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Serum malondialdehyde levels in lung cancer patients

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ABSTRACT

Lung cancer is a leading cause of cancer death internationally, with smoking being the largest single cause. This is the leading cause of cancer death in developed countries and is rising in alarming rates in developing countries. The aim of the present investigation was to measure the levels of malondialdehyde (MDA), a product of lipid peroxidation as supplementary marker in patients with lung cancer. Levels of serum MDA was determined spectrophotometrically in 35 lung cancer patients and equal number of healthy controls. The mean serum MDA level in the lung cancer group was found to be 3.8 ± 2.5 nmol/mL, whereas it was 1.4 ± 0.8 nmol/mL in controls. There was statistically significant increase in serum malondialdehyde levels in the lung cancer patients compared with the control group. The results of this study offer additional data of the association between lipid peroxidation and cancer and should add to the understanding studies in this area for future research. Our observations specify that increased lipid peroxidation levels are associated with cancer development, with and without smoking. Though, an enhanced raise of TBARS in smokers may be due to increased oxidants introduced by smoking.

1. INTRODUCTION

The lung is the organ with the highest exposure to atmospheric oxygen. Due to its large surface area and rich blood supply, the lung is susceptible to oxidative injury by large numbers of reactive oxygen species (ROS) and nitrogen species, as well as by free radicals. In situ lung injury due to ROS is strongly associated with oxidation of proteins, lipids and DNA. These oxidized biomolecules may also induce a variety of cellular responses with generation of secondary metabolic species [1]. Smoking is one of the major lifestyle factors influencing the health of human beings. It is known that cigarette smoke and tar phase contain a number of oxidizing compounds, ROS and carcinogens, which damage the genome, membranes and macromolecules of cells [2, 3, 4, 5]. Smoking may enhance oxidative stress not only through the production of reactive oxygen radicals in cigarette tar and smoke but also through weakening of the antioxidant defense systems, Moreover, approximately 60 known carcinogens and mutagens are present in the tobacco smoke. The action of oxidative agents and mutagens are accompanied by DNA damage, mutations in cancer-related genes, oncogenes activation, tumor suppressor inactivation and deregulation of gene expression. Some of these events can induce the development of malignant process [6].

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Free radicals with their structure, physicochemical properties, cellular origins, reactions and impacts have been implicated as important mediators in many clinical disorders. 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. Thiobarbituric acid reacting substance (TBARS) is formed as an end product of lipid peroxidation and acts as an indicator of it [7, 8]. There is evidence that oxidative stress is an important event in the development of smoking related diseases such as lung and oral cancer and chronic obstructive pulmonary disease [4].

The local production of free radicals and the role of oxidative stress in the pathogenesis of lung cancer have not been extensively studied. The present study aims to investigate potential changes in the antioxidant status induced by cigarette smoking in lung cancer patients compared to the healthy subjects, utility of oxidative stress parameter serum TBARS for estimating smoking-induced harm and probability of cancer incidence in healthy subjects.

2. MATERIALS AND METHODS

Thirty five male newly diagnosed lung cancer patients and equal number of age matched volunteers were involved in this study. Fasting venous blood samples were collected from two groups of males. Each of the main groups included two sub-groups such as smokers and non-smokers. Female patients, patients suffering from moderate or severe hypoxia and patients having

chronic systemic disease are excluded in this study. The group of healthy subjects consisted of 19 smokers and 16 non-smokers. The group of cancer patients included 20 smokers and 15 non-smokers. The mean ages of investigated groups were sufficiently close. The healthy smokers and non-smokers were of mean age 50 ± 1.5 years; the sub-group of smoking cancer patients was 55 ± 3.5 years and the group of non-smoking patients was 50 ± 2.3 years of age. After obtaining prior consent, venous blood was collected from the subjects under aseptic condition by vein puncture using 5 mL sterile disposable syringe and needle. Plasma was separated by centrifugation at 3000xg for 15 minutes. The samples were stored at 4ºC before analysis and all the samples were analyzed on the same day of the collection. The work was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

2.1 Estimation of Malondialdehyde

Serum MDA level was measured according to the method of Ohkawa et al [9]. MDA level was determined by thiobarbituric acid reactive substances (TBARS) in serum, based on the reaction between MDA and TBARS. Standard Malondialdehyde solution in 5 mL of volume was processed along with test samples. 1.5 mL of 0.8% of TBA was added to 1 mL of the serum sample. Then 0.4 mL of 8.1% sodium dodecyl sulphate and 1.5 mL of acetic acid was added. The mixture was finally made upto 5 mL with distilled water and placed in hot water bath at 95ºC for 1 h. After cooling, 1 mL of distilled water and 5 mL of the mixture of n-butanol and pyridine (15:1, v/v) was added. The mixture was vortexed and after centrifugation at 4000 rpm for 10 minutes, the absorbance of the organic layer (upper layer) was measured in UV-Vis spectrophotometer (Shimatzu) at 532 nm against blank using distilled water. TBA when allowed to react with MDA aerobically formed a colored complex [MDA-(TBA) 2 complex] which was measured with spectrophotometer. MDA concentration (measured as TBARS) was calculated as nmol/mL

2.2 Statistical Analysis

All the results were expressed as the mean value ±SD and statistical analysis was done by student's t-test. Data from the control subjects was compared with the lung cancer patients and a value of $P<0.05$ was considered as statistically significant.

3. RESULTS AND DISCUSSIONS

Lung cancer is a leading cause of cancer death internationally, with smoking being the largest single cause. Smoking is responsible for 85-90% of lung cancers, yet <20% of lifelong smokers develop lung cancer, suggesting that other factors including genetics may play a role. In smoking related lung diseases an excessive load of oxidative stress is produced, from the smoke itself and/or from the specific inflammatory process predominating in each disease. The general characteristics of the group studied are summarized in Table 1.

Table. 1: General characteristics of groups studied.

Study group		n	Age (years) Mean ±SD	Average number of cigarettes/ day
Control Group	Smokers	19	50 ± 1.5	18
	Non-smokers	16	50 ± 1.5	-
Cancer Patients	Smokers	20	55 ± 3.5	29
	Non-smokers	15	50 ± 2.3	-

The mean, standard deviation and 'P' value of serum MDA levels of lung cancer patients and the controls are summarized in Table 2. Assessment of serum MDA levels revealed a significant difference ($P<0.05$) between lung cancer patients with smoking (3.8 ± 2.5) and controls with smoking habit (1.4 ± 0.8). There was a significant elevated level of mean serum MDA found in all sub-groups (3.8 ± 2.5 and 2.9 ± 1.7) of lung cancer patients as compared to the control sub-groups (1.4 ± 0.8 and 0.6 ± 0.2). 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 [10]. In many diseases free radicals play a very important role [11]. Impairment in the antioxidant system in our body due to ROS triggers lipid peroxidation and DNA damage which can lead to carcinogenesis [12].

Table. 2: Serum MDA level in lung cancer patients and controls.

Study group		n	MDA (nmol/mL) (Mean ±SD)
Control Group	Smokers	19	1.4 ± 0.8
	Non-smokers	16	0.6 ± 0.2
Cancer Patients	Smokers	20	3.8 ± 2.5*
	Non-smokers	15	2.9 ± 1.7*

*****Significantly different, $P<0.05$

Extensive epidemiological [13] and statistical data [14] clearly establish role of cigarette smoking in the genesis of lung cancer and head and neck cancer. Several studies have revealed the extent of oxidation induced damage can be exacerbated by a decreased efficiency of the antioxidant defense mechanisms and that low levels of essential antioxidants in circulation are associated with an increased risk of cancer. In our prospective study, serum MDA level indicates the augmentation of oxidative stress in lung cancer patients and the control group smokers.

4. CONCLUSION

Elevated level of serum MDA levels was observed in lung cancer patients and smokers of control group. The increased level of oxidative stress in cancer patients probably represents the increased local production of free radicals. Cigarette smoke has been identified as a major risk factor for various diseases. It has the capacity to produce a highly diffusible ROS which cause oxidative damage in vital organs. By products of lipid peroxidation cause marked alteration in the structural integrity and function of cell membrane. The most characteristic of lipid peroxidation is to cause a considerable DNA-MDA adducts by interacting with cellular DNA and an imbalance between antioxidant defense

mechanism. The damage to the organs by cigarette smoke is evidenced by the elevation of biomarkers in serum. The study also demonstrated the potential applicability of the evaluated oxidative stress biomarker in the serum of healthy non-smokers and smokers to determine the risk of cancer incidence in these subjects.

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