

Effect of Vitamin E and C Supplementation on Oxidative Stress in Diabetic Patients

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Abstract Background: Diabetes is a metabolic epidemic that causes vascular complications. The presence of oxidative stress in diabetes patients and the preventive role of vitamins therapy have been reported by many researchers. Vitamins supplementation improves antioxidant defense system in diabetes patients. **Aim:** To study the effect of vitamin E and C supplementation on oxidative stress in diabetes patients. **Methods:** Subjects enrolled in the study received 500 mg of both vitamin C and vitamin E daily twice for 4 months under medical supervision. Fasting blood glucose, MDA, catalase, SOD and nitric oxide were determined. Serum vitamin E and plasma vitamin C were also measured. Statistical analysis was performed using SPSS 12.0. Numerical normally distributed and categorical data were compared using independent t-test. Data were presented as means \pm SD. **Results:** After supplementation with vitamin E and C in diabetic patients, a signify decrease in FBS, MDA levels and increase in serum nitrite, erythrocyte SOD, blood catalase, vitamin E and vitamin C levels were observed. A negative correlation between MDA and vitamins was observed. **Conclusion:** Vitamin E and C supplementation is useful for the treatment of oxidative stress related complications in diabetes patients. Prescribed medicines contain active ingredients that may cause effect on the patients in terms of side-effects. Controlled vitamin therapy for prolonged period may not cause any side-effects, as well as play effective role for the management of type 2 diabetic related oxidative stress.

Keywords Type 2 Diabetes, Oxidative Stress, Vitamin E and C

1. Introduction

Diabetes mellitus is metabolic epidemic that occur due to ineffective secretion of insulin from pancreas [1, 2] and the possibly the worldwide number of diabetic patient will be rise up to 439 million by 2030 [3, 4]. Vitamin E supplementation could improve glycemic control in diabetes

patients [5, 6]. Vitamin C supplementation is effective in prevention of non-enzymatic glycosylation of proteins [7] and it also serves as therapeutic agent for diseases that protects body from damage caused by free radicals [8].

The reactive oxygen species (ROS) can damage cellular bio-molecules (protein, nucleic acid etc.) as well as plasma membrane. The malondialdehyde (MDA) is reported as indicator of lipid peroxidation reactions [9]. Involvement of Nitric oxide (NO•) in smooth muscle relaxation, cytotoxic reactions, and neuronal transmission [10] is reported in the literature. Currently researchers are focusing on the role of NO• in the development of uremic symptoms [11, 12].

In addition, some vitamins can prevent the harmful effects of free radicals by non-enzymatic modes like in both vitamin E (α -tocopherol) and C (ascorbic acid). The α -tocopherol prevents damage to polyunsaturated fatty acids by free radicals in membranes [13].

The present study planned to investigate the possible alterations of oxidant - antioxidant status in diabetic patients and effect of vitamin E and C supplementation. This study included fifty diabetic patients and control, in the mean age of 52.3 ± 9.62 years.

2. Materials and Methods

Present study was performed at the Rama Hospital, Rama Medical College (NCR), UP [India], as a randomized controlled trial and with parallel design. According to ADA [14], the fifty patients with type 2 diabetic of mean age 52.3 ± 9.62 years were selected for the study. Alcoholics, Smokers, patients with chronic inflammatory conditions, or hepatic or respiratory diseases were excluded from the study.

The study was reviewed and approved by Ethics Committee, Rama hospital, Rama Medical College (NCR), UP [India]. Subjects enrolled in the study received 500 mg of vitamin C and 500 mg of vitamin E, daily twice for 4 months under medical supervision. The age, weight, sex, height, diabetes duration, blood pressure were examined ($\geq 130/\leq 80$ mm Hg) and recorded. Venous blood (10 ml) was collected by using standard laboratory methods at each study point and

used for various estimations.

Malondialdehyde (MDA) was estimated by the modified protocol of Mossa *et al.* [15]. MDA in serum was separated and determined as conjugate with TBA. The MDA-TBA complex was measured at 534 nm.

Catalase activity was determined by modified protocol of Goth [16]. Serum catalase activity is linear up to 100 kU/l.

The SOD activity was assayed by the modified method of Kakkar *et al.* [17]. The SOD activity was measured by the inhibition of the reduction of nitroblue-tetrazolium by Superoxide anion produced by potassium Superoxide (K^+ , O_2^-) dissolved in dimethyl sulfoxide.

Nitric oxide was determined by Cortas and Wakid method [18]. Nitric oxide is a labile and diffusible molecule, which forms stable metabolites (nitrite / nitrate, NO_2^- and NO_3^-), which are detected by Griess reaction.

Serum vitamin E was measured by their reduction of ferric to ferrous ion, which then forms a red colored complex with α - α' -bipyridyl in Baker and Frank method [19]. Plasma vitamin C was determined by DNPH method [20], where vitamin C is oxidized to diketogulonic acid, which reacts with 2,4 dinitrophenylhydrazine to form diphenylhydrazone.

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS 12.0, Chicago IL).

Numerical normally distributed data and categorical data were compared using independent t-test. Significance was considered to be $P < 0.05$. Results were given with their 95% CIs. Data were presented as means \pm SD. Numerical normally distributed data and categorical data were compared using independent t-test.

3. Results and Discussion

All the selected type 2 Diabetes mellitus patients were supplemented with 500 mg/day twice of both vitamin E and C for four months. After two months of supplementation all the parameters mentioned above were studied again.

Reduced vitamin E and C levels were lower and malondialdehyde levels (MDA) were higher in type 2 diabetes mellitus patients compared to healthy controls ($p < 0.001$). Table depicts, after vitamin supplementation, causes significant reduction in fasting blood sugar [FBS], significant increase ($p < 0.001$) in concentration of SOD, Nitrite, catalase in type 2 DM group as compared to controls. Significant fall ($p < 0.001$) in antioxidants like serum MDA was observed in type 2 DM as compared controls. $P^* < 0.05$ $** < 0.001$ compared to before treatment [Table 1, Figure 1].

Table 1. Levels of biochemical parameters in diabetic patients and controls before and after supplementation with doses of vitamin C and E [(Data are mean \pm SD)]

Parameters (Tests)	Control	Diabetic Group [Mean \pm SD]	Diabetic with Vitamin E and C Supplementation		P Value
			2 Month [Mean \pm SD]	4 Months [Mean \pm SD]	
FBS (mg/dl)	102 \pm 4.64	162.78 \pm 4.82	147.09 \pm 7.05*	49.73	0.00001
Serum MDA (μ mol / l)	1.95 \pm 0.54	3.72 \pm 0.30	3.00 \pm 0.41*	2.74 \pm 0.40	< .001
Erythrocyte SOD (U/ mg Hb)	1.54 \pm 0.62	0.82 \pm 0.10	1.02 \pm 0.18	1.20 \pm 0.38	< .001
Serum Nitrite (μ mol / l)	67.42 \pm 4.08	59.26 \pm 7.4	64.52 \pm 4.5	66.94 \pm 5.5	< .005
Blood Catalase (k/gm Hb)	291.55 \pm 13.47	210.08 \pm 23.78	233.62 \pm 19.54	244.58 \pm 21.81	< .001
Serum Vitamin E (mg/dl)	1.24 \pm 0.21	0.86 \pm 0.06	1.75 \pm 0.21	1.92 \pm 0.22	< .001
Plasma Vitamin C (mg/dl)	1.55 \pm 0.41	0.78 \pm 0.08	1.32 \pm 0.21	1.47 \pm 0.16	< .001

Values were expressed as Mean \pm SD, indicates $p < 0.001$ (unpaired 't' test)

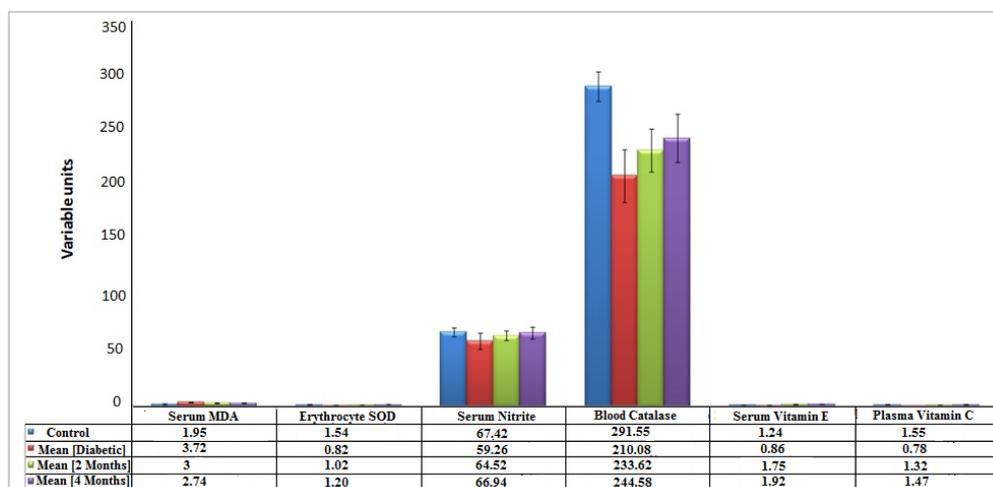


Figure 1. Standard deviation graph showing mean values and positive, negative error of serum MDA, erythrocyte SOD, serum nitrite, blood catalase, vitamin E and vitamin C of control group [blue bars], diabetic group before [red bars], after two months [green bars], and after four month [purple bars] of supplementation / treatment with doses of vitamin C and E [(Data are mean \pm SD)].

A negative correlation of MDA with vitamin E and vitamin C was found. Figure 1 describes about standard deviation showing mean values and positive, negative error of serum MDA, erythrocyte SOD, serum nitrite, blood catalase, vitamin E and vitamin C before and after supplementation / treatment with doses of vitamin C and E [(Data are mean \pm SD)].

Vitamin E ameliorates oxidative stress in type 2 diabetes mellitus patients and improves antioxidant defense system. However, vitamin E does not have any advantage for metabolic parameters [21]. In the present study, the increased serum MDA level in diabetic patients indicates that indeed there is oxidative stress. Further increase in serum MDA levels shows that the oxidative stress has increased in these patients. Oxidative stress may increase the synthesis of asymmetric dimethyl arginine (ADMA), which is an endogenous inhibitor of endothelial nitric oxide synthase [22].

The most probable explanation for decreased SOD activity is a possible direct inactivation of the enzyme by its product hydrogen peroxide, or by superoxide anion itself [23]. Decreased SOD activity could also be related to trace element deficiencies in patients [24].

Significant decrease in the activity of catalase could be due to less availability of NADPH [25]. Our study shows a significant decrease in catalase activity. This decrease could be due to increase in MDA [26]. Vitamin E is a lipophilic antioxidant. Vitamin E radical formed by free radical attack interact with vitamin C and regenerate vitamin E. In the process vitamin C is consumed and vitamin E is formed [27, 28].

4. Conclusions

Previous research findings explain the presence of oxidative stress in diabetes patients and the preventive role of vitamins therapy. Results of present study suggested that, vitamin E and C supplementation is useful for the treatment of oxidative stress related complications in diabetes patients. Prescribed medicines contain active ingredients that may cause effect on the patients in terms of side-effects. Controlled vitamin therapy for prolonged period not causes any side-effects, as well as play effective role for the management of type 2 diabetic related oxidative stress.

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Conflicts of Interest

There is no conflict of interest to declare.

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