Correlation between oxidative stress and antioxidant defence in south indian urban vegetarians and non-vegetarians

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Abstract. – Background and Objectives: The diet is a key environmental factor implicated in health and disease. Oxidative stress, antioxidant status and their relation to diet is a subject of interest in recent years. The objective of the study was to compare lipid peroxidation and antioxidant status in healthy vegetarians and non-vegetarians.

Methods: The present study comprises 100 healthy individuals (50 vegetarians and 50 non-vegetarians) residing in Belgaum urban area. All the participants were in the age group of 40-60 years of both sexes. This cross-sectional study was done in one year period from April 2007 to March 2008. Malondialdehyde (MDA) (lipid peroxidation product) was estimated by thiobarbituric acid method, glutathione peroxidase by Beutler's method, Vitamin A and Vitamin E by Bessay et al and Quife et al methods respectively.

Results: Our study revealed that the blood MDA level was significantly increased (p value < 0.001) in non-vegetarians compared to lacto-vegetarians and lacto-ovo-vegetarians. There was significant decrease in the level of enzymatic antioxidant glutathione peroxidase and non-enzymatic antioxidants Vitamin A and Vitamin E in non-vegetarians compared to lacto-vegetarians and lacto-ovo-vegetarians (p value < 0.001).

Conclusions: Results of this investigation present study indicate that there was an increased lipid peroxidation and a low antioxidant status in non-vegetarians compared to vegetarians. Vegetarian nutrition provides adequate antioxidants which effectively prevent free radicals generation.

Key Words: Antioxidants, Lipid peroxidation, Oxidative Stress, Vegetarian nutrition.

Introduction

Oxygen and diet are playing a vital role in human life. Oxygen is vital to provide energy through numerous metabolic reactions. A small fraction of the oxygen is diverted to form reactive oxygen species [ROS] either accidentally or deliberately1.

In recent years, oxidative stress due to ROS is implicated in the pathogenesis of wide variety of diseases like cancer, cataract, diabetes mellitus, rheumatoid arthritis, atherosclerosis, viral autoimmune diseases and aging2.

The association of vegetarian diets with lower risk for several chronic diseases has been well documented. The reduced risk of diseases found among vegetarians suggests that biologic processes are influenced by diet3,4. Therefore, much attention is currently focused on the beneficial effect of vegetarian versus non-vegetarian diet3,5-7.

The present study was planned to correlate the oxidative stress and antioxidant status in healthy vegetarians and non-vegetarians.

Subjects and Methods

100 age and sex matched healthy individuals (vegetarians and non-vegetarians) residing in Belgaum urban area in the age group ranging from 40-60 years of both sexes, were included in the present study. Volunteers involved in the study group were selected randomly from Belgaum urban population. Volunteers were divided into 3 groups.
1. Lacto-vegetarians (n=25) vegetarians since birth but consuming milk and its products.
2. Lacto-ovo-vegetarians (n=25) vegetarians since birth but including dairy products and eggs.
3. Non-vegetarians (n=50) consuming animal products such as meat, poultry, fish and other sea foods at least twice a week.

Exclusion Criteria
Individuals suffering from hypertension, diabetes mellitus, coronary artery disease, hyperlipidemia or any other systemic diseases (liver and renal diseases). Smokers and alcoholics were also excluded.

8 ml of blood was drawn by venepuncture from vegetarians and nonvegetarians and was collected in heparinized tube (5 units/ml of blood) under aseptic precautions. Parameters were analyzed from whole blood, plasma and hemolysate. Malondialdehyde (MDA) was estimated by thiobarbituric acid method, glutathione peroxidase by Beutler’s E9 Vitamin A (Retinol) by Bessey et al method10 and vitamin E (α-tocopherol) by Quaife et al method11. Analysis were carried out within four hours and in case of enzyme assay it was done within one hour of collection of blood sample.

The study was approved by the Ethical and Research Committee of J.N. Medical College, Belgaum.

Statistical Analysis
Mean and standard deviation (SD) for each of the outcome variable were computed. Comparison of gender distribution among groups was done by Chi-square test. Comparison of mean MDA, glutathione peroxidase, vit. A and vit. E levels among groups by analysis of variance (ANOVA) followed by Tukey HSD multiple comparison test.

Results
Age distribution of the participants and malondialdehyde (MDA) and antioxidant levels in vegetarians and non-vegetarians are presented in Table I and II respectively.

There was a significant increase in MDA levels in non-vegetarians as compared to lacto-ovo-vegetarians and lacto-vegetarians (p value ≤0.001). A significant decrease of glutathione peroxidase (p value ≤0.001), vitamin A (p value 0.007) and vit. E (p value 0.025) levels were seen in non-vegetarians as compared to lacto-ovo-vegetarians and lacto-vegetarians.

There was no significant difference in the levels of MDA (p value 0.795), glutathione peroxidase (p value 0.994), vit. A (0.063) and vit. E (p value 0.997) between lacto-vegetarian and lacto-ovo-vegetarian groups.

Discussion
In the present study there was a significant increase of MDA in non-vegetarians compared to lacto-vegetarians and lacto-ovo-vegetarians. Our results are in accordance with the findings of Krajcovicova et al12, Manjari et al13, Nagyova et al14 who observed significantly increased levels of MDA in non-vegetarians compared to vegetarians. Dierckx et al15 showed that intakes of iron and copper are related to MDA levels. They suggested that transition metals in the diet have important role in the initiation and progression of lipid peroxidation. They concluded that lipid peroxidation was significantly increased in non-vegetarians as their diet is good source of iron and copper. The findings in this investigation are in contradictory with the study of Szeto et al16 who shown that there is no change of MDA levels between lacto-vegetarian and lacto-ovo-vegetarian groups.

Glutathione Peroxidase:
It is a seleno-protein enzymatic antioxidant that removes H2O2 and organic hydroperoxides. In our study there was significant increase in glutathione peroxidase levels in vegetarians compared to non-vegetarians. Our findings are contradictory to Kováciková et al17, Bonnie Bruce et al18 who showed that activity of selenium dependent glutathione peroxidase was low in plasma and erythrocytes of vegetarians. They suggested it may be due to low dietary selenium intake/bioavailability in vegetarians which leads to low glutathione peroxidase activity. This may be due to higher content of phytic acid in vege-

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Table I. Age distribution. Data are expressed as means ± SD.

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<tr>
<th>Subject category</th>
<th>Years</th>
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<tr>
<td>Lacto-vegetarians (n=25)</td>
<td>44.20 ± 6.95</td>
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<tr>
<td>Lacto-ovo-vegetarians (n=25)</td>
<td>43.60 ± 7.53</td>
</tr>
<tr>
<td>Non-vegetarians (n=50)</td>
<td>46.38 ± 6.34</td>
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tarian diet which is a well known inhibitor of mineral absorption. Manjari et al, Haldar et al, Rauma et al have found that there was no significant difference in the activity of glutathione peroxidase between vegetarians and non-vegetarians.

Vitamin E: (α-Tocopherol)

It is a fat soluble vitamin which converts $O_2^\cdot-$ and $'OH$ and lipid peroxy radicals to less reactive forms and acts as a chain breaking antioxidant. In the present report plasma concentration of vitamin E was significantly decreased in non-vegetarians compared to lacto-vegetarians and lacto-ovo-vegetarians due to higher intake of vegetable oils e.g. cotton seed oil and sunflower oils.

Our findings are in accordance with Krajcovicová et al, Rauma et al, Nagyova et al and Millet et al who found significantly increased Vitamin E levels in vegetarians compared to non-vegetarians. They also found that serum vitamin E concentration was strongly associated with blood lipids. They suggested that reduced level of α-tocopherol in nonvegetarians may be due to enhanced lipid peroxidation and increased utilization of vit E. Rauma et al and Bruce et al found that vegetarian diet provided more than twice the amount of vitamin E than the non-vegetarian diet. This may be due to higher consumption of nuts and seed oils by vegetarians. They also suggested that flavonoids which occur naturally in fruits, vegetables and in some beverages such as tea might have reduced oxidative stress in vegetarians. Haldar et al found that there was no significant difference in the levels of α-tocopherol between vegetarians and non-vegetarians. Our results are contradictory to Szeto et al and Pronczuk et al who found that plasma α-tocopherol concentrations were lower in vegetarians compared to non-vegetarians.

Vitamin A

Is a fat soluble vitamin that scavenges $O_2^\cdot-$ and reacts directly with peroxy radical. In our study the plasma vitamin-A levels were significantly increased in lacto-vegetarians and lacto-ovo-vegetarians compared to non-vegetarians may be due to higher intake of carrot, fruits and vegetables.

Our results are in accordance with findings of Krajcovícová et al, Rauma et al, Ingrid Kiefer et al and Millet et al who found higher serum β-carotene concentration in vegetarians compared to non-vegetarians. They suggested that it may be due to higher dietary β-carotene intake in vegetarian diet and action of β-carotene in presence of other food factors such as non-nutritive phytochemicals could have played a role. They proposed that additive and synergistic effects of phytochemicals in fruit and vegetables are responsible for their antioxidant activity. Haldar et al have found that overall antioxidant status was similar between vegetarians and non-vegetarians. They also found that total intake of fruits and vegetable was positively associated with increased plasma levels of carotenoids.

Conclusions

The results of this investigation indicate that there is an increased lipid peroxidation in non-vegetarians compared to vegetarians, as evidenced by increased malondialdehyde (MDA) and low antioxidant status as evidenced by decreased levels of enzymatic and non-enzymatic antioxidants i.e. glutathione peroxidase, vitamin A and vitamin E levels. This may be due to higher and regular consumption of fruit and vegetables, dark and whole grain products, grain sprouts, plant oils and oil seeds rich in

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<th>Subject category</th>
<th>Malondialdehyde (MDA) nmol/ml</th>
<th>Glutathione peroxidase IU/g of Hb</th>
<th>Vitamin A [µg/dl]</th>
<th>Vitamin E [mg/dl]</th>
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<tr>
<td>Lacto-vegetarians n-25</td>
<td>3.76 ± 1.57</td>
<td>19.70 ± 2.60</td>
<td>35.61 ± 7.91</td>
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<td>Lacto-ovo-vegetarians n-25</td>
<td>3.97 ± 1.28</td>
<td>19.79 ± 2.62</td>
<td>34.36 ± 5.79</td>
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<td>Non-vegetarians n-50</td>
<td>7.29 ± 0.86</td>
<td>13.21 ± 3.11</td>
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ANOVA

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<td>105.301</td>
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<td>&lt; 0.001</td>
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<tr>
<td>5.645</td>
<td>= 0.005</td>
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<tr>
<td>5.440</td>
<td>= 0.006</td>
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trace elements like zinc, copper and selenium, mono and polyunsaturated fatty acids, antioxidant vitamins, fibers, complex carbohydrates and flavonoids by vegetarians. Results clearly indicate that vegetarian nutrition provides adequate antioxidants which effectively prevent free radical generation and thus responsible for better antioxidant status.

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References