is aspirated. A 20 or 22 gauge needle is passed along the line of anesthesia, while aspirating 30 ml of fluid. Pleural fluid and serum samples were collected from all patients on the day of their hospital admission. All samples were immediately analyzed for the following biochemical parameters: Malondialdehyde (MDA); glucose; total protein; lactate dehydrogenase (LDH) and cell count.

Methods

The amount of lipid peroxidation products (Oxidative Stress) present in the pleural fluid was estimated by the thiobarbituricacid reactive substances (TBARS) method (Carbonneau et al., 1991), which measures the malondialdehyde reactive products by using High Pressure Liquid Chromatography (HPLC, Shimadzu LC-8A Solvent delivery module, SPD-10AVP UV-Visible spectrophotometric detector, Class CR-10 Data Processor). Using Altec C18 column (25 cm length, 4.6 mm diameter, 5 µ m size) and developed with a mobile phase of methanol: water (70:100) containing 550 ml of H_3PO_4 with 80 nM of NaOH and 20 µl sample was injected.

Lactate dehydrogenase (LDH) is a Zinc containing intracellular enzyme concerned with reversible oxidation of pyruvate to lactate involved in glycolytic cycle. Estimation of LDH is based on the reaction velocity is determined by a decrease in absorbance at 340 nm resulting from oxidation of NADH (Varley et al., 1980).Total protein content was estimated by the method of Lowry et al., 1951, Proteins form chromophoric complex with phenol reagent, which was measured at 610 nm using UV–VIS spectrophotometer. Glucose was estimated by glucose – oxidase – peroxidase method (Trinder, 1969).

Statistical Analysis

Data are presented as the mean ± SD, unless otherwise mentioned. Comparisons of levels of MDA between two different groups were compared using unpaired *t* tests, as the data were normally distributed. Comparisons between more than two groups were performed with one-way analysis of variance. The influence of age, sex, smoking on MDA levels were checked with a Chi-Square test. Correlations between oxidative stress levels and other parameters were checked with the Pearson correlation coefficient. A p value of < 0.05 was considered to be statistically significant.

RESULTS

The demographic characteristics and the pleural fluid characteristics of the 90 patients who were studied are presented in Table-1. The mean oxidative stress expressed by MDA levels were higher in exudates compared to transudates (0.640 ± 0.09 vs 0.379 ± 0.08 mmol/L, respectively; $p < 0.001$). Similarly, LDH levels were higher in exudates compared to transudates (189.62 ± 24.7 vs 114.3 ± 16.65 IU/L, $p<0.001$). Serum glucose, total proteins and MDA levels were significantly raised in exudates compared to transudates (117.7 ± 44.59, 5.66 ± 1.06, 0.908 ± 0.12 vs 138 ± 14.14, 6.3 ± 1.21, 1.04 ± 0.06, respectively, $p < 0.001$). Total protein pleural fluid/protein serum ratio, LDH pleural fluid/LDH serum ratio and MDA pleural fluid/MDA serum ratio were raised in exudates compared to transudates (0.587 ± 0.09, 0.620 ± 0.061, 0.710 ± 0.08 vs 0.323 ± 0.09, 0.422 ± 0.122, 0.428 ± 0.07, respectively; $p < 0.001$). The optimum cutoff point for the differentiation between exudates and transudates ware evaluated by using chi-square test as the level of oxidative stress with the greatest sum of sensitivity and specificity. The cutoff point of pleural fluid MDA levels was 0.50 mmol/L. This cutoff point provides a sensitivity of 93.34%, a specificity of 86.67%, a positive predictive value of 97.26%, a negative predictive value of 82.33%, and an accuracy of 92.22% for the diagnosis of exudates. The cutoff point of pleural fluid and serum ratio of MDA levels was 0.56 mmol/L. This cutoff point provides a sensitivity of 98.72%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 92.85% and an accuracy of 98.82% for the diagnosis of exudates. The influence of age, sex and smoking on pleural fluid MDA, pleural fluid and serum MDA ratio were evaluated by using chi-square test. Sex was uninfluenced in both the cases but age was significantly influenced in these two cases, whereas smoking was influenced in the pleural fluid MDA but not in the ratio of PF/S MDA levels.

DISCUSSION

In this prospective study, we have shown that exudative pleural effusions present increased levels of oxidative stress (MDA levels) compared to transudative effusions. The increased levels of oxidative stress in exudates probably represent the increased local production of free radicals. The origin of this local oxidative burst is related to the nature of each disease entity. However, the local production of oxidants in the pleural cavity has not been extensively studied. There is *in vitro* evidence in animal models that reactive oxygen and nitrogen species may be implicated in the pathogenesis of asbestos-related pleural effusions (Choe et al., 1998).

In the diseases studied in our patients, there is little evidence in the literature regarding the local production of oxidative stress. Oxidants have been shown to play an important role in carcinogenesis; serving not only as tumor initiators but also as tumor promoters and regulators of gene expression (Upham and Wagner, 2001). TB has been associated with increased levels of several markers of oxidative stress and decreased antioxidant capacity (Madebo et al., 2003).Mesothelial cells are responsible for the release of oxidants in pleural space infections (Antony and Mohammed, 1999).

In blood samples, it has been shown that the method presents both an acceptable stability and an acceptable margin of error (Iamele et al., 2002). In pleural effusions, MDA levels proved to be detectable in all of the samples of our study and their concentration was significantly higher in exudative pleural effusions compared to transudative pleural effusions. The separation between exudative and transudative pleural effusions in our study was based on clinical presumption, after a thorough investigation of each patient, compared with the evaluation using the Light's criteria any patient with diagnostic uncertainty were excluded from further evaluation. In this group of well-characterized pleural effusions, the measurement of oxidative stress proved to be an excellent marker for the differentiation between exudates and transudates.

The method provided high sensitivity (93.34%), specificity (86.67%), and accuracy (92.22%) for the characterization of an effusion as an exudate, when a cutoff level of MDA of – 0.50mmol/L, which was provided by the chi-square analysis, was used. These results were superior to the results provided using the criteria of Light et al, 1972 and were comparable to those using other criteria from the literature (Light, 2002; Paramothayan and Barron, 2002).This, again, may be attributed to the different pathophysiology behind the production of exudates and transudates, which results in low levels of locally produced reactive oxygen species in the latter. Furthermore, with the application of the Light's criteria 15 to 30% of transudates in the literature are misclassified as exudates, and most cases of misclassifications occur in patients receiving diuretic therapy (Burgess et al., 1995).

Table 1—Demographic Characteristics, Serum and Pleural Fluid Measurements

Variables	Exudates (72)	Transudates (18)
Age, years	38.87 ± 16.92	40.75 ± 20
Smokers	25	7
Male	50	10
Female	22	8
Pleural fluid cells (cells/mm^3)	2812.89 ± 916.20	510.00 ± 60.24
Pleural fluid glucose (mg %)	82.6 ± 49.65	100 ± 5.65
Serum glucose (mg %)	117.7 ± 44.59	138 ± 14.14
Total protein pleural fluid, (g %)	3.38 ± 0.717	2.77 ± 0.753
Serum Protein fluid (g %)	5.66 ± 1.06	6.3 ± 1.21
Total protein pleural fluid/ total protein serum ratio	0.587 ± 0.094	0.323+0.096*
Pleural fluid LDH, (IU/L)	189.62 ± 24.75	114.38 ± 27.47
Serum LDH, (IU/L)	296.9 ± 42.35	235.33 ± 16.65
LDH pleural fluid/LDH serum ratio	0.620 ± 0.061	0.422 ± 0.122*
Pleural fluid MDA (mmol/L)	0.640 ± 0.092	0.379 ± 0.086
Serum MDA (mmol/L)	0.908 ± 0.126	1.04 ± 0.06
MDA pleural fluid/MDA serum ratio	0.710 ± 0.082	0.428 ± 0.070*

Values are given as the mean ± SD, * P<0.001

CONCLUSION

In this study we have reported that the level of oxidative stress (MDA) was higher in exudative pleural effusions compared to transudative pleural effusions, probably due to the production of more reactive oxygen species in the former. Pleural fluid /serum MDA ratio is much more accurate than PFMDA alone in the classification of effusions into transudates and exudates.MDA ratio it is nearly equivalent in reliability to light's criteria. Oxidative stress levels (MDA) were assessed with TBARS method that was highly repeatable in the patients studied and may serve as a marker for the differentiation between exudates and transudates in clinical practice.

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