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ORIGINAL ARTICLE

Comparison of Serum Levels of Vitamins E and C and Dietary Antioxidants Intakes between Patients with Inflammatory Bowel Disease and Healthy Subjects

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Inflammatory bowel disease (IBD) is a chronic gastrointestinal inflammatory disease of unknown etiology. Nutrition has been proposed as an important factor in etiology of IBD. The aim of this study was to compare dietary intake of antioxidants and serum levels of α -tocopherol and vitamin C in IBD patients with that of healthy subjects. Materials and Methods: Twenty-six IBD and twenty-eight sex and age matched healthy subjects were selected as the case and control groups from gastroenterology and other wards respectively, during the fall and winter of 2005. Subjects' serum vitamin C and alpha-tocopherol levels were analyzed using the HPLC method. Semi-quantitative food frequency questionnaires were completed by all subjects during interviews. Results: There was no significant difference in serum levels of vitamin C and α-tocopherol between the two study groups. Intakes of whole grain bread and fresh vegetables in healthy subjects were 2.5 and 1.5 times higher than those of patients, respectively ($p<0.05$). Although patients consumed fewer cups of tea daily ($p<0.05$), no significant difference was seen in the intakes of other dietary antioxidant sources between the two groups. Conclusion: Results of this study indicated that although there was no significant difference between vitamin C and α-tocopherol serum levels in the both groups, dietary intake of the main antioxidant sources in IBD patients was lower than healthy matched subjects. More advanced studies with higher number of subjects are needed to explore the antioxidant status in such patients.

Key Words: Inflammatory bowel disease, α-tocopherol, Vitamin C, Dietary antioxidants

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Introduction

Although the etiology of inflammatory bowel disease (IBD) has not been fully elucidated, the current concept of pathogenesis involves the interactions between immunologic abnormalities, genetic influences, and environmental agents.[1,2] Among the manifestations of IBD are increased inflammation and coagulability, impaired cellular membrane function, exaggerated nitric oxide production and impaired short-chain fatty acid production.[3] IBD is often associated with significant nutritional disturbances, such as protein-calorie malnutrition and vitamin and trace element deficits.[4]

In recent years, increasing attention has been given to the role of reactive oxygen metabolites in the pathogenesis of inflammatory bowel disease.[5-10] During the cascade of immunological events producing intestinal inflammation, activated neutrophils infiltrate the intestinal wall and produce reactive oxygen species (ROS).[11,12] These molecules are highly reactive and can induce tissue damage by attacking any cell component, thereby compromising cell integrity and function.[13] For example, the ROS can damage the cell membrane by attacking the double bonds in polyunsaturated fatty acids (PUFA), inducing lipid peroxidation[14] and more oxidative damage.[15,16] ROS can also enhance intestinal inflammation by increasing vascular and mucosal permeability and by promoting neutrophil adherence.[17]

Vitamin C, an important water-soluble antioxidant in biological fluids,[18,19] readily scavenges reactive oxygen and nitrogen species, such as superoxideand hydroperoxyl radicals, aqueous peroxyl radicals, singlet oxygen, ozone, peroxynitrite, nitrogen dioxide, nitroxideradicals, and hypochlorous acid,[20] thereby effectively protecting other substrates from oxidative damage. Vitamin C can also act as a co antioxidant by regenerating α-tocopherol (vitamin E) from the α-tocopheroxyl radical, produced via scavenging of lipid-soluble radicals.[21,22] This is a potentially important function because in vitro experiments have shown that α-tocopherol can act as a prooxidant in the absence of coantioxidants such as vitamin C.[21,22]

Examination of antioxidant concentrations in patients with diseases such as IBD may be important because deficiency of circulating antioxidants may predispose them to more severe oxidant tissue injury,[23] and may indicate ongoing oxidant stress.[24] Nutritional or other means of improving antioxidant status, therefore, could be of potential therapeutic value.[25] In this regard, some studies have reported low concentrations of circulating antioxidant vitamins in adults with IBD.[26,27]

This study hence aimed at evaluating vitamin C and vitamin E plasma levels and dietary intake of the main antioxidant sources (such as fresh vegetables, legumes, salad, whole grain breads and tea) in patients with chronic inflammatory IBD.

Materials and methods

This study was conducted during the fall and winter of 2005. Over a period of three months,twenty six patients,24 with ulcerative colitis (UC) and 2 with Crohn's disease (CD) were recruited from patients hospitalized in the gastroenterology ward of the University Golestan Medical Center, Ahvaz. Patients were diagnosed by an attending gastroenterologist (EH) using clinical, radiological, endoscopic, and histophatological findings. IBD patients were excluded from the study if they were smokers, pregnant, had known liver disease, or taking any micronutrient and herbal supplements. Medications prescribed were also recorded and matched.

Healthy individuals, who had no signs or symptoms of any specific disease and/or any illness history in active or remission stage of disease, made up the control group, selected from among the patients' relatives, visiting people and other healthy sex– and age-matched subjects who had referred to the hospital. All subjects gave their informed consent before participating in the study. Study protocol was approved by the University Medical Ethics Committee.

Fasting blood samples were collected and centrifuged at room temperature (18-24 ºC) and serums were prepared and stored at -20 ºC until further analyses. After defrosting, samples were prepared for determining α-tocopherol and ascorbic acid concentrations by a high performance liquid chromatography (HPLC) apparatus. All subjects completed the food frequency questionnaires, describing the frequency and number of portion size of each food item. Subject's weights and heights were measured using a platform digital scale (Seca, Germany) and non-stretchable wall me-

ter, respectively. For determination of α-tocopherol serum levels a reverse phase high pressure mi ×inj HPLC system (Jasco; Japan) with the following conditions was used: *Column:* Fire pack SIL-5 4.6 × 250mm. *Mobile phase:* n-hexane: 2-propanol(99.5:0.5). *Flow rate:* 1mL/min. Fluorescence detection (Ex.λ290nm–Em.λ330nm).

Method Validation: Recovery (accuracy) was 99-103٪ with R^2=0.9989 (linearity) and a precision of C.V< 3.26 (intra- and inter-day assays); the same instrument as above was used for determination of serum vitamin C levels. Chromatographic conditions were as follows:

Column: Eurosohere-100 C18 4 ×125 5μm particle size made by Knauver; Germany. *Mobile phase:* 0.1 M KH2 PO4, PH 7.8: Methanol(16:3) with flow rate of 0.8mL/min. Fluorescence detection was (Ex.λ355nm_Em λ 425nm) (pre-column derivation: with 1, 2-phenelendiamine). *Method validation:* recovery (accuracy) was 98-105٪ with R^2=0.9995 (linearity), and precision of C.V<3.7 (intra- and inter- day assays).

In order to perform statistical analysis, two tailed t and chi square tests were used for two-group comparison and stratified variables, respectively and statistical soft-ware was SPSS version #11.5 and P values <0.05 were considered statistically significant.

Study protocol was approved by the Ahvaz University Medical Ethics Committee. All subjects gave written consent.

Results

Table 1 demonstrates the basic characteristics and serum concentrations of vitamin C and α-tocopherol of both patients and controls. There were no significant differences in age, sex, vitamin C and α-tocopherol levels between the study groups; however, patients had lower mean BMI, compared to the patients (p<0.004). In Table 2, daily consumption of whole grain breads and fresh vegetables, good dietary antioxidants sources like lignans and carotenoids, was more common in controls than in patients (67٪ vs. 14٪, p<0.001; 70٪ vs. 22٪, p<0.04, respectively). On the other hand, more patients included legumes in their meals, on a weekly basis (p<0.001). Regarding consumption of fruits, no significant diffe-rences between patients and controls was observed. However, the amount of tea consumed daily among controls, as a rich source of polyphenols antioxidants, was about three fold that consumed in patients (p<0.05).

Table1. Basic characteristics and serum vitamin C and α-tocopherol levels of study groups*.

Variables	Patients	Controls
Age (yr)	33±14	33±8
Height (cm)	169±8	167±6
BMI (Kg/m^2)	22±4	26±4†
Sex (%M)	46	53
Serum vitamin C(μmol/L)	21.0±14.4	29.6±19.3
Setum α-tocopherol (μmol/L)	19±7	22±9

*values are mean± SD, † P<0.01

Table 2. Consumption of the main dietary antioxidant sources in study groups

Food groups	Servings	Patients Number (%)	Controls Number (%)
Fresh vegetables	1-2/D	5(22)	12(70)*
Fruits	1-2/D	10(45)	13(76)
Whole grain breads	1-2/D	3(14)	11(67)*
Legumes	3-5/wk	10(45)	5(18)†
Tea	1-3 cup/d	4(17)	14(50)*

* P<0.05, † P<0.01

Discussion

This study addresses the status of serum levels of vitamin C and α-tocopherol, as the most important antioxidant defenses in the human body, and the intakes of the main dietary antioxidant sources in patients with IBD. Serum levels of vitamin C and α-tocopherol of IBD patients were compared with those of healthy subjects and no significant difference between two study groups

was observed. Our patients showed no significant differences in their basic criteria compared with their matched controls, except for the mean body mass index, which could be, in part, due to the lower body weights of patients.

In CD, several mechanisms such as malabsorption and reduced oral intake can lead to decrease in antioxidant defense; another mechanism may be a sustained production of ROS from a chronic intestinal inflammation leading to an increased requirement for antioxidant micronutrients.[10,27] In the present study, the serum level of vitamin C was decreased, a finding similar to those of other studies documenting depleted levels of vitamin C in CD patients;[29-31] However, no statistically significant difference in these levels between our patients and controls was observed, similar to the finding reported by Kuroki et al.[26] Cox et al, on the other hand, reported vitamin C levels as being well correlated with the progression of the disease;[32] although there is no known explanation for this discrepancy, there is the possibility that our IBD patients had a less severe form of the disease than those in Cox's study. Vitamin C status seems to remain unchanged until the disease has severely progressed.[26] Another explanation for this finding could be due to the fact that majority of our patients had been diagnosed with UC, a condition in which limited findings are available on the antioxidant status. It needs to be mentioned that most IBD patients referring to gastroenterologists here are diagnosed with UC and according to a study by O'Keefe, 18-62 percent of these patients have nutritional deficiencies.[33]

Few studies have evaluated the serum α-tocopherol in patients with IBD, in whom decreased α-tocopherol levels are reported.[25,34-36] We found no significant difference in the serum concentrations of α-tocopherol between IBD patients and healthy controls, in agreement with the results of Hengstermann et. al.,[37] who reported similar serum α-tocopherol levels in both active and inactive IBD patients and their matched controls.

Our results showed that consumption of the main dietary antioxidant sources such as vegetables, tea (rich sources of catechins flavonoids) and whole grain breads (as good sources of lignins) in IBD patients were lower than in the control group, a finding supported by the reports of Mayberry et al.,[38] Thornton et al.[39] and Panza et al.[40] Moreover, fruits and vegetables are good sources of potassium, magnesium, vitamin C, fiber and also water. Thus to generalize, these findings indicate that low residue foods are regarded as contributing factors, and high fruit, vegetable, and fiber diets as protective factors.[41] However, it must be kept in mind that vitamin C, potassium, magnesium, and fiber are not exclusively limited to fruits and vegetables. Although the consumption of fruits among controls was 1.6 higher than patients, it was statistically insignificant, which may also be due to the number of subjects compared. Furthermore, the finding that intakes of fibers and/or fruits and vegetables were lower in IBD patients, could well be a response to the disease rather than an etiological factor. For example, patients may choose or be advised to follow a low fiber diet and consequently avoid fruits and vegetables.[42]

The lack of significant difference findings in vitamin C and α-tocopherol serum levels between IBD patients and healthy controls, in contrast to others may, in part, be due to number of subjects, severity of disease or duration of the study.

To conclude, the results of this study indicate that there is no significant difference in vitamin C and α-tocopherol serum levels between IBD patients and healthy controls. Further studies with more number of subjects evaluating tissue and WBC levels of vitamin C and α-tocopherol are needed to explore the status of such nutrients in IBD patients.

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