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KEY WORDS: Head and Neck Cancer, Antioxidants, Lipid peroxidation, Free radicals, Soya Bean, Red Kidney Bean.

Effect of Dietary Protein Supplementation on the Antioxidant Levels in Head and Neck Cancer Patients

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ABSTRACT

Head and neck cancers (HNCs) accounts for more than 550,000 cases and 380,000 deaths annually, worldwide. Objectives of the study are to evaluate variations in the levels of MDA (a marker of lipid peroxidation) and serum antioxidant (Enzymatic and non-enzymatic) status in cases with HNC. To investigate whether Dietary protein supplementation with Soya, Red Kidney Bean and combination can improve biochemical status of enzymatic and non-enzymatic antioxidant response in cases with Head Neck Cancer. A total of 60 new cases with HNC (Stage I and II) aged 30 to 60 years of both the sex were considered for the present study. Group A, Group B, and Group C each group consist of 20 cases. Soya bean supplemented (A), Red Kidney Bean supplemented group (B) and Soya+Red Kidney Bean supplemented group (C) were considered to record antioxidant values of head and neck cancer patients. The serum was analyzed for enzymatic & non-enzymatic antioxidants. Consent was taken from the cases. Permissions obtained from the Institutional Ethical Committee. The present study evidenced Soya bean and red kidney bean supplementation to head and neck cancer patients undergoing treatment had a beneficial effect in lowering the serum MDA levels by improving the antioxidant status, which directly reflects, education in the lipid peroxidation in cases. These dietary protein supplementations during treatment may serve as an adjuvant therapy in head and neck cancer patients offering a good protection to normal cells that may further reduce the risk of developing secondary cancers.

Introduction:

Head and neck cancers (HNCs) accounts for more than 550,000 cases and 380,000 deaths annually, worldwide (Fitzmaurice et al. 2017). In the United States, HNCs accounts for three percent of malignancies, with approximately 63,000 Americans developing HNCs annually and 13,000 dying from the disease (Siegal et al. 2017). It can disfigure the face, strip away the voice and rob one of his/her basic abilities to eat, drink and swallow. The psychosocial impact can be extremely upsetting. HNCs are a significant problem in India constituting approximately one-third of all cancer cases.

It is well known fact that cytotoxicity mediates tumor activity and antioxidants mediate anticancer activity by changing the cellular homeostatic redox balance. Free radical is a molecular fragment, contains an odd number of unpaired electrons in the valence shell (i.e. radical) and is capable of being existent freely. Lipid peroxidation is an indicator of free radical metabolism and oxidative stress in living beings. Molondialdehyde (MDA), a by-product of lipid peroxidation (LPO), which is inherent in carcinogenesis displays differences in serum and its, levels in radiation treated malignant cases (Upadhya et al. 2004). Free radical mediated lipid peroxidation was recently proposed as a basic mechanism of injury, responsible for a wide variety of diseases including atherosclerosis, cancer and malnutrition (Khanna et al. 2005).

Antioxidants in the context of cancer mainly comprise natural health products, dietary supplements and certain fruits and vegetables. The potential impeding effect of such antioxidants on treatment success and their interaction with various chemotherapeutic agents and/or in combination with radiation therapy with suspected oxidative methods of action have been the subject of an important discussion within the medical community (Lawenda et al. 2008; Nakayama et al. 2011; Prasad et al.2002).

Mammalian cells are constantly exposed to oxidative stress and to a wide-range of antioxidant defenses comprising of both enzymatic and non-enzymatic molecules (Haliwell. 2007). Endogenous (e.g. glutathione, catalase) and dietary (e.g. Carotenoids, Vitamin E, Vitamin C, and Polyphenols) low molecular weight antioxidants were known to neutralize ROS and reactive nitrogen species (RNS). Dietary antioxidants act complementarily to endogenous antioxidants in removing free radicals directly and also up-regulate different genes involved in the endogenous antioxidant defense (Yeum et al. 2004; Blomhoff. 2005).

In the present study, some of the enzymatic antioxidants (Superoxide Dismutase, Total thiols, and Catalase) non-enzymatic antioxidants (Vitamin A, E and C) and lipid peroxidation by product Malondialdehyde (MDA) were analyzed and were calculated before and after three types of diet supplementations. Soya bean supplemented (A), Red Kidney Bean supplemented group (B) and Soya+Red Kidney Bean supplemented group (C) were considered to record antioxidant values of head and neck cancer patients. Objectives of the study are To evaluate variations in the levels of MDA (a marker of lipid peroxidation) and serum antioxidant (Enzymatic and non-enzymatic) status in cases with HNC.

To investigate whether Dietary protein supplementation with Soya, Red Kidney Bean and combination can improve biochemical status of enzymatic and non-enzymatic antioxidant response in cases with Head Neck Cancer.

Methods:

A total of 60 new cases with Head and Neck Cancer (Stage I and II) aged 30 to 60 years of both the sex were considered for the present study and the group consist of mainly rural and agricultural laborers. Consent was taken from the cases. The cases were divided into three groups. Group A, Group B, and Group C each group consist of 20 cases. A group was supplemented with Soya Bean, B group with Red Kidney Bean, and C group with Soya and Red Kidney Bean combination. Ethical clearance for this study was also obtained from the Institutional Ethics Committee. Consent was also taken from the HNSCCs.

Soya and Red kidney bean recipe (liquid form) intervention study was performed. The recipe had to be taken in addition to the balanced high protein semisolid diet consumed, two times a day for four months on selected head and neck cancer patients. 90% of the cases had hospital high protein diet was taken. Hospital diet includes rice, vegetable and green vegetable curry, two eggs, sambar, butter milk and two bananas will be supplying for the patients two times a day along with morning breakfast. Patients received the supplementation in the morning one glass (250ml) and evening one glass (250ml), the investigator used to visit the hospital every day.

Sample collection:
Blood samples (6 ml) were drawn before and after supplementation from patients 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 & the packed cells were washed 3 times with physiological saline. The separated plasma was stored at light tight conditions at -20°c until analysis.

The different biochemical parameters considered in the present study were carried out by the following standard methods.

i. Superoxide Dismutase assay: By the modified Spectrophotometric method of Kakkar et al. (1984).
ii. Total thiols: By the method of Hu (1994).
iii. Catalase assay: by the modified method of Sinha (1972).
iv. Malondialdehyde (MDA): by the method of Ohkawa, Ohishi and Yagi(1979).
v. Vitamin A assay: A manual of laboratory techniques. NIN 2003. Vitamin A. Plasma. Page no 158-159.
vi. Vitamin E assay: by the method of Bieri et al (1964).
vii. Vitamin C assay: A manual of laboratory techniques. NIN 2003. Ascorbic acid. Plasma. Page no 160-161.

Statistical analysis:
Statistical analysis was carried out by student's t-test. A value of $P<0.05$ was considered as statistically significant. Results are presented as mean ± Sds.

Results:
Invitro experimental studies revealed that oxidative DNA damage is the root cause of carcinogenesis (Evans et al. 2004). ROS are constantly generated endogenously as byproducts from cell metabolism and in response to outside factors from lifestyle and diet. Oxidative stress is said to occur in the cell, if ROS formed in amounts exceed the capacity of antioxidant defense system of the cell. It again results in oxidative protein damages, lipid peroxidation and DNA lesions.

A. Enzymatic Antioxidants: Table 1 shows data on the enzymatic antioxidant levels in HNC cases before and after supplementation of each type. The different enzymatic antioxidants considered in the present study include Superoxide Dismutase (SOD), Catalase, total thiols and lipid peroxidation indicator Melanoldihyde (MDA). Soya bean supplementation significantly enhanced the SOD, total thiols and MDA content in HNSCC patients. Red kidney bean supplementation showed significant positive response on total thiols and MDA only. The combined supplement (Soya + Red kidney bean) also resulted in the similar way. The antioxidant catalase enzyme was not affected by the supplementation type. Soya bean supplementation appears to be relatively better supplementation the Red kidney bean in promoting enzymatic antioxidants.

B. Non-enzymatic Antioxidants: Vitamin A, Vitamin E and Vitamin C were estimated in HNSCC cases before and after providing three types of supplementations. The soya bean supplementation exhibited significant positive effect on the three antioxidant vitamins considered in the experimentation (Table 2). The Red kidney bean supplementation has found to exert positive influence only on Vitamin E and no effect on Vitamin A and C. The combined supplement of soya bean and red kidney beans resembled soya bean effect on the three vitamins.

Discussion:
In the present study, the soya bean supplementation has significant positive effect on enzymatic and non-enzymatic antioxidants. It has been also observed that soya beans effect was better over red kidney bean supplement. Byun et al (2010) reported that Superoxide Dismutase activity was higher in the groups fed with yellow soya beans and black soya beans. They observed no significant difference in catalase activity of groups fed with different soya beans. The present study results agree with above said reports. Tu-F Lien et al (2009) studied the effect of soya bean on lipid peroxidation. They reported higher activity of antioxidant enzymes. Soya foods and Soya beans are also reported as resistant to oxidative modifications (Eugene et al. 2011). The plasma thiol groups break the peroxidative chain and allow the repair of oxidatively damaged molecules (Giacomo et al. 2003).

Red kidney beans contain relatively high levels of phenolics and flavonoids. These compounds are strong antioxidants and activate detoxification enzymes that destroy harmful Reactive Oxygen Species (ROS) by blocking carcinogens and suppressing malignant cells (Penningston. 2002; Luthria. 2005; Heimler et al. 2005). The ability of an antioxidant to neutralize free radicals is known as Oxygen Radical Absorbance Capacity (ORAC). Red kidney beans possess better total antioxidant capacity (TAC) and also a rich source of mineral manganese an essential cofactor in number of enzymes that produce energy and antioxidant defense system. The key oxidative enzyme SOD requires manganese an important trace mineral, which is abundant in red kidney beans (Khanna et al. 2005). Shen et al (2003) reported that bioactive constituents such as flavonoids in red kidney beans induce apoptosis in cancer cell lines and possess antitumor cytotoxic properties. Purna Mukhopadhyay et al (1999) also reported that serum Vitamin A and E cancer patients improved following Soya bean supplementation. Nacharava et al (2009) reported that UV-irradiation of red kidney bean seed enhanced tocopherol an important antioxidant that play protective role against different types of stress. Arosike et al (2008) reported that soya bean supplementation enhanced serum Vitamin C in the test group. Ascorbic acid is one of the strongest antioxidants and source of electron donor for many enzymatic and non-enzymatic reactions (Blokhina et al. 2003).

The research reports made by Horn-Ross and Morris (2001) and Pamela et al (2002) provided proof that soya bean consumption reduces the risk of thyroid cancer. The present study results conform with the above said reports in that the soya bean and red kidney bean supplementations improve the antioxidant enzyme systems in HNSCC cases by promoting the production of enzymatic and non-enzymatic antioxidants.

Conclusion:
In the present study, the soya bean supplementation has significant positive effect on enzymatic and non-enzymatic antioxidants. Byun et al (2010) reported that Superoxide Dismutase activity was higher in the groups fed with yellow soya beans and black soya beans. They observed no significant difference in catalase activity of groups fed with different soya beans. The present study results agree with above said reports. Tu-F Lien et al (2009) studied the effect of soya bean on lipid peroxidation. They reported higher activity of antioxidant enzymes. Soya foods and Soya beans are also reported as resistant to oxidative modifications (Eugene et al. 2011).

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In order to ensure best possible course of supportive care for HNC patients, more information on outcomes such as survival cognitive function, diet modification and intervention is highly useful in future.

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Conflicts of Interest:
The authors have none to declare.

Table 1. Enzymatic Antioxidants Measured (meanSD) in HNC cases under before and after dietary protein supplementation:

Enzymatic Antioxidant	**Level of Antioxidant in HNSCC**			
	Group A	Group B	Group C	
SOD U/mg protein	Before	2.021±0.350	1.914±0.24	1.72±0.40
	After	2.564±0.379	2.05±0.24	1.93±0.31
	t value	*4.71	1.85	1.81
Catalese U / m g protein	Before	2.31±0.31	2.25±0.24	2.22±0.35
	After	2.38±0.33	2.39±0.30	2.43±0.26
	t value	0.65	1.67	2.12
Total Thiols	Before	60.9±12.8	62.6±18.3	58.5±12.5
µmol/mL	After	113.4±23.4	90.5±30.2	110.8±32.3
	t value	*8.81	*3.54	*6.75
MDA	Before	3.08±0.63	2.93±0.64	2.92±0.50
nmoles/ml	After	1.79±0.63	1.75±0.52	1.65±0.44
	t value	*6.43	*6.34	*8.47
Significant at 5% level; A- Group: Soya Bean; B-Group: Red Kidney Bean; C-Group: Soya + Red Kidney Bean				

Table 2. Non-Enzymatic Antioxidants Measured (meanSD) in HNC cases under before and after dietary protein supplementation:

Non-Enzymatic Antioxidant	**Level of Antioxidant**			
	Group A	Group B	Group C	
Vitamin A	Before	20.35±3.35	19.04± 4.48	20.58±4.83
µg/dL	After	33.69±7.09	20.71± 5.08	28.81±4.90
	t value	*7.60	1.10	*5.35
Vitamin E	Before	3.62±1.85	3.05± 1.10	3.20±1.32
µg/mL	After	7.78±2.67	4.14± 1.63	5.69±2.08
	t value	*5.72	*2.48	*4.50
Vitamin C	Before	0.383±0.24	0.531± 0.344	0.510±0.285
mg/dL	After	1.125±0.306	0.685± 0.366	1.064±0.433
	t value	*8.52	1.37	*4.78
* Significant at 5% level. A- Group: Soya Bean; B-Group: Red Kidney Bean; C-Group: Soya + Red Kidney Bean				

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