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ORIGINAL ARTICLE

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Impaired antioxidant status and decreased dietary intake of antioxidants in patients with systemic lupus erythematosus

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Abstract *Objective* This study compared plasma antioxidant/oxidant status and the dietary nutrient intake of patients with systemic lupus erythematosus (SLE) to those of healthy controls. *Patients and methods* The study included 97 consecutive patients with SLE and 97 age- and sex-matched healthy controls. Blood samples from 19 patients and 19 controls were subjected to analyses of plasma concentrations of α-tocopherol and malone dialdehyde and the activities of superoxide dismutase and glutathione peroxidase in the plasma. All patients and controls were interviewed using a semi-quantitative food-frequency questionnaire. *Results* The plasma α-tocopherol concentration was lower in patients, but this difference was not statistically significant. Plasma superoxide dismutase and glutathione peroxidase activities were significantly lower in patients than in controls, and the plasma malone dialdehyde level was significantly higher in patients than in controls. The intake of all major dietary antioxidants, including vitamin A, β-carotene, and vitamin C, but not retinol, was lower in patients than in controls, but this difference was significant only for vitamin A and β-carotene. *Conclusions* These results show that plasma antioxidant status is impaired and dietary antioxidant intake is decreased in patients with SLE.

Keywords Systemic lupus erythematosus · Antioxidant · Diet · Nutrition · Tocopherol

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Introduction

Systemic lupus erythematosus (SLE) is a prototypical chronic, relapsing, inflammatory, and complex multisystem rheumatic disease. The cause of SLE remains unclear, but it is generally accepted that it is an autoimmune disease associated with autoantibodies, immune complexes, and defective cellular and humoral immunity which is probably caused by genetic, environmental (e.g., viral, dietary, UV light), and hormonal influences. Oxidative stress and the related biological damage have been proposed to be critically involved in the development and maintenance of rheumatoid arthritis (RA) in human and animal models [1, 2, 3, 4]. The few studies that have investigated antioxidants in patients with SLE [4, 5, 6, 7] have suggested that damage by free oxygen radicals play a role in SLE. The finding in one prospective case-control study of a lowered antioxidant status prior to the recognition of SLE showed that oxidative damage might precede the onset of definitive SLE, and thus that a low antioxidant status might be a risk factor for SLE [4]. However, the number of cases was too small to allow definitive conclusions about the association of serum antioxidants with SLE. Furthermore, no studies have provided an explanation for the lower serum antioxidant levels found in patients with SLE.

The aim of our study was to compare plasma antioxidant/oxidant status and the dietary nutrient intake of SLE patients with those of healthy controls.

Patients and methods

Patients

The study included 97 consecutive patients (91 women, 6 men; mean age 34.2 ± 11.3 years) with SLE who attended the Hospital for Rheumatic Diseases, Seoul, Korea, between July 2000 and March 2001. We also selected 97 age- and sex-matched healthy controls (91 women, 6 men; mean age 33.6 ± 10.9 years). The demographic characteristics of the patients with SLE and the controls are presented in Table 1. All participants gave informed consent.

Table 1. Demographic characteristics of patients with systemic lupus erythematosus (SLE) and of sex- and age-matched controls

Variables	SLE ($n=97$)	Control ($n=97$)	P
Age (years)	34.2 ± 11.3	33.6 ± 10.9	NS
Sex			NS
Female	91 (94%)	91 (94%)	
Male	6 (6%)	6 (6%)	
Height (cm)	159.1 ± 6.6	161.4 ± 5.1	< 0.05
Weight (kg)	54.5 ± 9.2	56.5 ± 7.6	NS
Body mass index	21.5 ± 3.2	21.6 ± 2.6	NS
Education (years; %)			< 0.01
≤ 12	63	40	
> 12	37	60	
Income (US$/year; %)			NS
< 10,000	14	6	
10,000–20,000	31	24	
20,000–30,000	28	24	
30,000–40,000	18	24	
> 40,000	9	20	
Smoking (%)			NS
Smoker	8	2	
Former smoker	4	6	
Nonsmoker	87	92	
Alcohol consumption	41	76	< 0.001

SLE patients fulfilled the 1997 revised American College of Rheumatology (ACR) criteria for SLE [8] (Table 2).

Methods

Blood samples from 19 patients and 19 controls were subjected to analyses of plasma concentrations of α-tocopherol (major lipid soluble nonenzymic antioxidant) and malone dialdehyde (MDA, a degradation product of lipid peroxidation), and the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx; both are natural enzymic antioxidants) in the plasma. All patients and controls were interviewed to determine dietary intakes using a validated semiquantitative food-frequency questionnaire specifically formulated to evaluate the consumption of the major nutrient. Clinical information including disease activity (revised Systemic Lupus Activity Measure activity index, Fellows of Harvard College, 1988, revised 1991) and damage (Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index, SLICC/ACR) [9] was obtained from all SLE patients. Their mean disease duration was 4.5 ± 2.9 (range 0.5–12) years, mean SLAM-R score 6.67 ± 4.36 (range 0–23), and mean SLICC/ACR score 0.58 ± 0.78 (range 0–3) (Table 2).

Table 2. Disease duration, activity, and damage, and 1997 revised American College of Rheumatology specific criteria [8] of 97 patients with SLE (*SLAM-R* revised activity index of systemic lupus activity measure, *SLICC/ACR* Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index)

Disease duration (years)	4.5 ± 2.9
SLAM-R	6.67 ± 4.36
SLICC/ACR	0.58 ± 0.78
Malar rash (%)	33
Discoid rash (%)	6
Photosensitivity (%)	28
Oral ulcers (%)	30
Arthritis (%)	65
Serositis (%)	29
Renal disease (%)	37
Neurological involvement (%)	6
Hematological disease (%)	74
Immunological deficit (%)	82
Antinuclear antibody (%)	100

Plasma antioxidant/oxidant status analysis

Fasting blood samples were collected in heparin-containing tubes and left at room temperature for 1 h. The samples were centrifuged (2500 *g*) for 15 min at 4°C. The plasma was stored at –70°C until analysis. α-Tocopherol was extracted to hexane from the plasma and was then quantified by HPLC using a UV detector at 292 nm. A Waters MicroBondapak C18 column was used for separation. Plasma MDA content was determined according to the method of Yagi [10]. Briefly, 4 ml 1/12 N H_2SO_4 and 0.5 ml phosphotungstic acid were added to 100 µl plasma. The mixture was incubated at room temperature for 5 min and then was centrifuged at 2500 *g* for 3 min. The pellet was collected and washed with 2 ml 1/12 N H_2SO_4 and 0.3 ml phosphotungstic acid and then centrifuged at 2500 *g* for 3 min. Distilled water 2 ml and of 0.67% thiobarbituric acid solution 1 ml were added to the pellet, and the mixture was incubated for 1 h. The concentration of MDA was determined fluorometrically at excitation and emission wavelengths of 515 and 553 nm, respectively.

Plasma SOD activity was measured according to the method of Flohe et al. [11]. Xanthine 5 µmol in 0.001 N sodium hydroxide and 2 µmol cytochrome *c* in 50 mM phosphate buffer solution were mixed together. Of this solution 2 ml was mixed with 20 µl plasma, and the reaction was then started by adding 50 µl 0.2 U xanthine oxidase solution. The amount of cytochrome *c* reduction was determined at 550 nm, with 1 U SOD corresponding to 50% inhibition of cytochrome *c* reduction. Plasma GPx activity was measured according to the method of Flohe and Gunzler [12]. Of plasma 100 µl was added to the reaction mixture (0.1 M potassium phosphate buffer containing 1 mM EDTA, 2.4 U/ml glutathione reductase, and 10 mM glutathione) and was incubated for 10 min at 37°C. Of 1.5 mM NADPH solution 100 µl and of 12 mM *t*-butyl hydroperoxide 100 µl were added to the reaction mixture and then monitored at 340 nm for 3 min.

Estimation of nutrient intakes from the food-frequency questionnaire

We used a validated interviewer-administered semiquantitative food-frequency questionnaire to determine the nutrient intakes of the subjects. The questionnaire included a list of 103 food items. Selection criteria were: (a) most frequently consumed food items, (b) most consumed food items, and (c) major food items supplying each nutrient. The selection was based on the 1998 Korea National Health and Nutrition Survey Report [13]. Selected food items were

categorized according to food groups and subdivided by food preparation methods, nutrient content, and portion sizes. The number of food items in each category were as follows: cereals and starches (17), meats (16), fish and other seafoods (5), fruit (10), egg (1), legumes (6), vegetables (20), mushrooms (2), milk and dairy products (5), oils and seeds (4), seaweeds (2), hot and cold nonalcoholic drinks (7), and alcohol beverages (8). Subjects were asked to state their average frequency of consumption of each food item according to the following categories: none, 1/month, 2–3/months, 1/week, 2/weeks, 3–4/weeks, 5–6/weeks, 1/day, 2/days, or 3/days. The portions were classified according to fractions of standard serving sizes: 0.5, 1.0, or 1.5. The interviewer showed food models and photographs of the standard serving size, and asked the subjects to refer to those portions when thinking about the amount of food consumed. The food-frequency questionnaire was coded and analyzed by a computer-aided nutritional analysis program (CAN-PRO, APAC Intelligence, Seoul, Korea).

Statistics

The significance of differences in the means between groups was assessed using the Student's t test. A probability of randomness value of $P < 0.05$ was considered statistically significant. All analyses were performed using the Statistical Analysis System (version 6.12; SAS Institute, N.C., USA).

Results

Plasma antioxidant/oxidant status

Table 3 shows the values of plasma oxidative stress markers in patients and controls. The plasma α-tocopherol concentration was lower in patients, at 10.62 ± 3.61 mg/l compared to 12.41 ± 2.62 mg/l in controls, but the difference was not statistically significant. In patients the plasma SOD and GPx activities were 42.11 ± 11.26 U/ml ($P < 0.001$) and 0.103 ± 0.028 U/ml ($P < 0.01$), respectively, while those of control subjects were 58.67 ± 12.60 U/ml and 0.129 ± 0.021 U/ml, respectively. The plasma MDA level was higher in patients (4.14 ± 0.38 nM/ml) than in controls (3.88 ± 0.28 nM/ml; $P < 0.05$).

On the basis of disease activity (SLAM-R), damage (SLICC/ACR), renal involvement, and disease duration, plasma α-tocopherol concentration, SOD activity, GPx activity, and MDA level differences were not statistically significant between the two groups (Table 4).

Nutritional status according to the semiquantitative food-frequency questionnaire

Calorific intake was significantly lower in patients ($1,871 \pm 366$ kcal) than in controls ($2,028 \pm 446$ kcal; $P < 0.01$). The nutritional status values are shown in Tables 5 and 6; the values in Table 6 were obtained by normalizing calorie intake to 1,000 kcal. The intakes of protein, lipid, and niacin were significantly lower in patients than in controls.

The intakes of all major dietary antioxidants, including vitamin A, β-carotene, and vitamin C, were lower in patients than in controls (Tables 5, 6), but this difference was significant only in the case of vitamin A and β-carotene (vitamin A: 669.2 ± 351.9 vs. 783.5 ± 407.7 retinol equivalents; β-carotene: $2,798 \pm 1,752$ vs. $3,375 \pm 1,965$ μg; Table 5). We did not detect any significant differences in vitamin A, retinol, β-carotene, or vitamin C

Table 3. Plasma α-tocopherol concentration, superoxide dismutase activity, glutathione peroxidase activity, and malone dialdehyde level in patients and in their sex- and age-matched controls

	SLE ($n = 19$)	Control ($n = 19$)	P
α-Tocopherol (mg/l)	10.62 ± 3.61	12.41 ± 2.61	NS
Superoxide dismutase (U/ml)	42.11 ± 11.26	58.67 ± 12.60	< 0.001
Glutathione peroxidase (U/ml)	0.103 ± 0.028	0.129 ± 0.021	< 0.01
Malone dialdehyde (nM/ml)	4.14 ± 0.38	3.88 ± 0.28	< 0.05

Table 4. Plasma α-tocopherol concentration, superoxide dismutase activity, glutathione peroxidase activity, and malone dialdehyde level in patients according to disease activity, renal involvement, and disease duration. SLAM-R score and disease duration cutoff values (9 and 1.4 years, respectively) are based on their median values; all differences are statistically nonsignificant (*SLAM-R* revised activity index of systemic lupus activity measure, *SLICC/ACR* Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index)

	α-Tocopherol (mg/l)	Superoxide dismutase (U/ml)	Glutathione peroxidase (U/ml)	Malone dialdehyde (nM/ml)
SLAM-R				
< 9	8.39 ± 3.29	38.85 ± 13.40	0.10 ± 0.01	4.24 ± 0.41
≥9	11.72 ± 2.86	44.00 ± 9.94	0.10 ± 0.03	4.05 ± 0.35
SLICC/ACR				
0	10.68 ± 3.20	40.80 ± 13.30	0.10 ± 0.01	4.16 ± 0.45
≥1	9.57 ± 3.93	43.55 ± 9.04	0.09 ± 0.03	4.09 ± 0.26
Renal involvement				
No	10.24 ± 3.52	43.20 ± 9.39	0.10 ± 0.02	4.07 ± 0.42
Yes	10.31 ± 3.52	40.88 ± 13.53	0.10 ± 0.03	4.20 ± 0.34
Disease duration				
< 1.4 years	9.33 ± 3.47	44.80 ± 7.72	0.11 ± 0.02	3.99 ± 0.33
≥1.4 years	10.81 ± 3.33	42.28 ± 14.39	0.09 ± 0.01	4.23 ± 0.43

intakes on the basis of activity (SLAM-R), damage (SLICC/ACR), renal involvement, or disease duration (Table 7).

Relationship between plasma antioxidant/oxidant and dietary intake

There were no statistically significant correlations between plasma α-tocopherol concentration, SOD activity, GPx activity, MDA level and selected dietary intakes, vitamin A, retinol, β-carotene, vitamin C except α-tocopherol concentration and retinol (Pearson's correlation coefficient 0.622, $P = 0.023$; Table 8).

Discussion

We found that plasma antioxidant status is impaired in patients with SLE compared to that in healthy persons, and also that the consumption of major dietary antioxidants is lower in patients than in healthy

Table 5. Nutritional status in patients and in their sex- and age-matched controls using a semiquantitative food-frequency questionnaire (*RE* retinol equivalent)

	SLE ($n = 97$)	Controls ($n = 97$)	P
Calories (kcal)	1,871 ± 366	2,028 ± 446	< 0.01
Protein (g)	70.25 ± 19.99	78.91 ± 22.40	< 0.01
Lipid (g)	49.03 ± 16.69	58.13 ± 21.29	< 0.05
Carbohydrate (g)	285.0 ± 55.9	295.1 ± 59.6	NS
Ca (mg)	616.6 ± 283.3	625.3 ± 232.6	NS
P (mg)	1,165 ± 347	1,239 ± 334	NS
Fe (mg)	10.60 ± 3.03	11.76 ± 3.58	< 0.05
K (mg)	2,733 ± 321	2,988 ± 893	< 0.05
Vitamin A (RE)	669.2 ± 351.9	783.5 ± 407.7	< 0.05
Retinol (μg)	141.3 ± 83.45	149.0 ± 112.5	NS
β-Carotene (μg)	2,798 ± 1,752	3,375 ± 1,965	< 0.05
Vitamin B_1 (mg)	1.29 ± 0.31	1.34 ± 0.37	NS
Vitamin B_2 (mg)	1.27 ± 0.44	1.35 ± 0.42	NS
Niacin (mg)	15.14 ± 4.39	18.36 ± 6.82	< 0.001
Vitamin C (mg)	120.4 ± 45.54	124.2 ± 48.25	NS
Cholesterol (mg)	242.6 ± 129.3	232.6 ± 114.0	NS

Table 6. Nutritional status in patients and in their sex- and age-matched controls using a semiquantitative food-frequency questionnaire, normalized to 1,000 kcal (*RE* retinol equivalent)

	SLE ($n = 97$)	Controls ($n = 97$)	P
Calories (kcal)	1,000	1,000	–
Protein (g)	37.23 ± 5.76	38.89 ± 5.25	< 0.05
Lipid (g)	25.84 ± 5.72	28.20 ± 5.26	< 0.01
Carbohydrate (g)	153.3 ± 17.3	147.0 ± 15.5	< 0.01
Ca (mg)	325.0 ± 127.0	315.8 ± 94.2	NS
P (mg)	617.4 ± 113.4	615.0 ± 83.2	NS
Fe (mg)	5.64 ± 0.98	5.95 ± 1.20	NS
K (mg)	1,453 ± 283	1,521 ± 305	NS
Vitamin A (RE)	353.5 ± 163.5	402.3 ± 157.7	< 0.05
Retinol (μg)	72.94 ± 37.94	72.09 ± 47.33	NS
β-Carotene (μg)	1,484 ± 845	1,756 ± 804	< 0.05
Vitamin B_1 (mg)	0.69 ± 0.10	0.67 ± 0.11	NS
Vitamin B_2 (mg)	0.66 ± 0.18	0.67 ± 0.12	NS
Niacin (mg)	7.98 ± 1.65	9.00 ± 1.83	< 0.001
Vitamin C (mg)	63.82 ± 22.35	64.79 ± 22.62	NS
Cholesterol (mg)	125.5 ± 58.2	114.6 ± 38.7	NS

Table 7. Selected nutritional status in patients according to disease activity, damage, renal involvement, and disease duration. SLAM-R score and disease duration cutoff values (6 and 3.5 years, respectively) are based on their median values (*SLAM-R* revised activity index of systemic lupus activity measure, *SLICC/ACR* Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index)

	Vitamin A (RE)	Retinol (μg)	β-carotene (μg)	Vitamin C (mg)
SLAM-R				
< 6	385.1 ± 195.0	72.23 ± 39.77	1,680 ± 1,059	66.63 ± 21.92
≥ 6	331.1 ± 128.4	75.82 ± 35.44	1,338 ± 580	63.60 ± 21.22
SLICC/ACR				
0	354.8 ± 172.7	76.07 ± 41.15	1,493 ± 904	66.26 ± 23.62
≥1	357.9 ± 153.8	70.15 ± 33.93	1,501 ± 782	63.35 ± 18.46
Renal involvement				
No	362.8 ± 168.4	72.23 ± 39.77	1,546 ± 840	65.93 ± 22.65
Yes	344.2 ± 157.5	75.82 ± 35.44	1,407 ± 871	63.35 ± 19.42
Disease duration				
< 3.5 years	339.5 ± 149.8	78.12 ± 40.39	1,401 ± 736	66.93 ± 21.41
≥ 3.5 years	374.6 ± 178.3	68.39 ± 35.17	1,602 ± 956	62.86 ± 21.60

Table 8. Relationship between plasma α-tocopherol concentration, superoxide dismutase activity, glutathione peroxidase activity, malone dialdehyde level and selected dietary intake in patients with SLE ($n = 19$): Pearson's correlation coefficients

	Vitamin A (RE)	Retinol (μg)	β-Carotene (μg)	Vitamin C (mg)
α-Tocopherol (mg/l)	0.022	0.622*	0.336	–0.240
Superoxide dismutase (U/ml)	0.092	0.095	0.067	0.149
Glutathione peroxidase (U/ml)	0.162	0.332	0.109	0.371
Malone dialdehyde (nM/ml)	–0.353	0.085	–0.392	–0.286

*$P < 0.05$

controls. Weimann et al. [5, 6] showed that vitamin E beneficially affects the development of SLE-like disease in MRL/lpr mice, and that antioxidants such as vitamin C, vitamin E, and β-carotene also beneficially influence immune function in MRL/lpr mice and rats, suggesting a possible method for reducing human SLE. Both superoxide and hydrogen peroxide generation by peripheral leukocytes are enhanced in patients with SLE [7]. These observations – that damage by free oxygen radicals might play a role in humans and animals – are consistent with our finding of impaired antioxidant status in SLE patients. However, since ours was a cross-sectional study, it cannot give direct evidence regarding the development of SLE, unlike the prospective case-control study of Comstock et al. [4].

The mechanisms underlying lower antioxidant status in SLE are unclear but are perhaps related to decreased intake, absorption, transport processes, or excessive oxidation at the detriment of the antioxidant status due to the active inflammatory disease itself and/or a genetic predisposition. However, there have been no studies regarding this issue in SLE. We investigated the influence of disease activity, severity, and duration on the antioxidant status among SLE patients. Our findings suggest that active and severe disease status and disease longevity themselves do not influence the plasma level of oxidative stress markers and the dietary intake of antioxidants. Therefore the present study suggests that one of the causes of impaired antioxidant status in SLE is a decreased dietary intake of antioxidants. However, the sample size is too small to conclude that there is no association between antioxidant status and disease activity and severity, and that impaired antioxidant status is due to lower dietary antioxidant intake.

Energy intake was significantly lower in patients with SLE than in controls. Since we used the food-frequency questionnaire to determine nutrient intakes and did not include questions on food intolerance or intestinal symptoms, we cannot rule out any possible causes of lower calorie intake. However, we can assume that this difference might be caused by lower alcohol consumption, anorexia due to the intake of immunosuppressive drugs, nutritional intolerance, and/or intestinal symptoms caused by malassimilation.

Some limitations of our study require consideration. As noted above, the number of subjects was too small to provide definitive conclusions, and the study was performed cross-sectionally. Further studies using a larger number of subjects and designed with prospective and/or serial follow-ups are required.

In conclusion, these results suggest that plasma antioxidant status is impaired and dietary antioxidant intake is decreased in patients with SLE. Dietary education and/or supplementation of antioxidants might be an important strategy in the management of SLE. Intervention trials demonstrating preventive effects of antioxidant supplements would be required to confirm this.

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