

ORIGINAL ARTICLE

## Lipid Peroxidation Products and Nitric Oxide in the Evaluation of Benign and Malignant Pleural Effusion

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**Background:** Pleural effusion is a common complication of various diseases. Free radicals are known to produce damage in many biological tissues. Free radicals exert their cytotoxic effect by causing lipid peroxidation which is believed to be responsible for the exudation of fluid into the pleural space.

**Aim:** Evaluation of the association of benign and malignant pleural effusion with the free radical induced pleurisy by measurement of lipid peroxidation products and nitric oxide activity.

**Methods:** Case control study was conducted on 50 cases of benign pleural effusion, 50 cases of malignant pleural effusion and 15 cases of healthy controls. Malondialdehyde (MDA) levels were measured by spectrophotometric method with TBA. Nitric Oxide activity was measured by spectrophotometric method using Griess reaction.

**Results:** Our results showed significant increase of MDAs level in both groups of patients: benign and malignant in comparison with control group. Significant increase in the concentrations of nitrate /nitrite depicting total nitric oxide was observed in benign as well as malignant group in comparison to control healthy group.

**Conclusion:** These results suggest that determination of biomarkers of oxidative stress products may be useful in the diagnosis and treatment of patients.

*Key words: Pleural effusion, benign, malignant, free radical induced pleurisy*

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Pleural effusion is a common occurrence in a number of pulmonary and extra-pulmonary diseases, being often the only manifestation of the disease (Light et al.1973). Pleural effusion occurs in a variety of diseases pertaining to respiratory system, cardiovascular system, renal and other systems. Determining the cause of pleural effusion

is not always easy. The first and most important step in the evaluation of pleural effusion is to differentiate them as transudates and exudates (Storey et al. 1976; Guleria et al 2003). Recently, usefulness of MDA has been studied to differentiate transudates from exudates. MDA is a lipid peroxidation product, produced by oxidative

deterioration of unsaturated fatty acids. Two sources have been suggested for MDA pleural fluid (Gupta 2002). The first possible source is plasma proteins. When pleura are inflamed, there is increased leakage of plasma proteins into pleural space. Plasma MDA is mostly bound to plasma proteins. Due to increased leakage of plasma proteins into pleural space, MDA enters into pleural space, leading to increased concentration in pleural fluid. The second possible source is local production by inflammatory cells which are present in increased number in conditions like tuberculosis, malignancy etc (Gupta 2002).

Nitric oxide (NO), determined as its immediate metabolite nitrite, were measured by Agrenius et al, 1994 in pleural fluid, in order to investigate if they were involved in pleural inflammation and pleural fluid exudation.

An attempt has been made to evaluate the pleural fluid in benign and malignant effusion to have an insight on the correlation of MDA and nitric oxide in the disease process.

#### **MATERIALS AND METHODS**

Case control study comprising of 50 cases of benign pleural effusion and 50 cases of malignant pleural effusion was carried out in the Department of Medicine and Department of T.B and Respiratory Diseases, Institute of Medical Sciences, Banaras Hindu University. All the selected patients presented with pleural effusion of inflammatory origin, and of neoplastic origin diagnosed clinically, radiologically and cytologically were taken for the study. Exclusion criteria comprised of HIV positive patients, Diabetes Mellitus, Rheumatoid Arthritis and other immune mediated diseases, and patients who were consuming antioxidants for prolonged duration of more than four weeks. 15 age and sex matched healthy individuals were the subjects whose blood samples were taken as control

subjects. Blood samples and pleural fluids were taken from the study subjects with their informed consent.

#### **Estimation of Malondialdehyde - marker of lipid peroxidation**

Malondialdehyde (MDA) levels in the study cases and controls were assayed by thiobarbituric acid reactive substances (TBARS) technique of Philpot, 1963; Burge and Aust, 1978. MDA, which is a stable end product of fatty acid peroxidation, reacts with TBA at acidic conditions to form a complex that has maximum absorbance at 532 nm.

#### **Measurement of nitrite-nitrate concentration in pleural fluid**

The total nitrite concentration in pleural fluid of study subjects, an indicator of NO synthesis, was measured as described by Cuzzocrea et al (1998). The nitrate in the samples was first reduced to nitrite by incubation with nitrate reductase and NADPH at room temperature for 3 h. The total nitrite concentration in the samples was then measured using the Griess reaction, by adding Griess reagent to the sample. Optical density was measured at 550 nm. Nitrite concentration was calculated by comparing OD<sub>550</sub> with standard solutions of sodium nitrite prepared in distilled water.

#### **Statistical Analysis:**

Statistical analysis was done using statistical software SPSS version 10.0. Data was found to be normally distributed. Paired *t*' test, ANOVA test and SNK test were applied and the results were expressed as mean  $\pm$  SD and *p* value was considered significant at 5% level of significance.

#### **RESULTS**

The present study was conducted on the pleural fluid from 50 benign and 50 malignant cases treated in the Department of Medicine and Department of T.B and Respiratory Diseases, Institute of Medical

Sciences, Banaras Hindu University. Study also included 15 healthy individuals who acted as age and sex matched controls. Blood samples were collected from the antecubital vein of the healthy volunteers and the serum was separated from each sample for biochemical analysis. The consent of each individual was taken purely for research work.

The average age of the study subjects was  $48.7 \pm 13.9$ ,  $43.4 \pm 18.6$  and  $52.4 \pm 11.6$  years in the control, benign and malignant group respectively. The characteristics of the study subjects are given in table 1.

Most of the cases had tuberculosis. Disease wise distribution of the studied subjects in benign pleural effusion is depicted in table 2. Disease wise distribution of study subjects in malignant Pleural Effusion patients is depicted in table 3.

Status of malondialdehyde, nitrite/nitrate in patients of benign and malignant pleural effusion shows significantly higher values in comparison to serum concentrations in healthy control volunteers (Table 4). Raised level of malondialdehyde and nitrite/nitrate in patients is marker for higher levels of free radical damage and involvement of free radicals in pleurisy.

**Table 1** Characteristics of the Study Subjects

	Control	Benign	Malignant
<b>Number</b>	15	50	50
<b>Gender</b>			
<b>Male</b>	09	31	28
<b>Female</b>	06	19	22
<b>Age (Years)</b>	$48.7 \pm 13.9$	$43.4 \pm 18.6$	$52.4 \pm 11.6$
<b>Smokers</b>			
<b>Male</b>	08	16	19
<b>Female</b>	00	08	11

**Table 2** Disease wise distribution of study subjects in benign Pleural Effusion

Disease	No. of Patients	Sex	
		Male	Female
<b>Tuberculosis</b>	27	16	11
<b>Parapneumonic effusion</b>	09	05	04
<b>Empyema</b>	07	04	03
<b>Pancreatitis</b>	02	02	-
<b>Hemothorax</b>	01	01	-
<b>Liver Abscess</b>	04	03	01
<b>Total</b>	50	31	19

**Table 3** Disease wise distribution of study subjects in malignant Pleural Effusion

Disease	No. of Patients	Sex	
		Male	Female
LUNG TUMOUR	31	21	10
Histological subtypes			
• Small cell carcinoma	05	04	01
• Squamous cell carcinoma	12	10	02
• Adenocarcinoma	08	03	05
• Poorly differentiated or Large cell carcinoma	06	04	02
Other TUMOUR TYPES	19	07	12
• Mammary carcinoma	06	-	06
• Ovarian carcinoma	02	-	02
• Uterine carcinoma	02	-	02
• Stomach carcinoma	01	01	-
• Malignant melanoma	01	01	-
• Non-Hodgkin's carcinoma	02	02	-
• Unknown primary	05	03	02
Total	50	28	22

**Table 4** Status of Malondialdehyde, nitrite and nitrate in benign and malignant pleural effusions in comparison to controls

Group (Number)	MDA (mMol/L)	NO <sub>2</sub> (μMol/L)	NO <sub>3</sub> (μMol/L)
Control (15)	0.817±0.013	34.04±7.89	59.47 ± 13.79
Benign (50)	1.101 ± 0.178*	79.28 ±32.056*	125.96 ±41.07*
Malignant (50)	1.127± 0.161*	88.96± 37.16*	148.38 ± 5141*

Values expressed as Benign vs. Control; Malignant vs. Control, \*p < 0.001

## DISCUSSION

Numerous physiological and pathological processes such as ageing, excessive caloric intake, infections, inflammatory disorders, environmental toxins, pharmacological treatments, emotional or psychological stress, ionizing radiation, cigarette smoke and alcohol increase the bodily concentration of oxidizing substances, known as reactive oxygen species (ROS) or, more commonly, free radicals. These are chemical species which are highly reactive owing to the presence of free unpaired electrons. An increase in free radicals compromises the delicate homeostatic mechanisms which involve neurotransmitters, hormones, oxidizing substances and numerous other mediators. Owing to their structure, which is rich in double bonds,

polyunsaturated fatty acids (PUFAs) render cellular membranes vulnerable to damage from free radicals, causing peroxidation. The damage induced by lipid peroxidation renders the cell unstable, and therefore compromises fluidity, permeability, signal transduction and causes receptor, mitochondrial DNA and nuclear alterations (Halliwell and Gutteridge, 2007).

Nitric oxide, another free radical, is a poly-functional signaling molecule controlling processes of vasodilation, platelet aggregation, immunocytotoxicity and carcinogenesis could either mediate tumourocidal activity or promote tumour growth (Agrenius et al 1994). Nitric oxide is of importance in induced pleural inflammation. Inflammation and oxidative stress are pathogenic

mediators of many diseases. The intracellular redox state is a key determinant of cell fate. Oxidative stress plays a pivotal role in a variety of cellular responses.

In the present study, higher concentration of both MDA and total nitric oxide was observed in the pleural fluid of malignant patients than those in benign disease group. Comparison of mean concentration of MDA and total nitrate in cancer patients and control group; and also between benign and control group reveals a statistically significant difference. This points towards the existence and involvement of free radicals in pleurisy.

To assess whether cancer-induced pleurisy is associated with an alteration of nitric oxide (NO)-synthase activity, Alexander et al (2002) measured the levels of nitrate/nitrite (NO<sub>x</sub>) in blood serum (BS) and pleural effusion (PE) of cancer patients (secondary pleural metastases and mesotheliomas), patients with benign lung diseases, and in BS of healthy donors. Their results point up the diverse role of NO<sub>x</sub> in cancer patients and suggest that NO<sub>x</sub> acts as a signaling mediator during the formation of pleural metastases and might be considered as a non-specific marker in the corresponding PE.

Inflammatory and neoplastic diseases frequently involve the pleural space and walls (Kroegel and Antony, 1997). Pleural involvement in certain diseases is associated with the infiltration of a number of different types of immune cells, such as neutrophils, eosinophils or lymphocytes, in various proportions depending on both the course and the aetiology of the underlying disease. In addition to infiltrating cells, mesothelial cells have been demonstrated to actively participate in pleural inflammation *via* release of various mediators and proteins, including platelet-derived growth factor (PDGF), interleukin-8, monocyte chemotactic peptide (MCP-1), nitric oxide (NO), collagen, antioxidant enzymes and the plasminogen activation

inhibitor (PAI). Furthermore, several inflammatory mediators have been detected at increased concentrations within pleural effusions, including lipid mediators, cytokines and proteins (adenosine deaminase, lysosyme, eosinophil-derived cationic proteins, and products of the coagulation cascade). The presence of these mediators underline the concept of pleural inflammation and certain cytokines seem to characterize a specific aetiology of pleurisy. The understanding of these processes and the sequence of events leading to pleural loculation, pleural adhesion or repair are likely to provide the basis for early therapeutic intervention and reduce pleural-associated morbidity.

## CONCLUSION

The results of the study point the diverse role of lipid peroxidation products and nitric oxide in both benign and malignant pleural effusions. Malondialdehyde assay is the most widely used test in the appreciation of the role of oxidative stress in disease. Malondialdehyde is one of the several products formed during the radical induced decomposition of polyunsaturated fatty acids. The results obtained suggest that the combined analysis of increased levels of nitrate/ nitrite in pleural effusions and of histochemical properties of cancer and inflammatory cells may be useful in exploring the interrelationship of functionally important cellular characteristics. Thus reduction of endogenous and exogenous cause of oxidative stress is at present the best preventive option. In near future, new insights and interventions in the action of tumour suppressor genes, tumour cell apoptosis and the DNA repair mechanisms may lead to additional tools against carcinogenesis from oxygen free radicals and lipid peroxidation.

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