Malondialdehyde (MDA) and superoxide dismutase (SOD) levels – distinguishing parameters between benign and malignant pleural effusions

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ABSTRACT

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. Increased oxidative stress participates in the pathogenesis of both airways and parenchymal lung diseases. There is a critical balance between free radical generation and antioxidant defences. The local production of free radicals and the role of oxidative stress in the pathogenesis of pleural effusions have not been extensively studied. **Objective:** To assess the levels of malondialdehyde (MDA), superoxide dismutase (SOD), total proteins (TPR) and lactate dehydrogenase (LDH) in pleural fluid and serum, also to analyse that whether malondialdehyde and superoxide dismutase can be used as potential markers for distinguishing between benign and malignant pleural effusion. **Research Design and Methods:** 96 participants were included in this prospective study and were divided into two groups. Group-I consisted of 48 cases of malignant pleural effusion and group-II consisted of 48 cases of benign pleural effusion. **Results:** Mean serum levels of malondialdehyde, superoxide dismutase, lactate dehydrogenase and total proteins in malignant (group-I) were 131.88±12.17 nmol/ml, 39.28±5.28 U/ml, 285.20±33.06 IU/L, 6.0±0.67 gm % and in benign (group-II) were 72.83±5.58 nmol/ml, 34.35±3.61 U/ml, 227.69±10.01 IU/L, 6.13±0.58 gm % respectively. In pleural fluid mean levels of malondialdehyde, superoxide dismutase, lactate dehydrogenase and total proteins malignant pleural effusion (group-I) were 126.83±12.26 nmol/ml, 38.63±5.67 U/ml, 185.28±21.48 IU/L, 4.34±0.84 gm % and in benign pleural effusion (group-II) were 53.01±8.92 nmol/ml, 32.21±3.27 U/ml, 120.29±15.37 IU/L, 4.12±0.47 gm % respectively. This study showed statistically significant increase in malondialdehyde levels (p-value < 0.001), activities of superoxide dismutase enzymes (p-value < 0.001) and lactate dehydrogenase levels (p-value < 0.001) in malignant pleural fluids when compared to the benign pleural fluids. Non significant change was observed in total protein levels (p-value > 0.01) in malignant pleural fluids when compared to the benign pleural fluids. **Conclusion:** Oxidative stress is increased in malignant pleural effusion malondialdehyde and superoxide dismutase being oxidative stress markers, can be used for differentiating between malignant and benign pleural effusions.

Keywords: Pleural effusion, malondialdehyde, superoxide dismutase, lactate dehydrogenase.

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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. It heralds a serious prognosis if not diagnosed or treated properly. Approximately one million patients develop pleural effusion each year.[1] Free radical generation and antioxidant defences are well

balanced. It has been suggested that oxidative stress may be associated with many pulmonary disorders including malignancy.[2] Oxidative stress is defined as the imbalance between oxidants and antioxidants.[3]

Lipid peroxidation is a chain reaction providing a continuous supply of free radicals as it involves the oxidation of polyunsaturated fatty acids in membranes causing oxidative cell damage. MDA (thiobarbituric acid reacting substance) is formed as an end product of lipid peroxidation and acts as an indicator of it.[4,5] During pulmonary inflammation, increased amounts of Reactive Oxygen Species (ROS) and Reactive Nitrogen Intermediates (RNI) are produced as a result of phagocytic respiratory burst.[6] The primary target of ROS is presumed to be cellular DNA. ROS may act as carcinogenic agents by inducing structural changes in DNA and modulating expression of stress related genes.[7] In animal models, antioxidant molecules have been shown to inhibit experimental carcinogenesis. Not much is known about the respiratory tract antioxidant defences of patients with lung cancer, although superoxide dismutase (SOD) has been proven to prevent carcinogenesis in vitro.[8] Superoxide dismutase (SOD) is a primary enzymatic defence system, which catalyses dismutation of superoxide radicals to hydrogen peroxide and protects body against the potential damage from superoxide radicals.[9]

The local production of free radicals and the role of oxidative stress in the pathogenesis of pleural effusions have not been extensively studied. So the aim of the present study was to assess the levels of MDA, SOD, total proteins and lactate dehydrogenase (LDH) in pleural fluid and serum, also to analyse that whether MDA and SOD can be used as a potential marker for distinguishing between benign and malignant pleural effusion.

MATERIAL AND METHODS

This cross-sectional study was conducted in the Department of Biochemistry in collaboration with Department of Internal Medicine and Respiratory Medicine of Maharishi Markandeshwar Institute of Medical Science and Research, Mullana, Ambala for a period of one year. Total 96 patients with pleural effusion were included and were grouped based on the histo-pathological analysis:

1. Group-I Malignant pleural effusion.
2. Group-II Benign pleural effusion.

The present study was approved by the ethical committee of Maharishi Markandeshwar Institute of Medical Science and Research and the study was conducted after taking informed and written consent from the patients.

Inclusion criteria

All cases of pleural effusion which were admitted in Internal Medicine and Respiratory Medicine wards.

Exclusion criteria

1. Patients taking antioxidants i.e. Vitamin A, E and C.
2. Patient with known malignancies receiving cytotoxic drugs.
3. Traumatic pleural effusion.
4. Patients on anti-tubercular drugs.

Sample collection and analysis

The initial evaluation of the patients with pleural effusion consisted of history, physical examination, laboratory investigations and roentgenographic studies. Prior to aspiration, informed and written consent was taken before the thoracentesis was done. Aspiration of samples (pleural fluid) was done after proper analgesic/sedation (if required) and local anesthesia. Pleural fluid and serum samples were collected from all patients on the day of their hospital admission. All the samples were immediately analysed for biochemical parameters under study.

Methods

Lipid peroxidation products were quantified by the thiobarbituricacid (TBA) method. In this reaction, the pink colour obtained with MDA (an end product of lipid peroxidation) using TBA was determined at 532 nm wavelength. Tetramethoxypropane was used as an external standard.[10] SOD was determined by Kakkar et al method,[11] LDH by modified IFCC method 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[12] and total protein (TPR) was quantified by Biuret method.[13]

Statistical analysis

The data obtained was compiled and analyzed using SPSS 11.5 for Windows version. Means ± standard deviation were calculated and student t-test was applied to find out significance level. Statistical significance was defined as two-tailed $p < 0.05$ for all tests unless otherwise specified.

RESULTS

Out of 96 cases of pleural effusion, 61 were males and 35 were females. Their mean age was 45.12 ± 9.23 years. All the pleural fluid samples obtained from the patients were classified into two groups. Pleural fluid was collected from group-I which consist 48 patients (32 males, 16 females) and from group-II which consists of 48 patients (29 males, 19 females). The classification of pleural fluid according to histo-pathology is summarised in (Table-1). Mean serum levels of MDA, SOD, LDH and TPR in malignant pleural effusion (group-I) were 131.88±12.17, 39.28±5.28, 285.20±33.06, 6.0±0.67 and in benign pleural effusion (group-II) were 72.83±5.58, 34.35±3.61, 227.69±10.01, 6.13±0.58 respectively. In pleural fluid mean levels of MDA, SOD, LDH and TPR malignant pleural effusion (group-I) were 126.83±12.26, 38.63±5.67, 185.28±21.48, 4.34±0.84 and in benign (group-II) were 53.01±8.92, 32.21±3.27, 120.29±15.37, 4.12±0.47 respectively as shown in (Table-2). The results indicates there is a significant increase in MDA, SOD and LDH levels in both serum and pleural fluid in group-I patients $p < 0.05$ and there was non-significant increase in TPR levels in group-I in both serum and pleural fluid (Figures 1, 2).

Table 1 Patients with pleural effusion classified according to their histo-pathology

Malignant Pleural Effusion (Group-I)	Number of cases
Non-small cell carcinoma	32
Small cell carcinoma	4
Lymphoma	2
Metastatic lung carcinoma	10
Benign Pleural Effusion (Group-II)	
Congestive cardiac failure	10
Pulmonary tuberculosis	28
Pneumonia	6
Cirrhosis of liver	4

Table 2 Depicting mean, standard deviation and significance of pleural fluid and serum levels in two groups

Parameters	Malignant (Group-I) mean±SD	Benign (Group-II) mean±SD	Significance p-value
Pleural MDA (nmol/ml)	126.83 ± 12.26	53.01 ± 8.92	<0.001
Pleural SOD (U/ml)	38.63 ± 5.67	32.21 ± 3.27	<0.001
Pleural LDH (IU/L)	185.28 ± 21.48	120.29 ± 15.37	<0.001
Pleural TPR (gm %)	4.34 ± 0.84	4.12 ± 0.47	0.122
Serum MDA (nmol/ml)	131.88 ± 12.17	72.83 ± 5.58	<0.001
Serum SOD (U/ml)	39.28 ± 5.28	34.35 ± 3.61	<0.001
Serum LDH (IU/L)	285.20 ± 33.06	227.69 ± 10.01	<0.001
Serum TPR (gm %)	6.0 ± 0.67	6.13 ± 0.58	0.335

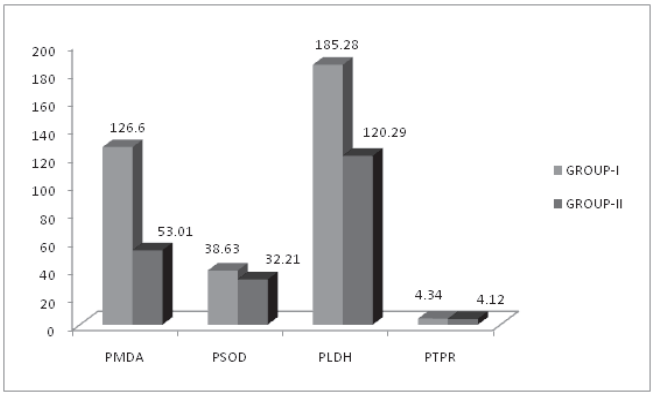

Figure 1. Showing pleural malondialdehyde (PMDA), pleural superoxide dismutase (PSOD), pleural lactate dehydrogenase (PLDH) and pleural total protein (PTPR) levels in group-I and group-II.

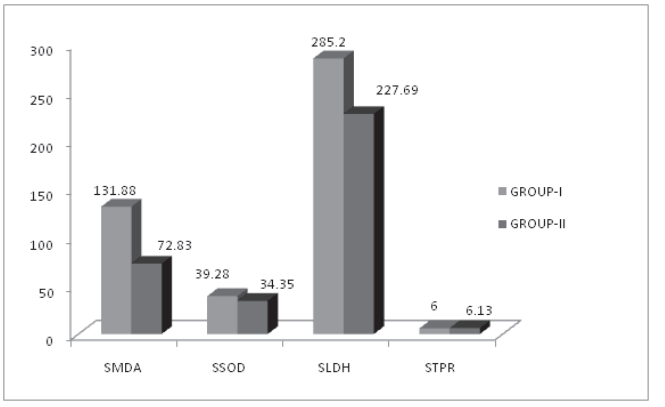

Figure 2. Showing serum malondialdehyde (SMDA), serum superoxide dismutase (SSOD), serum lactate dehydrogenase (SLDH) and serum total protein (STPR) levels in group-I and group-II.

DISCUSSION

In this prospective study, it was observed that malignant pleural effusions presents as increased levels of oxidative stress (MDA levels) compared to benign effusions. The increased level of oxidative stress in malignant pleural effusion probably represents the increased local production of free radicals. The origin of this local oxidative burst is related to the nature of each disease entity.[14] 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.[15] In many diseases free radicals play a very important role.[16] Impairment in the antioxidant system in our body due to reactive oxygen species (ROS) triggers lipid peroxidation and DNA damage which can lead to carcinogenesis.[17] Increased lipid peroxide products in abnormally proliferating cells due to the oxidative damage are thought to be released into the systemic circulation resulting in their increased levels in

serum or pleural fluids in case of cancer patients.[18] In our study, MDA content in malignant pleural fluids was higher than in benign pleural fluids. Earlier reports also described elevated plasma MDA levels in malignant pleural exudates when compared to those of non-malignant effusions.[19,20]

Free radicals like superoxide anion are highly reactive and can cause both morphological and functional damage in the cell.[21] The cells protect themselves against oxidative damage by enzymatic and non-enzymatic antioxidant system. Superoxide dismutase is the primary enzymatic antioxidant defence system in the cell.[22] This scavenging enzyme plays an important role in the protection of cell against the potentially harmful effects of superoxide anion generated by a wide variety of biological processes.[23] In our study there is significant increase in SOD activity in group-I (malignant) as compared to group-II (benign). Lizutci et al reported that SOD activity was significantly elevated in cancerous lung tissues when compared with those of normal uninvolved tissues.[24] In another study, increased SOD activity was also determined in serum from patients with ovarian cancer.[25]

We also found that there is a statistically significant increase in LDH activity and insignificant increase TPR in serum as well as pleural fluids of group-I (malignant) as compared to group-II (benign).

CONCLUSION

Elevated MDA levels and increased activities of SOD enzymes were found in malignant pleural fluids when compared to the benign pleural fluids. These results suggest that, in spite of an increase in the activity of SOD, there is an increase in lipid peroxidation products which could reflect the fact that oxidative stress had increased as compared to the capacity of the antioxidants enzymes in pleural fluid. As MDA and SOD levels are significantly increased in malignant effusions, so they might be helpful in differentiating between malignant and benign pleural effusions as compared to previous criteria.

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COMPETING INTERESTS

The authors declare that there were no competing interests associated with this study.

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