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Research article

EVALUATION OF ANTIOXIDANTS AND LIPID PEROXIDATION STATUS AMONG STUDENTS

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ABSTRACT: The study aims to estimate the changes in the plasma levels of lipid peroxidation product malondialdehyde (MDA), non-enzymatic antioxidants: vitamin C and E and enzymatic antioxidant: superoxide dismutase (SOD). The population used were healthy students (100 male, 100 female); mean age 22.4 years, range 18-25 years. The level of lipid peroxidation was found to be significantly increased among the students which were inversely related to the level of antioxidants ($p<0.05$). Increased antioxidant levels show a multiple link between fruit and vegetable intake among the study group. Diminished antioxidant status disturbed oxidant-antioxidant balance alleviating oxidative stress state in less fruit and vegetable intake group. Therefore, the alterations in the level of antioxidants in blood plasma could be used as biomarkers for nutritional tribulations.

Keywords - Antioxidants, superoxide dismutase, lipid peroxidation, thiobarbituric acid, oxidative stress.

INTRODUCTION

Oxidative stress is an imbalance between pro-oxidants and/or free radicals on one hand, and anti-oxidizing systems on the other (Delibas *et al.*, 2002; Saito *et al.*, 2005; Berr *et al.,* 2004; De Leo *et al.*, 1998; Maier and Chan 2002; Veurink *et al.*, 2003). A pro-oxidant will promote damaging oxidative changes to important cellular constituents and this may, in turn, lead to dysfunction and chronic diseases such as aging, cancer, cardiovascular disease and diabetes (Buring and Hennekens 1997; Kaneto *et al.*,1999) Therefore body antioxidant capacity is an important factor for the prevention of many chronic diseases. Severe oxidative stress progressively leads to cell dysfunction and ultimately cell death. Oxidative stress results from generation of oxygen free radicals, hydrogen peroxide, hydroxyl radical, hydroperoxide, dioxygen and nitric oxide, collectively termed as reactive oxygen species (ROS).

ROS are highly reactive toward protein, lipids and DNA molecules causing damage to these macromolecules and possibly leading to dysfunction or death of the cell (Bourdel-Marchasson *et al.*, 2001 and Meydani, 2001). There are many intrinsic free radical scavenger systems, which involve enzymatic and non enzymatic reactions. One of the enzymatic antioxidant defense systems is copper-zinc super oxide dismutase (Cu-Zn SOD) that converts super oxide radicals to hydrogen peroxide (H_2O_2). Glutathione peroxidase (GSH-Px) and catalase (CAT) will then convert H_2O_2 to a water molecule. Cu-Zn SOD, GSH-Px, and CAT together provide the primary antioxidant defense mechanism (Panter and Scott, 1991; Bourdel-Marchasson *et al.*, 2001 ; Rinaldi *et al.*, 2003; Matés *et al.*, 1999).

The non-enzymatic antioxidant defense system includes ascorbic acid (vitamin C), α- tocopherol (vitamin E), glutathione (GSH), β-Carotene, and vitamin A. There is a balance between both the activities and intracellular levels of these antioxidants that are essential for the survival of organisms and their health (Panter and Scott, 1991; Meydani, 2001; Palmer and Burns, 1994; Sigalov and Stern, 1998; Ozcankaya and Delibas, 2002). It is known that the brain bears relatively low antioxidant protection, and also contains high levels of polyunsaturated fatty acids that make it prone to increased lipid peroxidation (Saito *et al.*, 2005; Aksenov *et al.*, 2001; Ohkawa, *et al.*, 1979).

We assessed the extent of oxidative stress, the activities of antioxidants and lipid peroxidation among students to notice the activities much more keenly.

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MATERIALS AND METHODS

Study Population -The study group consisted of two hundred students (100 male and 100 female); between 18-25 years of age group. General and dietary data were obtained through questionnaires from each student. In order to evaluate the nutritional status, the participants were given 15 statements which could be replied as "true" or "false". Informed consent was obtained from each participant before obtaining the blood sample. The procedures were in accordance with the ethical standards of the Helsinki Declaration of 1975**.**

Blood Sample -Five ml of venous blood was drawn from all participants. Samples were centrifuged at 4000 rpm and the plasma was separated and stored at -20 °C until analysis.

Determination of lipid peroxidation -Plasma MDA was measured according to the standard method (Ohkawa et al., 1979). MDA level was determined by thiobarbituric acid (TBA) reactive substances (TBARS) in plasma, based on reaction between MDA and TBA. Standard MDA solution (100 nmoles) in 5ml vol was processed along with test samples. 1.5ml of 0.8% of TBA was added to 1ml of serum sample. Then 0.4ml of 8.1% of SDS and 1.5ml of acetic acid were added. The mixture was finally made upto 5ml with distilled water and placed in hot water bath at 95ºC for 1 hr. After cooling 1ml of distilled water and 5ml of the mixture of n-butanol and pyridine (15:1,v/v) were added. The mixture was vortexed and after centrifugation at 4000 rpm for 10 min, the absorbance of the organic layer (upper layer) was read by the UV-vis spectrophotometer (Shimadzu) at 532nm against blank using distilled water. TBA when allowed to react with MDA aerobically formed a colored complex [MDA-$(TBA)^2$ complex] which was measured by the spectrophotometer.

Estimation of SOD-Estimation of plasma SOD was done by the method of Kakkar *et al.,* 1984. 1.35 ml of double distilled water, 50 µl of plasma, 1.2 ml of sodium pyrophosphate buffer (pH 8.3), 0.1 ml of phenazine methosulphate (PMS) and 0.3 ml of nitroblue tetrazolium (NBT) were mixed. 0.2 ml of NADH solution was added to it to initiate the reaction. After incubation at 39°C for 90 s the reaction was terminated by adding 1 ml of glacial acetic acid. 4 ml of *n* -butanol was added and the mixture was centrifuged at 4000 rpm for 10 min and the absorbance of the upper butanol layer recorded at 560 nm. For the comparison, corresponding blank was prepared in the same way except addition of the plasma. One unit of SOD was defined as that amount of enzyme that inhibits the rate of reactions by 50% under specified conditions.

Estimation of vitamin C - Ascorbic Acid level was estimated by the method of Omaye *et al.*, 1973. Ascorbic acid is oxidized by copper to form dehydro-ascorbic acid and diketoglutaric acid. These products when treated with 2,4 Dinitrophenyl hydrazine (DNPH) form the derivative, bis-2-4-dinitrophenylhydrazine which undergoes rearrangement to form a product with 1.0 ml of the plasma was mixed thoroughly with 1.0 ml of ice cold 10% TCA and centrifuged for 20 minutes at 3500xg. To 0.5ml of the supernatant, 0.1ml of DTC reagent was added and mixed well. The tubes were incubated at 37ºC for 3 hrs. 0.75 ml of ice cold 65% sulphuric acid was added and the tubes were allowed to stand at room temperature for an additional 30 minutes. A set of standards containing 10-50 mg of ascorbic acid was processed similarly along with a blank containing 0.5ml of 10%TCA. The colour developed was read at 520nm.

Estimation of vitamin E - Plasma α tocopherol was estimated by the method of Baker and Frank, 1968 . The method involves the reduction of Ferric ions to Ferrous ions by alpha tocopherol and the formation of a red coloured complex with 2,2" dipyridyl. Absorbance of the chromophore was measured at 520nm. 0.5ml of plasma, 2 ml of petroleum ether and1.6ml of ethanol were added, mixed and centrifuged. To the supernatant 0.2ml of 2,2" dipyridyl solution and 0.2ml of Ferric chloride solution were added, mixed well and kept in the dark for five minutes. An intense red colour was developed. 4 ml of water was added to all the tubes and mixed well. Standard alpha tocopherol in the range of 10 -100 µgms were taken and treated similarly along with a blank containing only the reagent. The colour in the aqueous layer was read at 520nm.

RESULTS AND DISCUSSION

General characteristics of study group were shown in Table 1. Participants were composed of 100 male and 100 female students. In general, the mean age was 22.4 years, while the mean age of females was 19.66±1.51 and the mean age of males was 21.1±2.26. The mean height and weight of the male students were 160.5±9.46, 64.39±8.82 and females were 156.53±4.03, 49.73±4.41 respectively. The majority of the students were non-vegetarians (64%), while others vegetarians (32%).

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Table 1: General Characteristics of the study group.

Parameters	Students	
	Male(n=100)	Female(n=100)
Age (18-25)	21.1±2.26	19.66±1.51
Height (150-183cms)	160.5±9.46	156.53±4.03
Weight (40-90)	64.39±8.82	49.73±4.41
Vegetarian	14	50
Non- Vegetarian	86	50

Many factors impact a person's food choice, from age to culture, from income level to tradition, from location of residence to health knowledge, and the list goes on (Willoughby and Laitner, 2000). The busy lifestyle of a college student, especially in regards to eating habits, is very much based on ritual and convenience. As such, college students' eating habits may include more convenience foods, fast food, and out-of-home dining lacking nutritional foods like fruits and vegetables. The putative beneficial effects of an increased consumption of fruit and vegetables have been associated with antioxidant nutrients.

Cellular non-enzymatic antioxidants are also known as free radical scavengers that protect a cell against toxic free radicals. Vitamin C and E is the chief constituent of the aqueous and lipid soluble environment. Therefore, decreased vitamin C and E may reflect a depletion of non-enzymatic antioxidant reserves. On the other hand, they play prominent role in the antioxidant defense system, and in the reactions of catalysis, regulation, electron transportation and in preserving the correct structure of proteins.

Lipid peroxidation is a unique mode of oxidative injury which is triggered and promoted by different radical and non-radical members of the reactive oxygen species (ROS) family or by the catalytic decomposition of preformed lipid hydroperoxides. Antioxidant enzymes such as SOD, CAT and GPx, are important for cellular protection due to their ability to detoxify free radicals, such as ROS (Cosper and Wakefield, 1975).

According to our results, we found decreased antioxidant level in students and increased lipid peroxidation which was statistically significant ($p<0.05$). The level of SOD was found to be increased when compared to the other antioxidants vitamin C and vitamin E which was highly reduced. On splitting our subjects with respect to gender, we found a significant change in the oxidant and antioxidant parameters. The level of Vit- C and Vit-E in male and female were decreased when compared to that of super oxide dismutase level in plasma which was highly increased in both sexes (Table 2).

People who eat fruits and vegetables have a lower risk of diseases and there is evidence that some types of vegetables, and fruits in general, protect against some cancers. Since fruits and vegetables happen to be good sources of antioxidants, this suggested that antioxidants might prevent some types of diseases (Stanner *et al.*, 2004).

The nutritional status between the students was analyzed based upon the intake of fruits and vegetables (Table 3). Obtained values were graded as good, moderate and poor. In our study the values for moderate fruit and vegetable intake group (MDA: 5.4±0.24; SOD: 7.1±0.75; Vit C: 4.16±0.16 and Vit E: 6.43±0.23), poor fruit and vegetable intake group (MDA: 6.23±0.11; SOD: 5.01±0.34; Vit C: 3.34±0.10; Vit E: 5.69±0.43) and good fruit and vegetable intake group (MDA: 3.21±0.22; SOD: 10.1±1.75; Vit C: 6.16±0.19; Vit E: 9.94±2.01) respectively. The difference in the nutritional status directly related to the alteration in the enzymatic and non enzymatic antioxidant levels among the students which was found to be statistically significant ($p<0.05$).

Table 2: Levels of lipid peroxidation and antioxidants in plasma of healthy students.

Students	Lipid Peroxidation (nm/ml)	SOD (u/ml)	Vitamin C (mg/dl)	Vitamin E (mg/dl)
Male (n=100)	5.4±0.24	7.1±0.75	4.16±0.19	7.03±0.23
Female (n=100)	6.92±0.30*	8.3±1.75	5.03±0.89*	8.94±2.01

*p<0.05

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Table 3: Antioxidants and lipid peroxidation level among students based on their nutritional status.

Nutritional status (based on fresh fruit and vegetable intake)	Lipid Peroxidation (nm/ml)	SOD (u/ml)	Vitamin C (mg/dl)	Vitamin E (mg/dl)
Good	3.21±0.22	10.1±1.75	6.16±0.19	9.94±2.01
Moderate	5.4±0.24*	7.1±0.75*	4.16±0.16*	7.03±0.23*
Poor	6.23±0.11*	5.01±0.34*	3.34±0.10*	5.69±0.43*

*$p<0.05$

The results of the present study showed the presence of oxidative stress among the poor nutritional intake group students. From the study intake of more fruits and vegetables are recommended to the students community to reduce the oxidative stress because consumption of fruits and vegetables was directly associated with level of household support for healthy eating (Gadjeva *et al.*, 2005).

CONCLUSION

Free radicals have been implicated in the etiology of major diseases. They can adversely alter many crucial biological molecules leading to loss to form and function. Such undesirable changes in the body can lead to diseased conditions. Antioxidants can protect against the damage induced by free radicals activity at various levels. Dietary and other components of plants form major source of antioxidant. The traditional Indian diet is rich sources of natural antioxidants which includes variety of fruits and vegetables. Students should integrate more fruits and vegetables into their diets so that oxidative stress can be minimized.

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