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## Efficacy of Supplementary Vitamins C and E on Anxiety, Depression and Stress in Type 2 Diabetic Patients: A Randomized, Single-blind, Placebo-controlled Trial

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**Abstract:** Diabetes mellitus as one of the most prevalent endocrine disease is associated with high oxidative stress. Anxiety, stress and depression are common neuropsychiatric features in diabetic patients. Hyperglycemia leads to increased oxidative stress which in turn diminishes antioxidant defense system. On the other hand oxidative stress is the leading cause of depression and anxiety disorders. Thus, it seems that diabetes could accelerate the trend of psychiatric diseases. In this randomized single-blind study, evaluation of the effects of two antioxidants (vitamin C and vitamin E) was done on Stress, depression and anxiety levels in 45 diabetic patients for six weeks. The patients were randomly divided in three groups of vitamin E (400 IU day<sup>-1</sup>), vitamin C (1000 mg day<sup>-1</sup>) and placebo. DASS-21 (Depression Anxiety Stress Scales 21-item) questionnaire items were read to each patient and completed by the main investigator of this study before and after six weeks of supplementation. The scores of depression, anxiety and stress were evaluated separately based on the DASS questionnaire. The results showed a significant decrease in anxiety level ( $p = 0.005$ ) in vitamin C group compared to other groups but there were no significant differences between groups in terms of changes in stress and depression scores. In conclusion, this study suggests that short term supplementation of vitamin C is safe and beneficial for reducing anxiety levels in diabetic patients through alleviating oxidative damage.

**Key words:** Vitamin C and E, diabetes melitus, complications

### INTRODUCTION

Increased oxidative stress, imbalance in antioxidant defense systems and also atherosclerosis are the main features in diabetes mellitus and its complications. (Baynes and Thorpe, 1999; Feillet-Coudray *et al.*, 1999; Maritim *et al.*, 2003). Also it has been suggested that hyperglycemia itself leads to increased production of free radicals which in turn enhances lipid peroxidation (Ceriello *et al.*, 1999; Feillet-Coudray *et al.*, 1999).

Oxidative stress can be a pathologic cause for some neuropsychiatric diseases such as schizophrenia and major depressive disorder (Bilici *et al.*, 2001; Valko *et al.*, 2007; Bouayed *et al.*, 2009). The potential vulnerability of the brain to antioxidant imbalances through oxygen consumption and lipid rich constructs suggests that oxidative damage might have a role in depression disorders and elevated anxiety levels (Bouayed *et al.*, 2009). It has been reported that stress itself causes neurotoxic damage through reactive radical species and in this way could affect synaptic plasticity and dendritic morphology (Kashif *et al.*, 2004).

Anxiety is a pathologic emotional state which has been implicated in depression. A causal relationship has been found between cellular oxidative stress, regulation of anxiety and emotional stress (Bouayed *et al.*, 2009).

Vitamin E (alpha-tocopherol) is a lipid soluble antioxidant which protects the brain tissues from oxidative damage by scavenging free radicals. Lower serum levels of vitamin E have been reported in major depression. (Kashif *et al.*, 2004; Owen *et al.*, 2005).

Vitamin C (ascorbic acid) is also another water-soluble antioxidant that could be helpful in reducing oxidative stress indirectly via restoring the reduced form of vitamin E and thus supporting its antioxidant activity (Sies *et al.*, 1992).

According to studies patients with major depression had significantly lower levels of vitamin E (Maes *et al.*, 2000; Owen *et al.*, 2005) and also vitamin C (Khanzode *et al.*, 2003) compared to healthy individuals. In addition plasma alpha-tocopherol was inversely related to depression score according to Beck Depression Inventory (Owen *et al.*, 2005).

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So far, no clinical study has ever evaluated the effect of vitamin E and C supplementation on depression and anxiety levels, therefore, this study has been conducted to clarify the efficacy of these two important antioxidants on stress markers, anxiety levels and depression.

**METHODS AND MATERIALS**

This randomized single blind placebo controlled clinical trial was conducted on 45 type 2 diabetic patients who had the eligibility to participate in this study after screening of 423 patients from Diabetes Association of Shiraz, Iran. Participants were nonsmoker, receiving standard oral hypoglycemic agents, had no history of overt vascular disease and also no clinical evidence of acute or chronic inflammatory diseases. The patients who were treated with lipid lowering drugs, antioxidant supplements, diuretics, β-blockers and aspirin were excluded. Participants were also asked not to change their oral hypoglycemic drugs.

All subjects were aware of the purpose and procedures of the trial. The research protocol of this trial was in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and approved by the Ethics Committee on Human Experimentation of Shiraz University of Medical Sciences.

Diabetic participants were divided in three groups of study by block randomization with fixed blocked size of three:

**Group 1:** Patients received one vitamin C capsule, 1000 mg daily for six weeks

**Group 2:** Patients received one vitamin E capsule, 400 IU daily for six weeks

**Group 3:** Patients received one placebo capsule (acetate cellulose), 1000 mg daily for six weeks

Vitamin C and vitamin E capsules were prepared from General Nutrition Center (GNC) in the USA and the placebo capsules were prepared by Shiraz school of pharmacy.

The items of DASS-21(Depression Anxiety Stress Scales 21-item) questionnaire were read to each patient before and after the intervention period by face to face interview.

DASS-21 questionnaire had 21 separate items. It was essential that each patient be given a score between 1 to 5 for each item (score 1 = completely different from me, score 2 = somewhat different, score 3 = no idea, score 4 = somewhat the same and score 5 = completely the same).

Nine items of this scale were related to anxiety, seven items showed depression and also four items were indicative of stress. The total sum of item numbers in each category showed the total score of anxiety, depression and stress separately in each participant.

**Statistical analysis:** Statistical analyses were done using SPSS version 16 (SPSS Inc., Chicago, IL) statistical software package. One-way ANOVA tests were used to compare the mean difference between three groups after checking the normality of distributed data and also post hoc test, tam han, were used to compare the two groups. Student’s paired t-test was also used to analyze changes in each group of study after intervention. p-values<0.05 were considered statistically significant.

**RESULTS**

Baseline characteristics of patients in three groups are demonstrated in Table 1. During the treatment phase of study, four patients were excluded (1 in vitamin C group due to herpes zoster , 1 in vitamin E group because

Table 1: Baseline patients’ characteristics and biochemical profiles

Parameters	Groups			p-value
	Vitamin C*	Vitamin E*	Placebo*	
N (female/male)	14 (10/4)	14(11/3)	13 (9/4)	0.847
Age (years)	47±8.93	48±6.28	46.61±7.58	0.889
Duration of diabetes (years)	4.57±4.2	5.14±3.82	4.92±4.78	0.938
Body mass index (kg m <sup>-2</sup> )	26.94±4.34	29.22±6.62	28.81±4.04	0.470
Waist to hip ratio	0.96±.07	0.95±.05	.97±.07	0.737
Metformin (g day <sup>-1</sup> )	1.14±0.63	1±.85	0.73±0.75	0.365
Glibenclamide (mg day <sup>-1</sup> )	8.75±10.59	7.5±8.49	9.61±8.02	0.833
Glu (mg dL <sup>-1</sup> )	131.14±32.79	157.78±50.21	138±39.92	0.228
TG (mg dL <sup>-1</sup> )	174.85±110.35	168.78±66.77	147.15±37.38	0.634
Chol (mg dL <sup>-1</sup> )	189.42±38.09	208.64±29.3	200.15±17.54	0.244
LDL-C (mg dL <sup>-1</sup> )	135.2±24.17	142.47±25.33	133.32±15.01	0.218
HDL-C (mg dL <sup>-1</sup> )	31.25±12.69	32.41±9.83	37.4±5.6	0.247

\*Data expressed as Mean±SD except N (No. of participants)

Table 2: Stress, depression and anxiety scores before and after supplementation in three groups of study

Parameters	Vitamin C			Vitamin E			Placebo			
	Before	After	p-value	Before	After	p-value	Before	After	p-value	p-value
Stress	16.71±3.04	16.85±2.07	NS	14.0±4.930	14.92±2.92	NS	11.53±3.04	14.53±3.17	0.002	0.104
Depression	20.64±5.52	18.64±8.14	NS	21.92±6.54	23.78±6.11	NS	20.23±5.65	21.15±8.09	NS	0.318
Anxiety	29.85±7.83	26.92±7.75	0.013	31.07±6.24	34.28±7.54	0.048	28.69±9.40	27.92±8.73	NS	0.005

Values are Mean±SD, NS: Not significant

of non-compliance and also 2 cases in placebo group due to non-compliance). Moreover, no serious side effects have been seen in each group.

Table 2 shows the mean score of stress, depression and anxiety before and after intervention in each group of study. There was a significant difference in anxiety scores (mean difference) between three groups of study ( $p = 0.005$ ). Based on *post-hoc* test, a significant difference ( $p = 0.027$ ) in stress score was observed between vitamin C and placebo groups and also a significant difference ( $p = 0.007$ ) in anxiety score between vitamin C and vitamin E groups after intervention period. According to paired t-test in each group of study, there was a significant increase in stress score in placebo group ( $p = 0.002$ ) and also a significant decrease in anxiety score in vitamin C group ( $p = 0.013$ ). In vitamin E group significant increment in anxiety score was seen after intervention ( $p = 0.048$ ).

## DISCUSSION

Our study evaluated the effect of vitamin C and vitamin E supplementation on depression, anxiety and stress levels. When comparing the efficacy of vitamin C, vitamin E and placebo on anxiety levels of these diabetic patients, after 6 weeks the beneficial and statistically significant result has been seen only in the vitamin C group. No significant effect has been observed in each group regarding depression. Although, no statistically significant change has been found in stress levels when comparing three groups with each other, in placebo group stress level increased significantly after 6 weeks. It could be inferred that elevation of stress was somehow hindered in the vitamin E and vitamin C group.

The results of our study demonstrated the beneficial and statistically significant effect of vitamin C supplementation in palliating anxiety levels in diabetic patients. There are some reports on a possible causal relationship between elevated oxidative stress and high anxiety levels in depression (Bouayed *et al.*, 2009). Moreover, hyperglycemia causes lipid peroxidation in lipid rich tissues of the brain (Baynes and Thorpe, 1999; Feillet-Coudray *et al.*, 1999; Bouayed *et al.*, 2009). This process results in oxidative damage through free radical

production. Malondialdehyde (MDA) is a highly toxic and the most important product of lipid peroxidation (Sies *et al.*, 1992). Vitamin C as a potent water soluble antioxidant is involved in radical scavenging by donating its electrons or restoring the reduced form of vitamin E (Bilici *et al.*, 2001; Maritim *et al.*, 2003; Kashinakunti *et al.*, 2011). According to the results of one study in diabetic patients vitamin C supplementation (1000 mg day<sup>-1</sup>) reduced MDA levels after 6 weeks (Mazloom *et al.*, 2011). Also an inverse significant correlation between serum MDA level and vitamin C has been found in chronic smokers compared to non-smokers (Kashinakunti *et al.*, 2011).

So, vitamin C could have a positive role in diminishing anxiety through its antioxidant properties. This effect has not been seen in vitamin E group due to different reasons such as short duration of our study or low effective dose of vitamin E.

Although some few clinical trials have assessed the effect of vitamin C or vitamin E separately on major depression, no study has been conducted to compare the efficacy of these two antioxidants in the context of depression, anxiety and stress in diabetic patients.

In a randomized double-blind, placebo-controlled 14-day trial by Brody *et al.* (2002) efficacy of sustained-release ascorbic acid in 60 healthy young adults (3×1000 mg day<sup>-1</sup> Cetebe) and placebo (60 healthy young adults) was assessed. Based on the results ascorbic acid compared to the placebo alleviated the subjective response to acute psychological stress (According to Trier Social Stress Test, TSST, consisting of public speaking and mental arithmetic).

In another randomized double-blind, placebo-controlled 14 day trial, ascorbic acid supplementation caused a decrease in Beck Depression scores (Brody, 2002).

In an animal study conducted by Binfare *et al.* (2009) the antidepressant-like effect of ascorbic acid was evaluated in the Tail Suspension Test (TST) and in the Forced Swimming Test (FST) in mice in comparison to fluoxetine, imipramine and bupropion. Results revealed that ascorbic acid had an antidepressant-like effect in TS. Moreover, ascorbic acid caused a synergistic antidepressant-like effect in combination with conventional antidepressants.

In another study effect of alpha-Tocopherol was evaluated in an animal models of depression . In long term treatment with alpha-T (10 mg kg<sup>-1</sup>) increased the glutathione (GSH) antioxidant defense system, glutathione peroxidase and glutathione reductase activity in the hippocampus and in the prefrontal cortex (Lobato *et al.*, 2010).

The limitations of the present study were as follows: small sample size in each group, short duration of trial, inadequate doses of vitamin C and E, lack of the measurement of serum vitamin C and E at the baseline and also at the end of the trial.

### CONCLUSION

In conclusion, the present findings indicated that vitamin C as a potent water-soluble antioxidant could be helpful in reducing anxiety in diabetic patients.

However, further studies are needed to assess the effects of these antioxidants on oxidative markers and glycaemic control to determine the exact molecular mechanisms underlying antioxidant therapy in psychiatric disorders.

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