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Original Research Article

To Study the Effect of Vitamin C and Vitamin E on Fasting Blood Glucose Level and Lipid Profile in Type-2 Diabetes Mellitus Patients

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ABSTRACT

Introduction: Diabetes Mellitus (DM) is third commonest disease in the world and one of the leading causes of death. It is associated with several mechanisms, one of which is oxidative stress. Vitamins C and Vitamin E causes detoxification of free radicals directly.

Aim: We had studied the effect of vitamin C and E on fasting blood glucose and plasma lipid levels in individuals with type 2 diabetes mellitus as these vitamins are known for their helpful effects on plasma lipids and blood sugar level.

Material & Method: Fifty type 2 DM patients of the age group 40 to 80 years of both sexes were selected. Each patient received vitamins per day for twelve weeks. During the study period the treatment and diet was not changed. Fasting blood glucose and lipid profile were determined in baseline and after twelve weeks with receiving supplement.

Result: The mean fasting blood glucose, low density lipoprotein cholesterol, total cholesterol, serum triglyceride and serum high density lipoprotein values in type 2 DM patients before and after supplementation of Vitamin C and E were measured. When these values were compared, using 'Paired t test', it showed significant (P<0.05) reduction in glucose and lipid profile (TC, TG, LDL, VLDL) in concordance with a significant elevation in HDL (P<0.05).

Conclusion: Supplementation with vitamin C and E causes an improvement in blood glucose and lipid level in patients with Type 2 Diabetes.

Key words: Vitamins C, Vitamins E, Type 2 Diabetes Mellitus, Oxidative Stress, Lipid.

INTRODUCTION

Diabetes mellitus is a heterogeneous group of metabolic disease that is characterized by chronic hyperglycemia and instability in carbohydrate, lipid and protein metabolism resulting from defects in insulin secretion and/or insulin action. Systemically, these perturbations are accompanied with changes in a variety of

biochemical processes and are exacerbated by overweight and obesity, altered lipid profile, smoking and/or genetic profile. In these composite risk factors, gathering evidence suggests that reactive oxygen species (ROS) might play an important role in initiation, progression and complications of diabetes mellitus.⁽²⁾

The cluster of lipid abnormalities associated with type-2 diabetes is defined by increase in triglycerides (TG) and small, dense low-density lipoproteins (LDL) concentrations and decrease in high-density lipoprotein (HDL) cholesterol. ⁽³⁾ Lipid profile which is altered in diabetes state is one of the significant factors in development of cardiovascular diseases.⁽⁴⁾ Also oxidative modification of LDL is an important step in the development of atherosclerosis. Reactive oxygen species (ROS) can stimulate oxidation of low density lipoproteins (LDL). and oxidized low density lipoprotein, which is not recognized by the LDL receptors, can be taken by scavenger receptors in macrophages leading to foam cell formation and atherosclerotic plaques. ⁽⁵⁾

Hyperglycemia can directly cause increased reactive oxygen species (ROS) generation. Glucose can undergo autoxidation and generate free radicals. Reactive oxygen species can be eliminated by a number of enzymatic and nonenzymatic antioxidant mechanisms.⁽⁶⁾

Nonenzymatic antioxidants include vitamin A, C and E; glutathione; α -lipoic acid; carotenoids; trace elements like copper, zinc and selenium; and co-factors like folic acid, uric acid, albumin and vitamins B1, B2, B6 and B12. Alterations in the antioxidant defence system in diabetes have recently been reviewed.⁽⁷⁾

High doses of vitamins C and vitamin E have been shown to decrease blood glucose, plasma cholesterol and triglyceride in T2DM patients.⁽⁸⁾ Vitamin C is structurally similar to glucose and can replace it in many chemical reactions, and thus is effective in prevention of nonglycosylation of proteins. enzymatic Vitamin C has a hypocholesterolemic effect. By preventing oxidation of low density lipoprotein cholesterol, it decreases LDL cholesterol as well as total cholesterol. It also raises HDL cholesterol levels.

Vitamin E is a lipid soluble antioxidant and protects LDL cholesterol particles from oxidative attack. Vitamin E has benefits in preserving HDL antioxidant function in diabetic subjects.

With the above background the present study aims at evaluating the effect of Vitamin C and Vitamin E on fasting blood sugar and lipid profile parameters on type 2 diabetes mellitus patients.

MATERIALS AND METHODS

This is Open Label Randomized Prospective Clinical Trial also called 'Before and after' study carried out in the tertiary care centre. The study subjects were selected from diabetic outpatient department. Fifty known type 2 diabetes mellitus patients between the age group 40 to 80 years of both sexes on oral hypoglycemic drugs, with mean duration of disease 1 to 8 years and having blood glucose level less than 250 mg/dl were selected (n=50). Type 2 DM patients having blood glucose levels higher than 250 mg/dl, were excluded. Patients with rapidly progressive retinopathy, neuropathy. nephropathy, hypertension, hepatic and renal failure and patients taking insulin were excluded.

A detailed history, thorough general and systemic examinations were done. Age, body weight, standard height, body mass index (BMI) and blood pressure were measured. After written and informed consent, Tablet Limcee (500 mg bd) containing Vitamin-C or Ascorbic acid and Monovit EN 100 mg od (containing vitamin E 150 IU) was given for twelve week to every patient. During the study period the treatment plan was not changed. They were instructed not to change diet during the study period. They were interviewed regarding any changes in lifestyle or abnormal events such as disease or infection at each examination. None of the patients

used additional oral vitamins, either prior to or during the study period. After 12 weeks the investigations were performed.

Under all aseptic condition, 12 hour fasting venous blood samples were collected from all participants in plain and fluoride bulbs. All samples were taken in the morning to avoid the confounding effect of diurnal variation of oxidative stress parameters as reported previously. ⁽¹⁰⁾ Serum was separated after 1 hour by centrifugation at 3000 rpm for 10 minutes, and was tested for various parameters. The following investigations were done 'Before' and 'After' the supplementation of vitamin C and vitamin E.

- I. Blood glucose level
- II. Serum cholesterol
- III. Serum Triglycerides
- IV. Serum HDL
- I. Blood Glucose Level: Quantitative estimation done by glucose oxidase peroxidase end point method (GOD-POD) using commercial kits from ERBA diagnostics. ⁽¹¹⁾ Normal range: 70-110 mg/dl.
- II. Serum Total cholesterol: Quantitative estimation done by Cholesterol oxidase peroxidase end point method (CHOD-POD) using commercial kits from RECKON diagnostics. Normal range: Up to 200 mg/dl
- III. Serum HDL: Quantitative estimation done by Phosphotungstic Acid (PTA) end point method using commercial kits from RECKON diagnostics. Normal range: 30 - 70 mg/dl
- IV. Serum Triglyceride: Quantitative estimation done by Lipase/ Glycerokinase/ Glycerophosphate oxidase (GPO) end point method using commercial kits from

RECKON diagnostics. Normal range: Up to 150 mg/dl

V. Serum VLDL and LDL : Friedewalds formula VLDL (mg/dl) = TG/5

LDL (mg/dl) = Total Cholesterol - (HDL + VLDL)

RESULTS

In this study, fasting blood glucose and plasma lipid levels values in type 2 DM before and after supplementation of Vitamin-C (Tablet Limcee) 500 mg bd and vitamin E 150 IU daily for 12 weeks were estimated and compared. For this parameter, the mean value and standard deviation (SD) were calculated in study group. 'Paired t test' was applied to test whether the differences in means were statistically significant or not. P-value less than 0.05 (P < 0.05) was considered to be statistically significant. P-value of less than 0.001 (P < 0.001) was considered to be statistically highly significant. The results of the present study are as follows.

- The mean duration of Type 2 Diabetes Mellitus in the study subjects was 4.17± 1.74 years (Table-A).
- 2) The mean values for the age, body weight, height and body mass index in the study group were 54.03 ± 7.22 years, 69.31 ± 7.71 kg, 161.08 ± 8.32 cm and 26.65 ± 2.32 kg/m² (Table-A).

group		
Sr. No.	Parameter	Subjects
1.	Age(years) (Mean \pm SD)	54.03 ± 7.22
2.	Male : Female ratio	1:1.24
3.	Weight(kilograms) (Mean \pm SD)	69.31 ± 7.71
4.	Height(centimetres) (Mean \pm SD)	161.08 ± 8.32
5.	Body Mass Index (kg/m ²) (Mean \pm SD)	26.65 ± 2.32
6.	Mean Duration Of Disease (years) (Mean \pm SD)	4.17±1.74

Table A: Table showing the demographic data of the study group

Study Subjects	Fasting BSL	P Value
	(Mean \pm SD)	
Pre Vitamin-C and	146.9 ± 36.16	
Vitamin-E		P < 0.05*
Supplementation.		
Post Vitamin-C and	138.1±34.28	
Vitamin-E		
Supplementation		

 Table B: Table showing Fasting blood glucose level (mg/dl)

 before and after supplementation of Vitamin-C and Vitamin-E

P < 0.05*:- Statistically significant.

Table C: Table showing Serum Cholesterol Levels (mg/dl) before and after Supplementation with Vitamin C and Vitamin E.

	Mean	
Test	(Mean \pm SD)	P-Value
SCH- Pre	217.32±29.41	
SCH-Post	158.53±25.47	P < 0.001 **

P < 0.001**– Statistically highly significant. SCH- Pre- Serum cholesterol before supplementation with vitamin C & vitaminE.

SCH-Post - Serum cholesterol after supplementation with vitamin C & vitamin E

 Table D: Table showing serum triglyceride levels (mg/dl)

 before & after supplementation with vitamin C and vitamin E

		Mean	
	Test	(Mean \pm SD)	P-Value
	TG-Pre	234.66±55.41	
	TG-Post	163.17±34.32	P < 0.001 **
001.	* G	11 1 1 1 1	

P < 0.001 ** - Statistically highly significant.

TG-Pre - Triglycerides before supplementation with vitamin C & vitamin E.

TG-Post - Triglycerides after supplementation with vitamin C & vitamin E.

 Table E: Table showing serum high density lipoprotein levels (mg/dl) before & after supplementation with vitamin C and vitamin E

Test	Mean (Mean ± SD)	P-Value
HDL-Pre	37.25±4.3	
HDL-Post	42.19±3.6	P < 0.05*

P<0.05* - Statistically significant.

HDL-Pre- High density lipoprotein before supplementation with vitamin C and vitamin E.

HDL-Post - High density lipoprotein after supplementation with vitamin C and vitamin E.

Table F:Table showing serum low density lipoprotein levels (mg/dl) before & after supplementation with vitamin c and vitamin \underline{E}

Test	Mean	
	(Mean \pm SD)	P-Value
LDL-Pre	133.14±33.65	
LDL-Post	83.71±13.54	P < 0.001 **

P < 0.001 ** - Statistically highly significant.

LDL-Pre - Low density lipoprotein before supplementation with vitamin C and vitamin E.

LDL-Post - Low density lipoprotein after supplementation with vitamin C and vitamin E.

Table G: Table showing serum very low density lipoprotein		
levels (mg/dl) before & after supplementation with vitamin C		
and vitamin E		

Test	Mean	
	(Mean \pm SD)	P-Value
VLDL-Pre	46.932±11.02	P < 0.05*
VLDL-Post	32.634±6.86	SIGNIFICANT

VLDL-Pre- Very low density lipoprotein before supplementation with vitamin C and vitamin E.

 $\mbox{VLDL-Post}-\mbox{Very}$ low density lipoprotein after supplementation with vitamin C and vitamin E.

DISCUSSION

Type 2 diabetes is the commonest form of diabetes and associated with multiple metabolic derangements that result in the excessive production of reactive oxygen species (ROS) and oxidative stress. ⁽¹²⁾ Oxidative stress and resultant tissue damage are hallmarks of chronic disease and cell death. There is increasing evidence that, in certain pathological states, the increase production and / or ineffective scavenging of such reactive oxygen species may play a crucial role in determining tissue injury. Due to these events, the balance normally present in cells between radical formation and protection against them is disturbed. This oxidative leads to damage of cell components such as proteins, lipids and nucleic acids. There is a correlation between impaired glycemic control and enhanced lipid peroxidation.

Studies have demonstrated that antioxidant vitamins and supplements can help in lowering the markers indicative of oxidative stress and lipid peroxidation in diabetic subjects and animals. The most frequently studied antioxidant vitamins are Vitamin C and E. Vitamin C is a hydrophilic molecule that can scavenge radicals, among them the hydroxyl radical. Vitamin C is the strongest physiological antioxidant acting in the organism's aqueous environment. ⁽²⁾

Vitamin E plays a protective role in prevention of oxidation of unsaturated fats. In absence of vitamin E, the quantity of unsaturated fats in the cells becomes diminished, causing abnormal structure and function of such cellular organelles as mitochondria, lysosomes, and even the cell membrane. ⁽¹³⁾

 α -Tocopherol (Vitamin E) is reconstituted when ascorbic acid recycles the tocopherol radical; dihydroascorbic acid, which is generated and recycled by glutathione. Vitamin E, a component of the total peroxyl radical-trapping antioxidant system reacts directly with peroxyl and superoxide radicals and singlet oxygen and protects membranes from lipid peroxidation. (14)

The present study was undertaken to study the combined antioxidant effect of vitamin C and vitamin E on fasting blood sugar and lipid profile parameters before and after supplementation in type-2 diabetes mellitus patients.

The results obtained in the present study showed that with vitamin C and vitamin E there was significant (P<0.05) reduction in glucose and lipid profile (TC, TG, LDL, VLDL) in concordance with a significant elevation in HDL. (P<0.05)

Thus in the present study we observed that supplementation of 1000 mg of vitamin C and 150 IU of vitamin E daily for 12 weeks in type-2 diabetes mellitus patients causes a significant fall in their fasting blood sugar levels, serum cholesterol levels, low density lipoprotein-cholesterol, VLDL levels and serum triglyceride levels and elevation of high density lipoproteincholesterol levels.

Vitamin C and vitamin E are important antioxidant in humans, able of scavenging oxygen-derived free radicals, improved hyperlipidemia and decreased blood pressure. ⁽¹⁵⁾ Several studies showed increased oxidative stress, and decreased basal vitamin C and vitamin E levels in diabetic patients. ⁽¹⁶⁾

Decreased serum cholesterol levels after administration of vitamin C is related to decreased blood sugar levels rather than vitamin C directly acting on serum cholesterol. However more light on the effect of vitamin C on serum cholesterol and other lipid parameters can only be focused when Vitamin C administration will be studied in dyslipidemia or hypercholesterolemia.

The present study demonstrated that in people with diabetes, increased production of triglycerides and LDL cholesterol occurred in association with reduced levels of HDL cholesterol. This was similar with Budin et al (2009), who studied that both lipid accumulations particularly triglycerides and reduction in antioxidant activity contributed to the development of oxidative stress in diabetes. ⁽¹⁷⁾

Hyperglycemia found was to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxideresulting dependent pathway in the generation of free radicals. It may be that hyperglycemia could be an initiation step for induction of oxidative stress. ⁽⁶⁾ Vitamins C and vitamin E have an important function in glucose metabolism. Reduced levels of antioxidants such as vitamin C and vitamin E occurred in people with diabetes. ⁽¹⁸⁾

The findings of this study can be related with the other studies. Paolisso et al. (2007) also reported beneficial effects of oral vitamin C (1000 mg/day for 4 months) on glucose, lipid metabolism, and free radicals in T2DM. ⁽¹⁹⁾ In this work the efficiency of vitamin E against lipid peroxidation was apparent through the of susceptibility reduction the of erythrocytes to hydrogen peroxide-induced lipid peroxidation and a potent lipophilic agent that forms an important scavenger component of the cell membrane.⁽²⁰⁾ It may protect the safety of the membrane by reducing the production of lipid peroxides. ⁽²¹⁾ Other study suggested that vitamin E administration is associated with decreased glycosylation of hemoglobin, some serum

proteins, and decreased susceptibility of LDL to oxidation. Vitamin C is required for regeneration of α -tocopherol (vitamin E) and may thus prevent LDL oxidation, and transport of α -tocopherol in HDL may enhance and preserve these protective antioxidant effects of HDL. Vitamin E supplementation may have special benefits in preserving HDL antioxidant function in diabetic subjects. ⁽²²⁾ It has been shown decreased levels of lipid profile, lipid peroxidation and free radical production by vitamin E and vitamin C supplementation. ⁽²³⁾

V Peponis et al (2002) had given vitamin C (1000 mg/day) and vitamin E (400 IU/day) supplementation for 10 days to 50 patients with non-insulin dependent diabetes mellitus. Nitrite levels in tears were measured by photometric determination before and after vitamin supplementation. Nitrite levels were found to be significantly reduced after 10 days of vitamin C and vitamin E supplementation. Antioxidants, such as vitamin C and vitamin E, probably have an important role in reducing the oxidative damage produced by nitric oxide. (24)

Eriksson J et al (1995) observed that supplementation of 2 gm of vitamin C for 12 weeks in type-2 diabetes mellitus patients show an improvement in fasting blood sugar, cholesterol and triglycerides. The results suggested in his study that high-dose vitamin C supplementation may have a beneficial effect in type-2 diabetes subjects on glycemic control and blood lipids. ⁽²⁵⁾

The hypocholesteroleamic effects of vitamin C could be due to its direct effect as an antioxidant, in addition to its cholesterol lowering potential due to the effect on cholesterol metabolism directly in the liver. In support of this hypothesis the serum cholesterol decreased and in vitro activities of hydroxyl methyl-glutaryl-CoA reductase and sterol-o acyl transferse, the key enzyme

in cholesterol metabolism was inhibited by high dose of vitamin C. ⁽²⁶⁾ Also vitamin C may inhibit the absorption of cholesterol and bile acid in the intestine and increase the excretion with wastes. This leads to a reduction in cholesterol levels by the liver. ⁽²⁷⁾ As well as it may be due to an inhibition of TG synthesis by the increasing lipoprotein lipase activity, which is an insulin-dependent enzyme, since the lipoprotein lipase synthesis is defective in (28) diabetic patient. Also vitamin C contributes to the decreased synthesis of TG by liver through inhibiting fatty acid production.⁽²⁹⁾

Actually in diabetes, the oxidative stress is increased because of the deficiency in the antioxidant defense, so the intake of antioxidant such as vitamin C (powerful natural antioxidant) may reduce the oxidative stress associated with diabetes and hence help to restore the antioxidant defense system by reducing free radical. Vitamin C supplementation was able to normalize endothelial function and decrease oxidative stress to normal levels in type 1 and 2 diabetic patients. (30; 31)

Marc P. McRae (2006) studied the correlation between serum cholesterol and plasma vitamin C concentration. He found an inverse correlation (r = -0.500, p < 0.005) between the two. ⁽³²⁾ The findings of his study are consistent with the present study. Supplementation with high dose of vitamin plasma С increases the vitamin С leading concentration to subsequent decrease in serum cholesterol.

The above studies clearly showed that supplementation with high doses of vitamin C and vitamin E for a particular period will definitely improve the glycemia control and lipid profile in Type-2 diabetes mellitus patients.

In type-2 diabetes mellitus patients lipid abnormalities significantly contribute to complications of diabetes. Diabetes

mellitus is characterized by hyperglycemia together with biochemical alterations of glucose and lipid peroxidation. Some complications of diabetes mellitus are associated with increased activity of free radical-induced lipid peroxidation and accumulation of lipid peroxidation products. ⁽³³⁾ The major carrier of cholesterol and triglycerides in plasma is low-density lipoprotein (LDL). LDL can infiltrate the intimal layer of arteries and undergo oxidation locally, although the mechanism of oxidation is not fully understood. Oxidized LDL activates adhesion factor expression in endothelial cells. This induces monocytes to adhere to endothelium, where they are activated to differentiate into macrophages, in part via cytokines also induced by oxidized LDL. Macrophages accumulate oxidized LDL and remain in the vascular wall, developing into foam cells and subsequently into fatty streaks, the telltale lesion of atherosclerosis. If vitamin C and vitamin E reduces oxidants, LDL oxidation should be decreased.⁽²³⁾

The antioxidant balance is disturbed in diabetes due to hyperglycemia. When glucose is catabolised it converts into sorbitol by polyol pathway which leads to consumption of NADPH (a coenzyme in the production of reduced glutathione). Due to this depletion of NADPH reduced glutathione which itself is an antioxidant will not be available for breakdown of hydrogen peroxide to water and oxygen leading to increased oxidative stress. This reduced form of glutathione itself gets oxidised and converts oxidised vitamin C into reduced vitamin C which acts as an antioxidant and scavenge the free radicals.

Nonenzymatic glycation of lipids and proteins also contributes to increased oxidative stress in diabetes. Oxidative stress induced by reactive oxygen species (ROS), which is generated by hyperglycemia, is one of the major foci of recent research related to diabetes mellitus. ⁽³⁴⁾

The impact of supplementation of vitamin C and vitamin E on the lipid profile merits attention. It is well known that hyperlipidemia is a secondary consequence of hyperglycemia and diabetics are at great risk of developing coronary heart disease (CHD). ⁽⁴⁾ In light of this fact it is encouraging that vitamin C and vitamin E can act as a hypolipidemic agent reducing the risk of complications in diabetics. Thus vitamin C and E may be used as a supportive therapy for diabetics.

Apart from using vitamin C and vitamin E many other antioxidants including spices and natural products were also used in other studies to observe their effects on blood glucose and lipid profile in type-2 diabetes mellitus patients.

Antioxidant potency of vitamins is limited because these antioxidants work as scavengers for existing reactive species and this approach represents a symptomatic approach to oxidative stress associated clinical problems. If the antioxidants block the formation of free radicals due to hyperglycemia than it will provide a more targeted approach for use of antioxidants in the treatment plan of diabetes.

SUMMARY AND CONCLUSION

The present study was carried out to see the effect of antioxidant vitamin C and vitamin E on fasting blood glucose and lipid profile in Type-2 diabetes mellitus patients.

This study provides evidence that hyperglycemia plays a significant role in hyperlipidemia. Supplementation of vitamins C and vitamin E to Type-2DM patients might improve endogenous antioxidant capacity due to reducing blood glucose and lipid metabolites, and they may play a role in preventing complications in Type 2 diabetes. Derangements of lipid profile and blood sugar in diabetes patients leads to development of various types of complications. Hyperglycemia is the main cause of generation of free radicals leading to increased oxidative stress and complications in diabetes patients.

The lipid profile parameter which is most affected by oxidative stress is the LDL- cholesterol level. The LDL gets oxidized by free radicals and oxidized LDL is not recognized by LDL receptors. α tocopherol (vitamin E) is a lipid soluble antioxidant which protects LDL particles from oxidative attack. Vitamin C is required for regeneration of α - tocopherol and may thus prevent LDL oxidation.

In conclusion, supplementation with 1000 mg/day of vitamin C and vitamin E 150 IU/day in addition to the normal diet and treatment schedule may help in improving blood glucose and lipid profile in patients with type-2 diabetes.

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