International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Lipid Peroxidation, Free Radical Production and Antioxidant Status in Esophageal Cancer Patients

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Received: 02/08//2013 *Revised: 05/09/2013* *Accepted: 10/09/2013*

ABSTRACT

Purpose: To determine the deleterious effects of radiation and chemotherapy i.e. over production of free radicals and lipid peroxidation byproducts and disturbances in antioxidant defense system implicated in the pathogenesis of carcinoma esophagus. **Materials & Methods:** Study of this was undertaken involving 60 cases (Esophageal cancer patients) both male and female in the age group of 40 years to 60 years old. Another 20 healthy subjects were also selected as control group. Blood samples of cases and controls were also collected for the estimation of enzymatic and non enzymatic antioxidant levels and associated free radical damage leading to lipid peroxidation.
Results: Serum TBARS levels were found to be increased and decreased enzymatic & non enzymatic levels in cases compared to controls. Conclusion: The present study clearly demonstrates that oxidative stress, antioxidant status and environmental factors apart from life styles play an important role in the prevention, control and treatment of carcinoma of the esophagus. Our study can be helpful for establishing new biochemical markers in esophageal cancer patients.

Key Words: Esophageal Cancer, Lipid peroxidation, Oxidative stress, Antioxidants, Free Radicals, Nutritional Factors.

INTRODUCTION

It is estimated that 17,460 men and women (13,950 men and 3,510 women) will be diagnosed with and 15,070 men and women will die of cancer of the esophagus in 2012.[1] In India, rates for oral and esophageal cancers are some of the highest in the world. In contrast, the rates for colorectal, prostate and lung cancers are one of the lowest.[2] The causes for cancers can be both either internal factors like inherited mutations, hormones and immune conditions or environmental factors such as tobacco, diet, radiation and other infectious agents. A significant variation of cancer has been reported due to life styles and food habits.[3]

Both enzymatic and non enzymatic pathways leading to formation of reactive compounds by one electron reduction or

oxidation generate free radicals. Role of free radicals has been proposed in the pathogenesis of many diseases involving different organs such as breast, gastric, colon, multiple myeloma, ovarian and oral cancer.[4-9] Various natural antioxidants available in the cells like glutathione peroxidase (GPx), Glutathione S-transferase (GST), Supeorxide dismutase, catalese, vitamin E and reduced glutathione (GSH) etc., prevents free radical chain reaction. When these antioxidant defense system get exhausted or generation of free radicals exceed to their scavenging capacity, free radicals mediated damage results.[10] Antioxidants have been shown to display protective role against ROS. They reduces oxidative damage of DNA, proteins and lipids. Antioxidant enzymes enhance the cytotoxic ability of macrophages to scavenge free radicals. Changes in concentrations of enzymatic antioxidants were found in esophageal cancer,[11,12] gastric cancer,[13] colorectal cancer,[14] and breast cancer.[15, 16]

Nutritional factors may play an important role in the etiology of esophageal and gastric cancers. Higher intake of antioxidant vitamins including vitamin A, vitamin E and carotenoids may protect against cancer development. Vitamin E from foods and supplements may have different effects on the development of cancer because of the differences in dose, bioavailability and correlated intake of other nutritional factors. So, it is useful to study their effects separately.[17] The plasma level of vitamin C and carotenoids may serve as biomarkers of consumption of vegetables and fruits that are the primary dietary sources of these vitamins.[18- 20]

Aim of the Study:

To determine the deleterious effects of radiation and chemotherapy i.e. over production of free radicals and lipid peroxidation byproducts and disturbances in antioxidant defense system implicated in the pathogenesis of carcinoma esophagus.

METHODS & METERIALS

For this study, 40 esophageal cancer patients selected from department of Radiotherapy. The cases were categorized into two different groups of 20 each according to the age (group I: 40-50 years old & group II: 50-60 years old). 20 age-matched healthy males were also selected for the study as control group. The controls were not habituated to tobacco chewing and smoking and were of the same age, sex and socio-economic status as the cases.

Biochemical Analysis:

Sample Collection:

Blood samples (6 ml) were drawn from cases and controls by venous arm puncture in heparinized test tubes and the plasma was separated by centrifugation at 1000g for 20 minutes. After plasma separation, the Buffy coat was removed and the packed cells were washed 3 times with physiological saline. The separated plasma was stored at light tight conditions at -20^{o}c until analysis.

Catalase: The catalase activity in the liver, kidney and testis of control and all treated animals was assayed by the modified method of Sinha.[25] Determination of Thiobarbutiric Acid Reactive Substance (TBARS): TBARS determined by the method of Okhawa et al.[24] The estimation of vitamin A was done by spectrophotometric method referred in "A manual of Laboratory Techniques, 2003" page number 163-164.[22] Determination of plasma ascorbic acid (Vitamin C): Determination of ascorbic acid was done in plasma by the method described by Sullivan and Clarke-1955.[21] Plasma Vitamin E estimation: Vitamin E was estimated in plasma by the method of Bieri et al.[23]

Statistical Analysis:

Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by student's t-test.

RESULTS

The level of TBARS was significantly increased in plasma of esophageal cancer patients as compared to healthy subjects and was gradually increased from group-I to group-II of esophageal cancer patients (Table 1). This may be due to very low immunity, increased age and advanced disease condition in group-II. Dorota Diakowska., Krzysztof Grabowski., et al.[26] also reported the same results.

The levels of vitamins A, C and E (Table 2) in the plasma were significantly reduced in patients compared to healthy controls. Ascorbate is the first antioxidant to be depleted upon exposure to both environmental and inflammatory oxidants. Thus, it suggests that it is the ultimate antioxidant either by directly scavenging these oxidants or trapping their intermediates.

Table 1. TBARS levels in plasma of Controls and Cases.

Parameters	Controls	Cases	
		Group-I	Group-II
Plasma TBARS (n mol/ml)	1.88 ± 0.15	2.56 ± 0.24*	3.45 ± 0.78**
During Radio/chemotherapy:		3.35 ± 0.90*	4.90 ± 1.08**
Values are mean ± SD (n=20); P*<0.001, **<0.01 compared to controls			

Table-2: Non-enzymatic antioxidant levels in Cases and Controls. (Mean±SD).

Groups	Vitamin A (µg/dl)	Vitamin E (mg/dl)	Vitamin C (mg/dl)
Controls (no-10)	125.9± 45.2	1.45±0.12	1.45±0.12
Group-I (no-10)	55.25±22.85	1.12±0.11	1.05±0.02
Group-II	48.10±19.80	1.10±0.07	0.86±0.04
During Radio/Chemotherapy			
Group-I	40.02±15.10	0.98 ± 0.07*	0.75 ±0.03**
Group-II	38.10±12.80	0.85± 0.05*	0.71 ±0.01**
No significant change (Student's unpaired 't' test)			

In our study, the catalase levels were lower in both patient groups compared to healthy subjects (Table 3). With advanced age, immunity levels slowly decreasing, and it may be due to this fact that there is a considerable difference in the values of catalase between groups I and II. Vitamin C is also an important physiological antioxidant[27] and has been shown to regenerate other antioxidants within the body including alpha-tocopherol (vitamin E).[28] Ongoing research is examining whether Vitamin C by limiting the damaging effects of free radicals through its antioxidant activity might help prevent or delay the development of certain cancers, cardio-vascular disease and other diseases in which oxidative stress plays a causal role.

Table 3. Plasma Catalase levels in Cases and Controls.

Parameters	Controls (n-20)	Cases	
		Group-I (n-20)	Group-II (n-20)
Catalase (Uc/l)	0.63 ± 0.03	0.42 ± 0.02*	0.36 ± 0.05**
During Radio/chemotherapy:	0	0.37 ± 0.04*	0.33 ±0.03**
Values are mean ± SD (n=20); P*<0.001, **<0.01 compared to controls			

DISCUSSION

In the study, all the patients were agricultural laborers with income level less than Rs.2,000/- ($ 36.36) per month and with the family sizes greater than 4 members. Lack of awareness on their nutritional matter may be a major contributory factor to be noted in the

prevalence of Carcinoma Esophagus, despite living in the pollution free rural areas with fruits and green vegetables both abundant and affordable. Hence, low income may not be a major contributory factor. However, exposure to environmental toxicants pesticides may be a major factor. Typical air pollution level here is equaling to the pollution at larger polluted city. However, if they are exposed to high amounts of pesticides, the air could certainly carry aerosols of such pesticides. Also ground/river water could be highly contaminated from either these pesticides or something upstream.

The present study showed enhanced oxidative stress in patients diagnosed with carcinoma esophageal cancer patients. Lower antioxidant levels of vitamins A, C and E and higher lipid peroxidation products TBARS with activated immune response as indicated by levels show marked oxidative stress in patients compared to control subjects.

Study of other investigators conform that concentrations of serum SOD, GPx and CAT significantly decreased in cerebral cancer, cervical cancer and oral squamous cell carcinoma.[29-31] Normal human cells produce small amounts of ROS which are reduced by enzymatic & non enzymatic antioxidants. An imbalance between oxidants and antioxidants can lead to oxidative stress. Permanent oxidative stress can accelerate the development of cancer which was observed in colonic cancer tissues, [14] gastric cancer tissues [32] and esophageal cancer tissues. [33, 34] We suggest that ROS production which increased with clinical progression of different types of cancers involved increase of lipid peroxidation. Mayo et al.,[35] demonstrated that products of lipid peroxidation can inactivate antioxidant enzymes- catalase and reduce the effectiveness of cells to defend against free radical damage. Lipid peroxidation products can react with DNA bases to form exocyclic DNA adducts such as etheno (ε) - DNA adducts. They are promutagenic in early stages of the carcinogenesis process. Inflammatory mediators such as cytokines inhibit DNA repair (impaired repair DNA adducts) and reduce antioxidant levels.[36]

CONCLUSION

The present study clearly demonstrates that oxidative stress, antioxidant status and environmental factors apart from life styles play an important role in the prevention, control and treatment of carcinoma of the esophagus. In the present study, the levels of circulating TBARS and antioxidants were found to be associated with the esophageal cancer patients. Measurement of lipid peroxidation and antioxidants in circulation of oral cancer patients may be helpful in assessing the defense system in esophageal cancer patients. Further studies are required about the enzymatic & non-enzymatic serum antioxidant levels both in the healthy controls and in different cancer patients.

ACKNOWLEDGEMENT

One of the authors (Ch. Kereena) is thankful to University Grants commission, New Delhi for awarding the fellowship under the scheme Moulana Azad National Fellowship for Minority Students.

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How to cite this article: Chukka K, Vishnuvardhan Z. Lipid peroxidation, free radical production and antioxidant status in esophageal cancer patients. Int J Health Sci Res. 2013;3(11):24-30.

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