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Original Article

EVALUATION OF NON-ENZYMATIC ANTIOXIDANTS IN LUNG CANCER PATIENTS

MOHAMMED RAFIQKHAN[1], SUDHA SELLAPPA[1]*

[1*]**Molecular Diagnosis and Drug Discovery Laboratory, Department of Biotechnology, School of Life Sciences, Karpagam University, Coimbatore, Tamilnadu, India Coimbatore 641021.**
Email: sudhasellappa@yahoo.co.in

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ABSTRACT

Objective: Cancer is one of the leading causes of morbidity and mortality throughout the world and it is the second leading cause of death in most of the developed countries. 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. The aim of the present investigation was therefore to evaluate the levels of non-enzymatic antioxidants in lung cancer patients and controls.

Methods: The non-enzymatic antioxidants included were estimation of reduced glutathione, vitamin C and vitamin E. All the results were expressed as the mean value ±SD and statistical analysis was done by student's t-test.

Results: Assessment of non-enzymatic antioxidants revealed a significant difference ($p < 0.05$) between lung cancer patients with smoking and controls with smoking habit. The impaired antioxidant system may favour accumulation of free radicals. In the present study, decreased level of non-enzymatic antioxidants was observed.

Conclusion: 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 non-enzymatic antioxidants might be used to reduce lung cancer malignancy. Despite active investigation, knowledge is lacking concerning the local and systemic effects of free radical-generating treatments in lung cancer.

Keywords: Lung cancer, Non-enzymatic antioxidants, Reduced glutathione, Vitamin C.

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]. 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 [2]. Tobacco smoking is well established as the major etiological risk factor for lung cancer, contributing to a tenfold increase in risk in long-term smokers compared with non-smokers [3].

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 [4]. Human antioxidant defenses have evolved to protect biological systems against reactive oxygen and nitrogen species, and a sophisticated cooperative array of antioxidant defense mechanisms is found in biological systems [5]. To control the influence of ROS, aerobic cells have developed their own antioxidant defense system, which includes both enzymatic and non-enzymatic components [6]. There have been increasing interest in the role of free radicals and antioxidants in cancer during recent years. Damages to DNA, protein, cell membrane and mitochondria are involved in carcinogenesis, although no specific biochemical marker has been identified yet. In addition, information on the biochemical alterations in tissue and blood, particularly of antioxidant status, and its correlation with the clinical staging of the disease, is lacking. The objective of the present study is to investigate potential changes in the non-enzymatic antioxidant status induced by cigarette smoking in lung cancer patients compared to the probability of cancer incidence 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. 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 group of cancer patients included 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 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.

Estimation of Reduced Glutathione (GSH)

The GSH content was determined by the method of Ellman (1959) [7]. 1.0 ml of plasma was treated with 0.5 ml of Ellman's reagent (19.8 mg of 5, 5'-dithiobisnitrobenzoic acid – DTNB in 100 ml of 0.1% sodium nitrate) and 3.0 ml of 0.2M phosphate buffer (pH 8.0). The absorbance was read colorimetrically at 412 nm

Estimation of Vitamin C

Vitamin C (Ascorbic acid) was determined by the method of Omaye *et al* (1979) [8]. To 0.5 ml of plasma, 0.5 ml of 2, 4-dinitrophenyl hydrazine (DNPH) reagent and 4% thiourea (in 9N sulphuric acid) was added and incubated for 3 h at room temperature. After

incubation 2.5 ml of 8.5% sulphuric acid was added and the colour developed was read colorimetrically at 520 nm.

Estimation of Vitamin E

Vitamin E (α-Tocopherol) was determined by the method of Desai (1984) [9]. Vitamin E was extracted from plasma by addition of 1.6 ml ethanol and 2.0 ml of petroleum ether to 5.0 ml of plasma and centrifuged. The supernatant was separated and evaporated. To the residue, 0.2 ml of 0.2% 2, 2-dipyridyl, 0.2 ml of 0.5% ferric chloride was added and kept in dark for 5 min, an intense red colour layer obtained on addition of 4 ml butanol was read colorimetrically at 520 nm.

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 DISCUSSION

The demographic characteristics of the group studied are summarized in Table 1. Assessment of non-enzymatic antioxidants revealed a significant difference ($p < 0.05$) between lung cancer patients with smoking and controls with smoking habit.

The mean GSH in lung cancer patients with smoking habit was found to be lowered (1.18 ± 0.78), compared to controls (2.37 ± 0.54) with smoking habit. Mean Vitamin C was also found to be decreased in lung cancer patients sub-groups as 0.64 ± 0.90 in smokers and 1.08 ± 0.03 in non-smokers respectively, compared to control sub-groups (1.82 ± 0.51 and 2.33 ± 0.87). The activity of Vitamin E was significantly decreased in the lung cancer patients 0.49 ± 0.17 with smoking habit compared to the controls sub-group smokers as 1.31 ± 0.20. The non-enzymatic antioxidant status assessed is summarized in Table 2.

Cancer is one of the leading causes of morbidity and mortality throughout the world and it is the second leading cause of death in most of the developed countries. 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. The impaired antioxidant system may favour 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 [10].

The multifunctional properties and the versatility of the antioxidant network highlight the efficiency of the dynamic interactions between the components of the network in protecting plasma from radical attack driven by heterogeneous sources. Non-enzymatic antioxidants such as glutathione, alpha-tocopherol (Vitamin E) and ascorbic acid are mostly chain breaking antioxidants which interrupt the autocatalytic spread of radical reactions [11]. GSH, a widely distributed cellular reductant is a metabolic regulator and putative indicator of health. Blood glutathione levels are believed to be predicators of morbidity and mortality [12]. GSH plays a key role in protecting cells against electrophiles and free radicals. GSH can act directly as a free radical scavenger by neutralizing HO•, or indirectly by repairing initial damage to macromolecules inflicted by HO•. Vitamin C acts as a co-antioxidant by regenerating α-tocopheroxyl radical produced during scavenging of reactive oxygen metabolites.

Table 1: 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	-

Table 2: Non-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)
GSH (mg/dL)	2.37 ± 0.54	2.84 ± 0.63	1.18 ± 0.78*	1.34 ± 0.95*
Vitamin C (mg/dL)	1.82 ± 0.51	2.33 ± 0.87	0.64 ± 0.90*	1.08 ± 0.03*
Vitamin E (mg/L)	1.31 ± 0.20	1.96 ± 0.32	0.49 ± 0.17*	0.92 ± 0.02*

*Significantly different, $p < 0.05$

Kimmick *et al* [13] opinions that Vitamin E is thought to be an important chain-breaking antioxidant which plays an important role in various stages of carcinogenesis through its contribution to immunocompetence, DNA repair and decreasing oxidative DNA damage. Antioxidant vitamins have a number of biological activities such as immune stimulation, inhibition of nitrosamine formation and an alteration of metabolic activities of carcinogens [14]. They can prevent genetic changes by inhibiting DNA damage induced by the reactive oxygen metabolites [15]. A statistically significant reduction in the activity of these enzymes in patients with advanced clinical stage was also observed by Namyslowski *et al* [16] and by Manoharan *et al* [17]. Our earlier study on serum malondialdehyde [18], enzymatic antioxidants [19] in lung cancer patients provides an evidence that lipid peroxidation is to cause a considerable DNA-MDA adducts by interacting with cellular DNA and an imbalance between antioxidant defense mechanism causing a major risk factor in carcinogenesis. ROS in a controlled sphere are physiologically relevant in exerting a variety of biochemical reactions that regulate many important physiological functions including defense against microorganisms, cell signalling, vascular control, cell generation and degeneration, control of cellular homeostasis and presumably many other unknown essential functions [20, 21]. 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. In our prospective study, decreased level of non-enzymatic antioxidants was observed in lung cancer patients compared to the controls. The results of the present investigation suggest that normalization of the levels of these non-enzymatic antioxidants might be used to reduce lung cancer malignancy. Furthermore, research studies are in progress in order to establish the role of smoking in association with oxidative stress, antioxidants in wide range of population and the exact molecular mechanism behind the cause which helps in good prognosis.

CONCLUSION

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. 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. The high incidence and poor prognosis of lung cancer make it a major health problem worldwide. The potential importance of the antioxidant network in cancer prevention is a relatively new topic of research. Although there are still aspects that need to be further investigated, evidence is mounting that total antioxidant capacity measurement might represent a promising tool to monitor the non-enzymatic antioxidants.

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