

Effects of Vitamins C and E on Serum Resistin, Hs-CRP, Lipid Profile and Insulin Levels in Patients with Type 2 Diabetes

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ABSTRACT

Background and Objective: Vitamins C and E are the two main antioxidants involved in prevention of type 2 diabetes, by reducing oxidative stress. The aim of this study was to evaluate the effects of vitamins C and E supplementation independently, on serum levels of insulin, high sensitivity C-reactive protein (hs-CRP) and resistin in people with type 2 diabetes.

Methods: In this study, 38 patients with type 2 diabetes (17 men and 21 women) received 1,000 mg/day vitamin C, and 40 patients with type 2 diabetes (21 men and 19 women) received 400 IU/day vitamin E orally. Fasting blood glucose and lipid profile were measured using enzymatic method. Hs-CRP was measured by immunoturbidimetric method, and serum insulin and resistin levels were measured by ELISA.

Results: total cholesterol, triglycerides, hs-CRP, insulin and resistin significantly were reduced after vitamin C supplementation ($P < 0.001$). Moreover, the level of total cholesterol ($P = 0.018$), low-density lipoprotein, triglycerides and hs-CRP significantly changed after vitamin E supplementation ($P = 0.001$).

Conclusion: The daily intake of 1,000 mg of vitamin C and 400 IU of vitamin E may be useful in reducing diabetic complications by decreasing serum levels of hs-CRP and lipid profile in people with type 2 diabetes.

Keywords: Type 2 Diabetes Mellitus, resistin, C-reactive protein, vitamin C, vitamin E.

INTRODUCTION

Type 2 diabetes is the most common metabolic disorder in the world (1). The number of people with type 2 diabetes in Iran is estimated to be about 5.1 million (2). Diabetes causes many complications such as cardiovascular, neurological, kidney and eyes diseases. The cause of these complications has not been determined yet, but oxidative stress and protein glycation have been given a lot of attention recently (3). Vitamin C (ascorbic acid) and vitamin E (alpha-tocopherol) are among the most important antioxidants in human plasma. In vitro studies have shown that these two vitamins protect the body against free radicals-induced damage by reducing free radicals and recirculating body's antioxidant reserves (4-6). Experimental studies on animal models have demonstrated that the administration of antioxidants delays the onset of diabetes (7, 8). Thus, it is suggested that dietary antioxidant supplementation may prevent type 2 diabetes in humans. Epidemiologic and observational studies have reported a significant negative correlation between the antioxidants' concentration and several biomarkers of insulin resistance and glucose intolerance in healthy individuals (9). Resistin is an adipose tissue-secreted hormone, which is involved in insulin resistance and obesity-induced diabetes. Concentrations of antioxidants such as vitamin C (9-11) and vitamin E (12, 13) are usually significantly lower in blood of people with type 2 diabetes compared to non-diabetic controls. Evidence from prospective cohort studies also supported the inverse association between the incidence of type 2 diabetes and plasma, serum or dietary concentrations of vitamin C (14) and vitamin E (6-9) in non-diabetic individuals. Some small randomized clinical trials (15-17) on patients with type 2 diabetes demonstrated that high doses of oral vitamin C or E supplementation has beneficial effects on risk factors associated with insulin resistance and diabetes including oxidative stress, hypertension (15) lipid metabolism (16), endothelial function (17) and insulin-mediated glucose uptake (16). However, some other studies have indicated otherwise (18-20). Nevertheless, few large clinical trials with longer period of treatment have mainly shown the prevention of type 2 diabetes. In a randomized clinical trial, no evidence was found regarding the beneficial effects of

vitamin E supplements on the incidence of type 2 diabetes in apparently healthy women (21). To our knowledge, there are no previous clinical trials examining the effects of vitamin C and E in prevention of type 2 diabetes. It is still unknown whether vitamins C and E, independently or in combination, prevent type 2 diabetes. Given the high prevalence of type 2 diabetes in Khuzestan province, the beneficial effects of vitamins C and E on various factors in diabetic individuals and safety of supplementation of these two vitamins with standard doses, this interventional study aimed to evaluate the effects of four-week administration of vitamin C (1,000 mg/day) and vitamin E (400 IU/day) independently, on lipid profile, insulin, high-sensitivity C-reactive protein (hs-CRP) and resistin levels.

MATERIAL AND METHODS

In this interventional study, type 2 diabetes patients referred to diabetes clinic of Golestan Hospital in Ahvaz were enrolled after obtaining written consent. Baseline characteristics of the subjects including age, gender, body mass index (BMI), smoking, duration of diabetes, medications used and history of different diseases were investigated and recorded. The Patients with liver disease, kidney disease, hypothyroidism or hyperthyroidism, myocardial infarction or blood disorders who were taking medications containing estrogen, progesterone, diuretics, antihypertensive and vitamins C and E were excluded from the study. The study population included 80 type 2 diabetic patients aged between 35-65 years who were randomly divided into two groups of vitamin C (40 type 2 diabetes patients with daily intake of 1,000 mg vitamin C) and vitamin E (40 type 2 diabetes patients with daily intake of one capsule containing 400 IU of vitamin E). The sampling was conducted in the Golestan Hospital diabetes clinic under supervision of endocrinologist. The diagnostic criteria for diabetes were based on the American Diabetes Association guideline (fasting blood glucose of greater than or equal to 126 mg/dl in at least two separate occasions or a history of illness and consuming hypoglycemic drugs) (22). The participants were weighed with minimum clothing and without shoes using a digital scale (Seca). Height was measured in standing

position without shoes, using a tape measure while the shoulders were in normal position. BMI was calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2). Five mL of venous blood for baseline measurement, and after four weeks vitamins C and E intervention, was taken from subjects who were at least 10 to 12 hours fasting and no taking the glucose-lowering drugs. The samples were immediately centrifuged at 4000 rpm for 5 minutes, and then kept at $-70\text{ }^\circ\text{C}$ until time of testing. Blood glucose was measured by glucose oxidase method. Total cholesterol and triglycerides levels were measured by cholesterol oxidase and glycerol phosphate oxidase enzymatic methods, respectively. High-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured by using commercial available kits. For HDL-C measurement, first of all, LDL VLDL and chylomicrons were blocked by antibody, and then concentration of HDL-C was measured by enzymatic method. For LDL measurement, first all lipoproteins other than LDL were removed by using specific reagents, and then concentration of LDL-C was measured by using enzymatic method. hs-CRP levels were measured using immunoturbidimetric assay with Diazyme kit (USA). In this method, CRP in the serum of patients and anti-CRP polyclonal antibody coated on latex particles, cause turbidity by forming a complex. The amount of created turbidity is directly related to the amount of CRP in patients' samples. The serum resistin concentration was measured using ELISA technique and Mediagnost kits (USA). The insulin concentration was measured by ELISA, using DiaPlus kits (USA) with $0.5\text{ }\mu\text{IU}/\text{ml}$ sensitivity. Insulin resistance index (HOMA-IR) was calculated according to the formula: fasting insulin concentration (microU/L) x fasting glucose concentration (nmol/L)/22.5 (23). All quantitative values in this study were reported as mean \pm standard deviation (SD). Paired t-test was used to assess the significance of differences in mean values

measured in the two groups. Kolmogorov-Smirnov test was used to evaluate normality of distribution. The non-parametric Wilcoxon test was used for non-normally distributed cases. Statistical analysis was done using SPSS version 15. P-value less than 0.05 was considered statistically significant.

RESULTS

The study included 80 type 2 diabetic patients referred to Golestan Hospital Diabetes Center. Two subjects in the vitamin C group were excluded from the study. The vitamin C group included 38 patients (17 men and 21 women) with mean age of 48.7 ± 7.1 years and BMI of $28.9 \pm 4.9\text{ kg}/\text{m}^2$. The mean duration of diabetes in this group was 1.9 ± 0.96 years. The vitamin E group included 40 patients (21 men and 19 women) with mean age of 40.46 ± 7.17 and BMI of $27.07 \pm 4.46\text{ kg}/\text{m}^2$ (Table 1). Table 2 demonstrates the mean serum levels of fasting blood glucose, LDL-C, HDL-C, triglycerides, total cholesterol, hs-CRP, insulin and resistin before and after the intervention. Most data were normally distributed except serum concentrations of insulin, glucose, HOMA-IR and hs-CRP. Non-parametric tests were used for statistical analysis of these variables. There was no significant difference between the two groups in terms of mean age, BMI, height and weight at baseline and the end of study. None of the variables among men and women changed significantly after the intervention. The concentrations of HDL, LDL and blood glucose increased in the vitamin C group, but this difference was not statistically significant (Table 2). After supplementation, serum concentration of resistin significantly reduced in the vitamin C group. In addition, serum concentrations of total cholesterol, triglycerides, hs-CRP and insulin significantly were reduced after taking vitamin C (Table 2). The serum concentration of resistin was decreased after treatment with vitamin E, but this reduction was not statistically significant. On the other hand, mean concentrations of total cholesterol, LDL, insulin and hs-CRP significantly reduced in the vitamin E group.

Table 1- Anthropometric and biochemical characteristics before the intervention

Variables	Vitamin E	
	Vitamin C	Vitamin E
Gender (m/f)	17/21	19/21
Age (year)	48.78 ± 7.15	46.40 ± 7.17
Height (cm)	165.89 ± 9.35	163.92 ± 11.25
Weight (kg)	79.42 ± 13.95	72.30 ± 10.12
BMI (kg/m ²)	28.99 ± 4.9	27.07 ± 4.46
Systolic Blood Pressure (mmHg)	115.52 ± 8.91	113.50 ± 10.51
Diastolic Blood Pressure (mmHg)	74.73 ± 7.25	72.75 ± 10.12
Total cholesterol (mg/dl)	186.92 ± 48.94	184.30 ± 47.08
LDL-C (mg/dl)	93.42 ± 33.17	100.22 ± 32.16
HDL-C (mg/dl)	56.47 ± 14.07	52.65 ± 12.16
Triglyceride (mg/dl)	208.65 ± 62.56	194.47 ± 68.37
Fasting blood glucose (mg/dl)	166.83 ± 76.91	181.40 ± 99.76
Resistin (ng/ml)	9.2 ± 1.37	8.48 ± 3.1
Insulin (μIU/ml)	18.37 ± 7.07	18.57 ± 14.12
HOMA-IR	8.17 ± 6.03	5.67 ± 3.88
hs-CRP (mg/dl)	3.62 ± 3.02	2.97 ± 2.38

Continuous variables are presented as mean ± SD and were compared by independent sample t-test

Table 2- Comparison the mean of variables ,before^a and after the intervention

Variables	After intervention	Mean difference	95%CI	P-value
Total cholesterol (mg/dl)^b				
Vitamin C group	146.44 ± 45.26	-40.47	-56.64 ; -24.29	< 0.001
Vitamin E group	161.55 ± 61.20	-22.75	-41.36 ; -4.13	0.018
LDL-C (mg/dl)				
Vitamin C group	92.71 ± 30.11	-0.71	-10.74 ; 9.32	0.887
Vitamin E group	82.92 ± 25.73	-17.30	-26.29 ; -8.30	< 0.001
HDL-C (mg/dl)				
Vitamin C group	63.7 ± 24.9	7.28	-1.17 ; 15.75	0.089
Vitamin E group	52.7 ± 12.7	-0.03	-3.1 ; 3.04	0.982
Triglyceride (mg/dl)				
Vitamin C group	144.0 ± 81.31	-64.65	-92.91 ; -36.40	< 0.001
Vitamin E group	146.45 ± 64.77	-48.02	-74.13 ; -21.73	0.001
Fasting blood sugar (mg/dl)				
Vitamin C group	163.47 ± 76.23	-3.36	-21.29 ; 14.56	0.839
Vitamin E group	175.82 ± 80.26	-5.57	-29.38 ; 18.23	0.920
Resistin (ng/ml)				
Vitamin C group	7.5 ± 0.97	-1.77	-2.25 ; -1.28	< 0.001
Vitamin E group	8.08 ± 2.3	-0.39	-1.55 ; 0.76	0.494
Insulin (μIU/ml)				
Vitamin C group	9.26 ± 9.33	-9.10	-12.81 ; -5.40	< 0.001
Vitamin E group	17.67 ± 15.07	-1.02	-7.65 ; 5.60	0.686
HOMA-IR				
Vitamin C group	3.30 ± 2.96	-4.86	-6.87 ; -2.85	< 0.001
Vitamin E group	7.15 ± 5.85	1.47	-0.85 ; 3.80	0.207
hs.CRP (mg/dl)				
Vitamin C group	3.04 ± 2.74	-0.58	-0.85 ; -0.32	< 0.001
Vitamin E group	2.25 ± 2.01	-0.71	-0.97 ; -0.45	< 0.001

Data are presented as mean ± SD; ^a the mean of measured variables before the intervention are presented in Table 1; ^b paired *t*-test was used for statistical analysis; ^c non-parametric Wilcoxon test was used for statistical analysis

DISCUSSION

The results of this study clearly showed short-term supplementation of vitamin C significantly reduces serum levels of cholesterol, triglycerides, hs-CRP, insulin and resistin in people with type 2 diabetes compared with the controls. These findings are in agreement with the results of Errikson et al. study in Finland that showed intake of vitamin C significantly reduced serum total cholesterol in noninsulin-dependent diabetes mellitus patients (24). Afkhani et al. showed that consumption of 500 or 1000 mg of vitamin C for six weeks may reduce lipids and glucose, and risks of diabetic complications in patients with type 2 diabetes (25). Paolisso et al. showed that consumption of vitamin C significantly reduced plasma levels of LDL, total cholesterol, triglycerides and insulin. They also reported that vitamin C as an antioxidant, may reduce insulin resistance by improving endothelial function and reducing oxidative stress (16). In agreement with the present study, study of Chen et al. showed that vitamin C intake did not significantly change serum glucose concentrations (18). In the present study, intake of vitamin C reduced total cholesterol and increased HDL levels. The study of Abdullah Zadeh et al. showed that consumption of 250 mg/day vitamin C for 12 weeks significantly altered total cholesterol level, although no significant difference was observed in HDL levels (26). Tofler et al. also showed that taking one gram/day of vitamin C reduced the total cholesterol (27), which is consistent with the results of the present study. According to the results of the present study, vitamin C caused significant reduction in serum levels of hs-CRP, which is consistent with the results of Block et al. (28). They showed that taking 1,000 mg/day of vitamin C for two months significantly reduced the serum level of hs-CRP. However, study of Tousoulis et al. showed that taking 2,000 mg/day of vitamin C for four weeks caused no significant reduction in serum level of hs-CRP in people with diabetes type 2, which is not consistent with our results (29). The present study showed that taking one gram of vitamin C for four weeks significantly reduced serum level of insulin. This is consistent with the study of Paolisso et al. that showed that taking 500 mg of vitamin C twice a day for four months caused a significant reduction in serum levels of insulin. As an antioxidant, vitamin C

might improve vascular endothelial function and reduce oxidative stress by reducing insulin resistance (16). Afkhani et al. showed that taking 1,000 mg of vitamin C for six weeks significantly reduced plasma insulin level in people with type 2 diabetes, which is consistent with our results. However, intake of 500 mg of vitamin C did not cause any significant change in serum insulin levels (25). Study of Chen et al. also showed that daily intake of 800 mg of vitamin C for four weeks brought about no significant change in serum level of insulin in people with type 2 diabetes, which is not consistent with the findings of the present study. This inconsistency may be due to low dose of vitamin C used in the mentioned study (18). Our study also showed that daily intake of 400 IU of vitamin E for four weeks significantly reduced serum level of total cholesterol in people with type 2 diabetes. This finding is consistent with the findings of Paolisso et al. that indicated that daily consumption of 900 mg of vitamin E for two months significantly reduced total cholesterol (30). In this study, vitamin E supplementation significantly reduced serum levels of hs-CRP in patients with type 2 diabetes. This is consistent with the results of Wu et al., which showed that taking 500 mg/day of vitamin E for six weeks significantly reduced serum levels of hs-CRP in patients with type 2 diabetes (31). Study of Jiala et al. showed that vitamin E decreases serum levels of hs-CRP (32), which is consistent with the study of Upritchard et al. that showed taking 800 IU/day of vitamin E for four weeks significantly reduced serum levels of CRP in people with type 2 diabetes (33). In the study conducted by Devaraj et al., high-dose of vitamin E (1200 IU per day for three months) reduced systemic inflammation and significantly decreased plasma hs-CRP. They concluded that vitamin E reduced inflammation in diabetic people and can act as adjuvant therapy for prevention of atherosclerosis (34). Gomes et al. showed that uptake of vitamin E (1200 IU per day for two months) significantly altered total cholesterol and triglyceride levels in diabetic individuals compared to placebo (35). Khabbaz et al. showed that uptake of 800 IU of vitamin E for three months can reduce triglyceride levels in patients with type 2 diabetes, but has no significant effect on total cholesterol levels

(36). It has been suggested that vitamin C causes changes in LDL-C and HDL-C levels through two mechanisms. Through its antioxidant effect, it can reduce oxidation of LDL-C and increase recognition by its receptor. Due to structural similarities with glucose, vitamin C decreases glycosylation of lipoproteins, which in turn increases catabolism of LDL-C and reduces its serum levels. It also reduces excretion of HDL-C and consequently increases its serum level (37). It has been suggested that vitamin C is involved in improvement of glucose metabolism by scavenging oxygen free radicals (38). Vitamin C can also maintain the appropriate physicochemical integrity of cell membranes and active glucose carriers by increasing reduced glutathione (39).

CONCLUSION

Oral supplementation of 1000 mg of vitamin C and 400 IU of vitamin E can significantly reduce serum level of total cholesterol, triglyceride, hs-CRP, insulin and resistin in patients with type 2 diabetes. On the other

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hand, serum level of other biochemical variables may also change. Further studies with larger sample sizes are required to investigate the role of antioxidants in patients with type 2 diabetes and oxidative stress processes to determine the exact mechanism of effects of vitamins C and E.

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CONFLICT OF INTEREST

We have no conflict of interest to declare.

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