Research Article

EVALUATION OF ENZYMATIC ANTIOXIDANTS IN LUNG CANCER PATIENTS

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Abstract: 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, reactive oxygen species and carcinogens, which damage the genome, membranes and macromolecules of cells. Cells living in an oxygen-rich environment are inundated with various endogenous and exogenous sources of reactive oxygen species. As a result, cells have evolved numerous defense mechanisms to counteract and limit the levels of reactive oxidants and the cellular damage that can ensue. The primary target of reactive oxygen species is presumed to be cellular DNA. Reactive oxygen species may act as carcinogenic agents by including structural changes in DNA and modulating expression of stress relating genes. The aim of the present investigation was therefore to evaluate the levels of antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase among lung cancer patients compared to controls. Results revealed statistically significant ($p < 0.05$) decrease in the enzymatic antioxidant levels in the lung cancer patients compared with the control group. The present investigation suggests further efforts are necessary to fully elucidate the importance of antioxidant enzymes as a biomarker for an early and timely diagnosis of lung cancer.

Keywords: Antioxidant enzymes, Biomarker, Lung cancer, Reactive oxygen species, Smoking

INTRODUCTION

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, so far <20% of lifetime smokers develop lung cancer, signifying that additional factors, including genetics, may play a role (Shopland et al., 1991). In the beginning of the century, lung cancer was considered to be unusual. But now it has reached epidemic proportions. This is the top cause of cancer death in developed countries and is increasing in alarming rates in developing countries. Cigarette smoke contains reactive oxygen species (ROS) that are implicated in the pathology of many neoplastic and non-neoplastic diseases. Due to the direct exposure to carcinogens and very high amounts of oxygen, the lung needs antioxidants for a defense mechanism against possible oxidative injury, to cope with undesirable oxygen activation.

Free radicals are highly reactive molecules or atoms which contain one or more unpaired electrons in their outer orbitals (Singhal et al., 2000). They are constantly formed during cellular processes like energy production and activation of phagocytosing cells, and by auto-oxidation of different molecules (Halliwell and Gutteridge, 2007). Oxidative stress is thus an inevitable consequence of aerobic life. The implication of free radical reactions in the pathogenesis of various diseases is nowadays generally accepted (Cross et al.,

1994). Oxidants also have multifactorial effects on cell proliferation and synthesis of growth factors and proteases that have fundamental effects on tumor angiogenesis and invasion (Maulik et al., 2002; Toyokuni et al., 1995). To control the influence of ROS, aerobic cells have developed their own antioxidant defense system, which includes both enzymatic and non-enzymatic components (Bakan et al., 2003). However, oxidative stress may occur if the production of ROS exceeds the antioxidant capacity of the cell (Powell et al., 2005).

There has been increasing interest in the task of free radicals and antioxidants in cancer during recent years. Oxidative stress has been recommended to play a key role in carcinogenesis (Cerutti, 1994; Lu, 2007). However the exact role of free radicals especially during cancer treatments is still largely unknown. The objective of the present study is to investigate potential changes in the enzymatic antioxidant status induced by cigarette smoking in lung cancer patients compared to the chance of cancer occurrence in healthy subjects.

MATERIALS AND METHODS

Study Subjects:

Thirty two male patients who were diagnosed to have lung cancer were studied. Fasting venous blood samples were collected from two groups of males.

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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 control group consisted of 17 smokers and 15 non-smokers. The groups of cancer patients were 19 smokers and 13 non-smokers. The mean ages of investigated human groups were sufficiently close. The control smokers and non-smokers were of mean age 48 ± 2.8 years; the sub-group of smoking cancer patients was 49 ± 3.2 years and the group of non-smoking patients was 51 ± 2.7 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 3000 rpm for 15 minutes. The samples were stored at 4°C 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.

The blood samples obtained from the subjects were used for the evaluation of enzymatic antioxidants which included the estimation of Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx).

Estimation of Superoxide dismutase (SOD):

SOD was assayed utilizing the technique of Kakkar *et al* (1984) based on inhibition of the formation of nicotinamide adenine dinucleotide, phenazine methosulfate and amino blue tetrazolium formazan. A single unit of enzyme was expressed as a 50% inhibition of NBT (Nitroblue tetrazolium) reduction/min/mg Hb.

Estimation of Catalase (CAT):

CAT was assayed colorimetrically at 620 nm and expressed as μmol of H_2O_2 consumed/min/mg Hb as described by Sinha (1972). The reaction mixture (1.5 ml) contained 1.0 ml of 0.01M phosphate buffer (pH 7.0), 0.1 ml of hemolysate and 0.4 ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio).

Estimation of Glutathione peroxidase (GPx):

GPx levels were estimated by the standard method (Rotruck *et al.*, 1973). To 0.2 ml of sample, 6.2ml of buffer, 0.2 ml EDTA, and 0.1 ml sodium azide were added. To this mixture added 0.2 ml of glutathione, followed by 0.1 ml of hydrogen peroxide. The contents were mixed well and incubated at 37°C for 10 minutes along with a control sample, After 10 minutes the reaction was stopped by the addition of 0.4 ml of 10% TCA. The tubes were centrifuged and the supernatant assayed for glutathione content by using Ellman's

reagent. The activity of GPx was expressed as μmoles of GSH utilized/min/mg protein.

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.

RESULTS AND DISCUSSIONS

Smoking is known to contain a number of oxidizing compounds, ROS and carcinogens, which damage the genome, membranes and macromolecules of cells. 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. ROS generated during metabolism can enter into reactions that, when unrestrained, can influence certain processes leading to clinical manifestations. Direct effects comprise peroxidative changes in membranes and other cellular components, including oxidative DNA damage. The demographic characteristics of the group studied are summarized in Table I.

Table.I: General characteristics of groups studied

Study group		N	Age (years) Mean ±SD	Average number of cigarettes/ day
Control Group	Smokers	17	48 ± 2.8	16
	Non-smokers	15	48 ± 2.8	-
Cancer Patients	Smokers	19	49 ± 3.2	27
	Non-smokers	13	51 ± 2.7	-

The enzymatic antioxidant status assessed was summarized in Table II. Assessment of enzymatic antioxidants revealed a significant difference ($p < 0.05$) between lung cancer patients with smoking and controls with smoking habit. The mean SOD in lung cancer patients with smoking habit was found to be lowered (1.32 ± 0.89), compared to controls (2.4 ± 1.04) with smoking habit. Mean CAT was also found to be decreased in lung cancer patients sub-groups as 1.94 ± 1.08 in smokers and 2.17 ± 0.35 in non-smokers respectively, compared to control sub-groups (3.2 ± 1.95 and 3.8 ± 0.67). The activity of GPx was significantly decreased in the lung cancer patients 8.27 ± 1.63 with smoking habit compared to the controls.

Table.II: Enzymatic antioxidant status in Lung cancer patients and control subjects

Parameter	Controls		Lung cancer patients	
	Smokers (n=17)	Non-smokers (n=15)	Smokers (n=19)	Non-smokers (n=13)
SOD	2.4 ± 1.04	2.97 ± 1.73	1.32 ± 0.89*	1.48 ± 0.92*
CAT	3.2 ± 1.95	3.8 ± 0.67	1.94 ± 1.08*	2.17 ± 0.35*
GPx	13.2 ± 1.08	13.9 ± 1.34	8.27 ± 1.63*	9.16 ± 1.02*

*Significantly different, p < 0.05; SOD (unit/min/mg Hb), CAT (μmolH$_2$O$_2$ consumed /min/mg Hb), GPx (μmol GSH utilized/ min/mg protein)

The impaired antioxidant system may favor accumulation of free radicals. It has been found that low levels of essential antioxidants in the circulation are associated with an increased risk of cancer (Diplock, 1991). Alternatively, it is possible that the antioxidant system is impaired as a consequence of an abnormality in the antioxidative metabolism due to the cancer processes. Enzymes such as SOD, CAT and GPx are considered to be the primary antioxidant enzymes, as they are involved in the direct elimination of active oxygen species. Secondary antioxidant enzymes (e.g., GST) help in the detoxification of ROS by decreasing peroxide levels or by maintaining a steady supply of metabolic intermediates for the primary antioxidant enzymes. Antioxidants have been shown to inhibit initiation and promotion in carcinogenesis, and counteract cell immortalization and transformation (Halliwell and Gutteridge, 2007).

Cigarette smoking, the most important cause of lung cancer, has been shown to be associated with depletion of some plasma antioxidants including vitamin C, α-tocopherol, carotenoids, glutathione-S-transferase and GPx (Liu *et al.*, 2002). In addition, an age-dependent adaptive response to antioxidants has been suggested, leading to significant reduction of plasma GPx activity only in older smokers, who could no longer sustain a counteracting effect to oxidative stress (Hulea *et al.*, 1993). It is believed that ROS may cause enzyme deactivation in the course of carcinogenesis (Dursun *et al.*, 2006; Dincer *et al.*, 2007). A statistically significant reduction in the activity of these enzymes in patients with advanced clinical stage was also observed by Namyslowski *et al.* (2003) and by Manoharan *et al.* (2005). Similarly, our present investigation observed a significant decrease in the activities of SOD, CAT, and GPx in the lung cancer patients compared to the control subjects.

CONCLUSION

Cigarette smoke has been identified as a major risk factor for various cancers. It has the capacity to produce a highly diffusible ROS which cause oxidative damage in vital organs. Oxidants have been shown to play an important role in carcinogenesis; not only serving as tumor initiators but also as tumor promoters

and regulators of gene expression. The cells protect themselves against oxidative damage by enzymatic and non-enzymatic antioxidant defense system. Those abnormalities appeared in the cellular regulation and expression of antioxidant enzymes play a vital role in cell division cycle and in the balance of life. The data indicate that smoking weakens antioxidant defense mechanism could a major risk factor in carcinogenesis. The results of the present investigation suggest that normalization of the levels of these enzymatic antioxidants might be used to reduce lung cancer malignancy. Further research should attempt to establish the role of smoking in association with oxidative stress and antioxidants in wide range of population.

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