ASSESSMENT OF ANTIOXIDANT NUTRIENT INTAKE AND MALONDIADEHYDE PLASMA LEVEL IN RHEUMATOID ARTHRITIS

Yousef Shaabani(1), Maryam Foroughi(2), Reza Rastmanesh(3), Ahmadreza Jamshidi(4), Narges Tajik(2), Omid Assadi(5)

Abstract

BACKGROUND: Elevated free radical generation in inflamed joints and impaired antioxidant systems have been implicated in rheumatoid arthritis (RA). The present study was performed to evaluate dietary nutrient intake and plasma oxidant status in RA patients.

METHODS: This case-control study comprised 75 RA patients and equal number of age- and gender-matched controls. Nutrient intake was estimated by using a semi-quantitative food frequency questionnaire. Blood samples were obtained from each group, and as an indicator of oxidant status, plasma concentrations of malondiadehyde (MDA) were measured.

RESULTS: The mean calorie intake of RA patients was lower than that of the healthy controls. Energy-adjusted intake of fat, vitamin A and β-carotene were significantly lower in patients than in control . Plasma MDA concentration was significantly higher in RA patients than in controls (4.9±1.8 vs 2.1±0.6 nmoles/ml respectively, P < 0.01).

CONCLUSION: These results suggest proper antioxidant nutrient intake management may reduce free radical generation and improve antioxidant status in RA patients.

Keywords: rheumatoid arthritis, antioxidant, malondialdehyde.

Introduction

Rheumatoid arthritis (RA) is a chronic syndrome of unknown etiology and is characterized by non-specific inflammation of the peripheral joints with joint swelling, morning stiffness, destruction of articular tissues and joint deformities. It affects nearly 1% of the population worldwide. Studies have indicated that the development of RA is partly related to the excess production of reactive oxygen species and a lowered ability to remove oxidative stress. A recent study indicated that pro-inflammatory cytokines such as IL-1ß and TNF-α are involved in the formation of toxic peroxynitrite by increasing the activity of nitric oxide synthase.

Clinical evidence has suggested oxidative stress is elevated in RA patients. Plasma malondialdehyde, a degradation product of lipid peroxidation, level was significantly higher in the synovial fluid and serum of RA patients than that of control subjects.

Also, in children with juvenile rheumatoid arthritis, plasma and red blood cell alpha-tocopherol concentrations were lower compared to those of healthy children. In a Finnish cohort study, low Alpha-tocopherol status was suggested as a risk factor for RA. RA patients show not only low levels of antioxidants in the blood, but altered activity of blood antioxidant enzymes including glutathione peroxidase (GPx), CuZn superoxide dismutase (SOD) and catalase, although study results are not consistent.

Based on previous reports, diets high in major dietary antioxidants such as vitamin E, vitamin C, β-carotene and phenolic compounds have been suggested to alleviate RA symptoms, possibly by reducing disease-related oxidative stress. However, few studies have been conducted to evaluate the nutrient intake in RA patients. The objective of this study was to evaluate dietary intake of major nutrients including antioxidants and measure plasma oxidant status in rheumatoid arthritis patients and their age, gender-matched controls.
Materials and Methods

Subjects
Rheumatoid arthritis patients (n = 75) and age, gender-matched healthy controls (n = 74) were recruited into the study. All patients fulfilled the revised American College of Rheumatology (ACR) criteria for RA.12 The study was approved by the Ethics Committee of Shaheed Beheshti University of medical sciences (SBMU), and informed consent was obtained from all subjects. The patients were chosen for the study after having a preliminary evaluation consisting of a brief medical history and physical examinations. Patients with any history of liver diseases, diabetes mellitus, respiratory disorders and cardiovascular diseases were not included in the study.

Estimation of Nutrient Intake
We used an interviewer-administered semi-quantitative food frequency questionnaire to estimate nutrient intake of the subjects. The questionnaire included a list of 66 food items. Selection criteria were most frequently consumed food items, food items consumed in greatest amounts, and major food items supplying each nutrient, especially antioxidant vitamins. The selection was based on the latest National Health and Nutrition Survey Report of NNFTRI (national nutrition and food technology research institute). Selected food items were categorized according to food groups and subdivided by food preparation methods, nutrient content and portion sizes. Categories and numbers of food items in each category were vegetables-22, cereals and starches-7, fruits-10, legumes-6, meats-6, fishes & other seafoods-2, eggs-1, milk and dairy products-6, oils-2, hot beverages & soft drinks-4. Subjects were asked to state the average frequency of consumption of each food item according to the categories of frequency, none to three times a day. The portion sizes were set as follows: a 1/2 serving size, a serving size, and a 1.5 serving size. The interviewer showed the photographs of the standard serving size, and asked the subjects to refer to those portions when selecting the amount of food consumed. The food frequency questionnaire was coded and analyzed for nutrient intake by NUTRITIONIST 4 (N4) food analyzer.

Malondiadehyde analysis
Fasting blood samples were taken from the patients and controls. Then, the blood samples were collected in heparin-containing tubes (15 IU/cc) and left at room temperature for one hour. The samples were centrifuged for 15 minutes on 3000 g. Plasma malondiadehyde concentrations were determined by a colorimetric assay using CayMan kit (MDA-586, Oxis International, Foster City, CA, USA), according to manufacturer's instructions. CV was < 6%.

Statistical Analysis
The results are given as mean ± SEM values. The significance of the mean difference between groups was assessed by the Student’s t-test.

Results

Demographic Characteristics
Table 1 shows the demographic characteristics of RA patients and their age-gender matched controls. The mean weight and body mass index of the RA patients were not significantly different from those of their controls.

Table 1. Demographic Characteristics of Patients with RA and their Age-Gender Matched Controls.

<table>
<thead>
<tr>
<th></th>
<th>RA (n = 75)</th>
<th>Control (n = 75)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47 ± 10</td>
<td>46 ± 13</td>
<td>N.S</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>66 (88%)</td>
<td>66 (88%)</td>
<td>N.S</td>
</tr>
<tr>
<td>Male</td>
<td>9 (12%)</td>
<td>9 (12%)</td>
<td>N.S</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.6 ± 11</td>
<td>75.4 ± 12.7</td>
<td>N.S</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>28.4 ± 3.4</td>
<td>29 ± 2.9</td>
<td>N.S</td>
</tr>
</tbody>
</table>

1 Values are mean ± SEM.
2 Not significant
3 (no. of subjects/total no. of subjects in the group) x 100

Nutrient Intake of Study Subjects
The daily nutrient intake is shown in Table 2. The mean energy intake of the RA patients was significantly lower than that of the control subjects. Also, RA patients consume less carbohydrate, protein, fat, vitamin A, vitamin E and β-carotene than control subjects. Since the patients had less calorie intake, the mean intake of each nutrient was adjusted for calorie consumption to compare net nutrient intake between the two groups (Table 3). Results indicate that intake of fat, protein, vitamin A, vitamin E and β-carotene per 1,000 kcal in RA patients was significantly lower compared to that of the control subjects. The mean intake of vitamin A, vitamin E and β-carotene, major antioxidant nutrients, in the patients was 46.6%, 38.7%, and 32.9% of the intake in control subjects, respectively.
Table 2. Daily Intake of Energy and Nutrient Assessed by the Semi-Quantitative Food Frequency Questionnaire

<table>
<thead>
<tr>
<th>Groups</th>
<th>RA (n = 75)</th>
<th>Control (n = 75)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calorie (kcal)</td>
<td>1325 ± 186</td>
<td>1875 ± 204</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>39.4 ± 0.87</td>
<td>68.7 ± 0.43</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>41.2 ± 13.3</td>
<td>59.6 ± 9.8</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Carbohydrate(g)</td>
<td>195.7 ± 2.3</td>
<td>265.8 ± 5.9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Vit A (µg RE)</td>
<td>252.4 ± 35.18</td>
<td>751.7 ± 15.6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Vit E (mg)</td>
<td>2.6 ± 1.2</td>
<td>8.3 ± 1.9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>ß-Carotene (µg)</td>
<td>168.2 ± 25.4</td>
<td>482.5 ± 70.4</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

Table 3. Daily Nutrients Intake per 1000 kcal Calorie Intake Assessed by the Semi-Quantitative Food Frequency Questionnaire

<table>
<thead>
<tr>
<th>Groups</th>
<th>RA (n = 75)</th>
<th>Control (n = 75)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calorie (kcal)</td>
<td>1000</td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>Protein (g)</td>
<td>30.7 ± 0.52</td>
<td>39.4 ± 0.28</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>28.69 ± 9.7</td>
<td>38.16 ± 5.1</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Carbohydrate(g)</td>
<td>153.2 ± 1.4</td>
<td>148.37 ± 3.5</td>
<td>N.S.</td>
</tr>
<tr>
<td>Vit A (µg RE)</td>
<td>196.8 ± 24.9</td>
<td>422.36 ± 7.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Vit E (mg)</td>
<td>1.9 ± 1.4</td>
<td>4.9 ± 0.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ß-Carotene (µg)</td>
<td>124.6 ± 27.2</td>
<td>378.1 ± 35.7</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

Plasma Malondialdehyde
Plasma MDA concentration was significantly higher in RA patients than in controls (4.9 ± 1.8 vs 2.1 ± 0.6 nmoles/ml, respectively, P < 0.01).

Discussion
The present study was performed to evaluate nutrient intake especially of antioxidants in patients with RA and to assess oxidative stress markers in the blood. Etiopathogenesis of RA still remains obscure despite extensive research. Several different pathways can lead to increased formation of reactive oxygen species in inflamed joints. This enhanced oxidation plays a significant role in the tissue damage and inflammation perpetuating process in rheumatoid symposium. Oxygen free radicals lead to lipid peroxidation, which is defined as the oxidative deterioration of unsaturated fatty acids. Lipid peroxidation is thus a process which is determined by the peroxide forming mechanisms and peroxide removing antioxidants. Although lipid peroxidation affects many cellular components, the primary site involves membrane associated polyunsaturated fatty acids and protein thiols. During health, when reactive oxygen species production is low, lipid peroxidation is inhibited by the combined activities of various antioxidants present in the plasma. The failure of antioxidant defense mechanism to keep pace with oxidant generation may either be due to decrease in antioxidant defense or increased generation of oxidants as is the case in Rheumatoid arthritis. From the literature reviewed, it is apparent that patients of RA are exposed to oxidative stress and are more prone to lipid peroxidation. Also, recent investigations have consistently indicated that the inflammatory process produces oxygen radicals and decreased antioxidant levels, which may worsen the symptoms of the rheumatoid arthritis.

Plasma malondialdehyde level, a degradation product of lipid peroxidation, were found to be significantly (P < 0.05) elevated in the patients with RA compared to controls. In a study conducted by Kalavacherla et al., the concentrations of plasma MDA in cases of RA were significantly higher than the concentrations estimated in controls. Gambhir et al. reported markedly increased concentrations of MDA in patients as compared to controls. Similar results have been observed in this report and by others. Increased serum MDA concentrations in RA suggest the role of free radicals in the pathogenesis of this inflammatory disease. However, few studies have been conducted to examine antioxidant nutrient intake of patients.

Results from this study indicate that daily intake of total vitamin A and ß-carotene was significantly lower in the patients compared to that of the controls. The consumption of major macronutrients and total calories was also lower in patients although only fat and protein intake showed significance after calorie adjustment was made. Roubenoff et al. showed protein-energy malnutrition (PEM) among RA patients. The increased production of cytokines is known to induce anorexia in cancer patients. Therefore, the increased production of inflammatory cytokines may be a possible cause of PEM in RA patients.

A number of studies have indicated that the blood markers of antioxidant nutrient status in RA patients are significantly lower than those of controls. To the present, the decreased antioxidant status of RA patients has been explained by excessive need for antioxidants due to the inflammatory process itself. However, results from this study imply that the decreased antioxidant nutrient intake of RA patients is another possible contributing factor to decreased antioxidant status. A study conducted by Morgan et al. also indicated that the antioxidant nutrient intake and plasma levels are not optimal in RA patients. Stone et al. reported calcium, folic acid, vitamin E, zinc and selenium intake did not meet RDI in an observational...
study of forty-eight patients. However, nutrient intake of juvenile arthritis patients was not different from that of their healthy counterparts. 28

Also, previous investigations indicated that plasma markers of antioxidant status in RA patients are poor. Although definite evidence for the cause-effect of antioxidant levels in RA is not available, Araujo et al. 29 implied a decreased level of vitamin E is a possible cause of disease development. Also, serum concentrations of α-tocopherol, retinal and β-carotene were suggested as possible risk factors for developing RA in a 15-year follow-up study conducted by Comstock et al. 30 Shabani et al showed vitamin E and omega-3 fatty acids supplementation (100 mg/day- 1/2 g/day, respectively) decreased plasma MDA level compared with omega-3 alone. 31 In another study, Edmonds et al. 32 showed vitamin E supplementation (600 mg/day) improved clinical symptoms of RA patients. A possible mechanism by which vitamin E alleviated RA symptoms is reduced formation of prostaglandins, major molecules produced during the inflammation process. 33

It is not possible to conclude from this study that the increased levels of plasma MDA are due to either the lower antioxidant nutrient intake or the active inflammatory disease itself. Mechanistic studies on the relationship among oxidative stress, antioxidant defense and RA development will give better insights into a cause-effect relationship. Also, a large-scale cohort study is required to define the role of antioxidants in RA management. Nevertheless, this study indicates that proper antioxidant nutrient intake management may be important in alleviating RA symptoms.

References


