

The Effects of Vitamins E and D Supplementation on Erythrocyte Superoxide Dismutase and Catalase in Atopic Dermatitis

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Abstract

Background: Atopic dermatitis is a public health problem worldwide. Increment of reactive oxygen species (ROS) production may be one of the contributing factors of tissue damage in atopic dermatitis. The present study was designed to determine the effect of vitamins E and/or D on erythrocyte superoxide dismutase and catalase activities in patients with atopic dermatitis.

Methods: In a randomized, double blind, placebo controlled clinical trial 45 atopic dermatitis patients were divided into four groups. Each group received one of the following supplements for 60 days: group A (n=11) vitamins E and D placebos; group B (n= 12) 1600 international unit (IU) vitamin D3 plus vitamin E placebo; group C (n=11) 600 IU synthetic all-rac- α tocopherol plus vitamin D placebo; group D (n=11) 1600 IU vitamin D3 plus 600 IU synthetic all-rac- α tocopherol. Erythrocyte superoxide dismutase (SOD) and catalase activities, serum 25 (OH) D, plasma α -tocopherol were determined. The data were analyzed by analysis of variance (ANOVA) and paired *t*-test.

Results: After 60 days vitamin D and E supplementation, erythrocyte SOD activities increased in groups B, C and D ($P=0.002$, $P=0.016$ and $P=0.015$, respectively). Erythrocyte catalase activities increased in groups B and D ($P=0.026$ and $P=0.004$, respectively). The increment of erythrocyte catalase activity was not significant in group C. There was a positive significant correlation between SOD activity and serum 25 (OH) D ($r=0.378$, $P=0.01$).

Conclusions: It is concluded that vitamin D is as potent as vitamin E in increasing the activities of erythrocyte SOD and catalase in atopic dermatitis patients.

Keywords: Atopic dermatitis, Vitamin E, Vitamin D, Superoxide dismutase, Catalase

Introduction

Atopic dermatitis (AD) is a highly pruritic, chronic, and relapsing inflammatory skin disease characterized by typically distributed eczematous skin lesions (1). Atopic dermatitis is a public health problem worldwide. Its lifetime prevalence is 10-20% and 1-3% among children and adults, respectively. Prevalence of AD has increased by two to three folds during the past three decades in industrialized countries. Atopic dermatitis is the most common cause of occupational skin disease in adults (2).

Skin inflammation in AD is characterized by an intense infiltration of lymphocytes and eosinophils. These cells release pro-inflammatory cytokines, superoxide radical, hydrogen peroxide and peroxynitrite (3). Oxidative stress contributes to adverse effects on the skin, in form of erythema, edema, wrinkling, inflammation, hypersensitivity and keratinization abnormality (4). It seems that reactive oxygen species (ROS) are involved in the pathogenesis of allergic inflammation; therefore, increased ROS production may be one of the contributing factors of tissue damage in atopic derma-

titis (5-7). Defense mechanisms exist to cope with oxidative stress. These systems are consisted of two main groups; the group of antioxidant enzymes and the group of the non-enzymatic antioxidants. Some of antioxidant enzymes include superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), and glutathione reductase (GR) (3). Superoxide dismutase (SOD) is an antioxidant enzyme which catalyses the dismutation of superoxide anion to O₂ and H₂O₂. H₂O₂ is then deactivated to H₂O by catalase (8).

Some non-enzymatic antioxidants include α -tocopherol and β -carotene which are located in the cellular membranes and defend against lipid peroxidation, as well as interacting with free radicals. The increased membrane lipid peroxidation may stimulate immune and inflammatory response (6). Although considerable effort has been put into studying the antioxidant effects of vitamin E and its protective effects on oxidative damage, the role of vitamin D as a potential antioxidant has not been fully investigated. Vitamin D is well recognized for its action on calcium and phosphorus metabolism, and its beneficial effects on bone metabolism (9). A few studies have been conducted on antioxidant effects of vitamin D (10). Cholecalciferol (vitamin D₃) is likely to act as a membrane antioxidant by stabilizing the membrane against lipid peroxidation via an interaction between its hydrophobic ring (10). The other antioxidant activity of vitamin D seems to be by its effect on antioxidant enzyme. The aim of this study was to determine the effect of vitamin D and vitamin E on antioxidant enzymes specially superoxide dismutase and catalase in erythrocytes.

Materials and Methods

This study was part of a randomized double blind placebo controlled clinical trial on 52 atopic dermatitis patients, 13 to 45 yr old. Atopic dermatitis was diagnosed based on Hanifin and Rajka criteria (11). The patients were recruited from private clinics and Razi Hospital (Tehran, Iran). The including criteria were: 1) not taking any vitamin, mineral and fatty acids supplements, oral con-

traceptive pills, steroid hormones (oral or parenteral), antiepileptic agents, as well as anticoagulant drugs, 2) not having any hepatic and renal problems, 3) not being pregnant or nursing. However, the patients were allowed to take the routine treatments of atopic dermatitis including emollients, topical corticosteroids and oral anti-histamines.

The patients were fully interviewed about past medical history, medications, age and smoking habits. Body weight was measured to the nearest 0.1 kg with minimal clothing and without shoes, using a beam balance. Height was measured with a stadiometer to the nearest 0.5 cm. Body mass index (BMI) was calculated using the following formula: weight (kg)/ height (m)².

After 12 to 14 h overnight fasting, 10ml blood from the antecubital vein was drawn into two tubes, with and without EDTA as anticoagulant before and after the trial. Samples were centrifuged at 3000 rpm for 10 min. The separated plasma and serum were transferred to polystyrene tubes that were immediately stored at -80 °C until analysis. The erythrocytes were washed three times with isotonic saline solution and then stored at -80 °C until analysis.

The subjects were allocated to one of four groups in permuted block design. The randomization codes were only broken at the end of the study.

The groups consisted of, group A: taking vitamin D and E placebos, group B: taking 1600IU cholecalciferol (vitamin D₃) and vitamin E placebo, group C: taking 600IU synthetic all-rac- α tocopherol in two capsules (400 and 200IU) and vitamin D placebo, group D: taking 1600IU cholecalciferol (vitamin D₃) and 600 IU synthetic all-rac- α tocopherol in two capsules (400 and 200 IU). Therefore, each patient took three capsules for 60 d. The supplements and placebos had identical appearance. The patients were advised to take vitamin D and E supplements with meals and take them separately.

All of the analysis was performed in the same bench, to minimize bench-related variations.

Superoxide dismutase (SOD; EC.1.15.1.1) activity was measured with RANDOX kits (Cat.

No. SD 125; Randox Labs Ltd., Crumlin, UK) based on the method developed by McCord and Fridovich (12). This method employs xanthine and xanthine oxidase to generate superoxide radicals, which reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The superoxide dismutase activity was then measured by the degree of inhibition of this reaction. The activity was measured at 37 °C by a UV spectrophotometer, and absorbance was measured at 30 S and 3 min at 505nm. The unit of activity is defined as the amount of enzyme that inhibits the rate of the formazan dye formation by 50%. The activity of SOD was expressed as units/g hemoglobin (U/gHb).

Catalase (CAT; EC.1.11.1.6) activity was measured in hemolysates by UV spectrophotometer according to Aebi's method (13). The decomposition of the substrate H₂O₂ was monitored spectrophotometrically at 240 nm at 0 and 30 S. Catalase activity was expressed as U/gHb.

Hemoglobin was determined according to Van's method (14).

Serum 25-Hydroxyvitamin D₃ (25OH D₃) was determined by radioimmunoassay with BioSource kit (Cat no.KIP19161 BioSource Europe S.A. Belgium) following manufacturer's instructions (15).

Plasma α -tocopherol was determined by high performance liquid chromatography (HPLC) as described by Sanz (16).

Ethical consideration

Patients were fully informed about the purposes, procedures and probable hazards of the trial. They were free to leave the trial at any time. All of subjects signed written informed consent form before entering the trial. In the case of under 18 yr olds, one of parents signed written informed consent form. Research protocol was approved by Ethics Committee of Tehran University of Medical Sciences.

Statistical analysis

All values are expressed as mean \pm standard error (SE). Kolmogorov-Smirnov test was performed on variables to test their normal distribution. We compared four groups by one-way analysis of

variance (ANOVA) in the case of continuous variables and chi square test for categorical data. Post hoc comparisons were performed with Tukey test. To test the differences between variables before and after trial in each group, paired t-test was employed. A value of $P < 0.05$ was considered to be statistically significant. Statistical analysis was carried out with the Statistical Package for the Social Sciences (SPSS) (Version 16, SPSS Inc, Chicago, IL, USA).

Results

Forty-five of 52 patients completed the 60 d trial. One subject was excluded due to phototherapy, two patients due to oral or parenteral corticosteroids and two more patients due to immunosuppressive drugs. Finally, two patients were not willing to continue the trial. Table 1 shows the demographic and anthropometric data of the 45 patients who completed the study. As table 1 shows, the four groups were not different in age, sex, height, weight, and BMI at baseline. There was only one smoker in each group except group A. the smokers were all male. During intervention, none of patients underwent significant changes in weight and BMI.

Baseline levels of plasma α -tocopherol, serum 25-hydroxyvitamin D₃ (25OH D₃), erythrocyte superoxide dismutase (SOD) and catalase activities in male and female patients are shown in Table 2. There was not a significant difference between two sexes in plasma level of α -tocopherol, and erythrocyte SOD and catalase activities. However, serum levels of 25(OH) D of female subjects were significantly lower than males (9.6 versus 17.7, $P = 0.001$). At baseline non of the patients had plasma level of α -tocopherol lower than deficient level ($< 5 \mu\text{g/ml}$) (17). In the case of serum 25 (OH) D, only six people (13.3%) had greater than deficient level (20ng/ml) (9). Serum 25(OH) D of 17 patients (37.7%) were between 10 to 19.9 ng/ml. Twenty-two subjects had serum 25(OH) D levels lower than 10ng/ml. At the beginning of the trial, four groups had similar plasma levels of α -tocopherol, serum level

of 25(OH) D, erythrocyte SOD and catalase activities as showed by ANOVA in Table 3. After 60 d supplementation, groups taking vitamin E (groups C and D) had significant increase in plasma α -tocopherol ($P= 0.011$ for group C and $P< 0.001$ for group D (paired t -test)). Groups B and D who had taken vitamin D had significant increase in serum 25(OH) D ($P< 0.001$ for groups B and D (paired t -test)) (Table 3).

In posthoc analysis with Tukey test, we found significant differences in plasma α -tocopherol after intervention between groups A and C ($P= 0.016$), A and D ($P< 0.001$), C and B ($P= 0.002$), D and B ($P< 0.001$). In addition, we considered significant differences in serum 25(OH) D at the end of the trail between groups A and B ($P= 0.005$), A and D ($P= 0.006$), B and C ($P< 0.001$), C and D ($P= 0.001$).

In spite of taking placebo, erythrocyte SOD activity decreased significantly ($P= 0.02$, paired t -test) in group A. Moreover, plasma α -tocopherol decreased significantly ($P= 0.001$) in group A.

In multiple comparisons by Tukey test on after intervention data, erythrocyte catalase activity was significantly different between groups A and D ($P= 0.012$) and erythrocyte SOD activity was significantly different between groups A and B ($P= 0.047$), A and C ($P= 0.005$). Erythrocyte catalase activity increased in group C but it was not significant.

Baseline erythrocyte SOD activity was significantly correlated with serum 25(OH) D ($r= 0.378$, $P= 0.01$, two tailed). In the case of erythrocyte catalase activity, we did not find such significant correlation. Age had no significant correlation with erythrocyte SOD and catalase activities. Nevertheless, a significant correlation between height and serum 25(OH) D was found ($r= 0.349$, $P= 0.01$, two tailed). Plasma level of α -tocopherol and erythrocyte SOD and catalase activities had no significant correlation.

Table 1: Demographic, anthropometric characteristics of the four study groups at the beginning of trial

	Group A	Group B	Group C	Group D
n	11	12	11	11
Male/female	1/10	3/9	3/8	3/8
Age (years)	26.1±2.8	21.2±1.6	29.0±2.09	27.5±2.3
Height (cm)	158.2±2.0	161.6±2.8	165.5±2.8	160.5±2.5
Weight (kg)	59.4±2.7	63.8±4.1	72.9±3.7	64.1±3.7
BMI (kg/m ²)	23.7±1.07	24.2±1.2	26.6±1.2	24.8±1.2
Smokers (n)	0	1	1	1

Data are means±S.E. There were no significant differences between groups by ANOVA or χ^2 .

Table 2: Baseline plasma α - tocopherol, serum 25 hydroxyvitamin D3 (25OH D3), erythrocyte superoxide dismutase (SOD) and catalase activities in male and female separately:

	Male	Female	P value
n	10	35	
Plasma α - tocopherol (μ g/ml)	11.1±1.06	12.37±0.4	0.2
Serum 25OH D3 (ng/ml)	17.7±1.8	9.6±1.1	0.001
SOD (U/gHb)	1229.1±84.2	1116.0±28.4	0.11
Catalase (U/gHb)	197.4±19.2	201.4±9.08	0.8

Values are expressed as means ± SE. Values in different sexes were compared with independent sample t-test.

Table 3: Plasma α -tocopherol, serum 25 hydroxyvitamin D3 (25OH D3), erythrocyte superoxide dismutase (SOD) and catalase activities

	Group A	Group B	Group C	Group D	P value
n	11	12	11	11	
Plasma α -tocopherol (μ g/ml)					
Before	13.8 \pm 0.8	10.8 \pm 0.7	11.9 \pm 0.5	11.8 \pm 0.8	0.055
After	11.3 \pm 0.6	10.1 \pm 1.2	17.1 \pm 1.5	22.3 \pm 1.5	<0.001
P value	0.001	0.438	0.011	<0.001	
Serum 25OH D3 (ng/ml)					
Before	13.9 \pm 2.6	10.4 \pm 1.6	12.1 \pm 2.4	9.3 \pm 1.7	0.462
After	12.1 \pm 3.1	23.5 \pm 1.9	9.5 \pm 1.7	23.5 \pm 2.2	<0.001
p value	0.093	<0.001	0.15	<0.001	
SOD (U/gHb)					
Before	1181.4 \pm 45.3	1124.7 \pm 54.6	1187.5 \pm 82.9	1072.2 \pm 45.6	0.48
After	1074.1 \pm 49.1	1357.3 \pm 32.6	1458.1 \pm 129.5	1277.8 \pm 55.1	0.007
P value	0.02	0.002	0.016	0.015	
Catalase (U/gHb)					
Before	206 \pm 14.4	183.1 \pm 14.2	197.0 \pm 16.1	217.8 \pm 20.4	0.49
After	190.3 \pm 10.9	219.5 \pm 15.9	232.8 \pm 15.9	267.7 \pm 22.2	0.021
P value	0.173	0.026	0.09	0.004	

Values are expressed as means \pm SE. Before and after data were compared with paired t-test. Baseline and after intervention differences between groups were tested by ANOVA

Discussion

In this study, it was found that vitamin E and/or D supplementation could increase erythrocyte SOD and catalase activities in patients with atopic dermatitis after 60 d. Although, the patients who took vitamin E did not show significant increase in erythrocyte catalase activity, its changes between groups were significant in post hoc analysis of the changes of erythrocyte catalase activity by tukey test. There was a significant positive correlation between serum 25(OH) D and erythrocyte SOD activity. A positive correlation between height and serum 25(OH) D is also found. There was no correlation found between age, sex and erythrocyte SOD and catalase activities.

It seemed that vitamins D and E had synergistic effect on erythrocyte catalase activity but it was not true about erythrocyte SOD activity.

However, there is a negative correlation between BMI and serum 25(OH) D(9) in normal population, we did not find such a relationship. A positive correlation between height and serum 25(OH) D is found. In our study, there was no relationship between height and BMI. It seems that such

a relationship was due to males who had further serum 25(OH) D and being taller.

There was no relationship between age and erythrocyte catalase and SOD activities. However, some studies reported a negative relationship between age and erythrocyte catalase and SOD activities in normal population (18-19), some studies did not find such relationship about catalase (18, 20). Due to small sample size, we could not reject such a relation and it needs a study with more sample size and peoples of different age groups. There was no relationship between sex and erythrocyte catalase and SOD activities which was against other's report (18). However, the results reported by Ozbay's (19) is consistent with our findings. The number of males in our study was only ten and it was not enough to draw a conclusion. Our study showed no relationship between age and plasma α -tocopherol. Faure showed that age affects on plasma α -tocopherol (21). They observed such a relationship studying on 12741 volunteers. Our study showed that serum 25(OH) D of females were significantly less than its concentration in males, which can be attributable to more sunlight exposure in male compared to female.

The effects of vitamin D on SOD and catalase were comparable to the effects of vitamin E. This finding shows the antioxidant effects of vitamin D. Our result is consistent with Sardar's finding (22). However, they studied hepatic enzymes. Vitamin D does most of its effects on cells, through its nuclear receptor but there is no nucleus in RBC. Therefore, this finding supports the antioxidant activity of vitamin D molecule. This result is consistent with findings reported by others (10, 23-25). In researches on cancers, the prooxidant activity of vitamin D was revealed, however, these subjects were not healthy (26). Because free radicals have a role in pathogenesis of atopic dermatitis, it seems these vitamins could alleviate the disease through deactivating free radicals by antioxidant enzymes. To confirm such a conclusion, more study is needed.

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