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Research Article

EFFECT OF ORAL SUPPLEMENTATION OF VITAMIN C ON GLYCEMIC CONTROL AND LIPID PROFILE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Objective: Diabetes mellitus is one of the most common metabolic disorders which cause micro and macro vascular complications. The present study was designed to investigate the effect of vitamin C oral supplementation on glycaemic control and lipid profile in patients with type 2 diabetes mellitus.

Methods: Sixty two patients with type 2 diabetes mellitus were enrolled from Government Headquarters Hospital, Ootacamund, India, in a prospective, open label, randomized controlled trial. Patients were randomized into control and intervention groups. The control group received only oral hypoglycaemic agents whereas the intervention group received vitamin C (500 mg/day) oral supplementation along with oral hypoglycaemic agents for a period of 3 months. HbA_{1C}, fasting blood sugar, blood pressure, body weight, lipid profile, urea nitrogen, creatinine, total protein, ascorbic acid and oxidative stress were measured at the base line and at the end of 3 months by using specific methods.

Results: The results reveal that 3 months of vitamin C supplementation significantly improves the total cholesterol (mean ± SD 196.66±35.54 vs 178.16±26.61; $p<0.05$), LDL cholesterol (mean ± SD 113.67±27.64 vs 96.42±27.80; $p<0.05$) and urea nitrogen (mean ± SD 33±5.92 vs 29.71±7.27; $p<0.05$). Though not statistically significant, promising effects are also observed with respect to other variables.

Conclusion: Supplementation of vitamin C oral supplementation is found to be effective and has potential implications for the prevention of further complications in patients with diabetes mellitus.

Keywords: Diabetes Mellitus Type 2, Vitamin C, Glycaemic control, Lipid profile

INTRODUCTION

Diabetes mellitus is a chronic and progressive disease state of worldwide significance. It can affect children, young and adults of all ages. India is becoming the diabetes capital of the world[1] with 50.8 million in 2010[2] and set to increase 70 million by 2025.[3]

Diabetes mellitus is primarily a metabolic disorder arising from a lack of or resistance to insulin,[4] which results in the impairment of uptake and storage of glucose and its reduced glucose utilization for energy purposes leading to the condition called hyperglycaemia. Prolonged exposure to elevated glucose induces repeated acute changes in intracellular metabolism and cumulative long-term changes in the structure and function of biological macromolecules.[5,6]

Due to environment, stress and other factors, our own body metabolism generates free radicals. This can cause oxidative injury to the living beings by attacking the biological macromolecules. There is a critical balance in the generation of these free radicals and antioxidant defence systems used by organisms to deactivate and protect themselves against free radical toxicity under normal physiological conditions.[7-9]An imbalance in the oxidant/antioxidant equilibrium leads to a condition called oxidative stress which is known to be responsible for molecular and cellular tissue damage mechanisms in a wide range of human diseases including diabetes.

It has been suggested that free radical activity is high in diabetes leading to increased oxidative stress. Various pathways which lead to oxidative stress include increased non-enzymatic glycosylation, auto-oxidative glycosylation, metabolic stress resulting from changes in energy metabolism, alterations in sorbitol pathway, changes in the level of inflammatory mediators and the status of antioxidant defence systems.[10]

Vitamin C is an important antioxidant for human, capable of scavenging free radicals. Vitamin C is structurally similar to glucose and can replace it in many chemical reactions. Thus it is effective in the prevention of non-enzymatic glycosylation of protein. People with type 2 diabetes have comparatively vitamin C deficiency, despite adequate dietary intake, and increase in the level of its primary oxidative product due to inadequate intracellular delivery of vitamin C which depends on insulin.[11] Chronic vitamin C deficiency in people with type 2 diabetes can lead to a range of complications.

Patients with diabetes mellitus have 2 to 4 fold increase in the prevalence of cardiovascular disease compared to non-diabetes mellitus patients[12] and thus very often leads to premature death. Vitamin C has been shown to be effective therapeutic reducing total cholesterol, which is one of predictive measures of vascular diseases. Supplementation with vitamin C can, therefore, exert beneficial effects in diabetes and associated complications.

Although antioxidant like vitamin C is known to have beneficial effect in type 2 diabetes mellitus, only limited studies have dealt with its effect on glycaemic control and vascular risk factors in patients with diabetes mellitus of Indian population. The present study was, therefore, designed to investigate the effect of vitamin C oral supplementation on glycaemic control and lipid profile in patients of south Indian population with type 2 diabetes mellitus.

METHODOLOGY

Study design and participants

The design was prospective, open label, randomized controlled study involving type 2 diabetic patients. A total of 65 patients with type 2 diabetic mellitus were enrolled after explaining the objectives of the study at the screening visit and verifying the inclusion criteria. The main inclusion criteria were; patients with known type 2 diabetes mellitus, aged between 30 to 70 years, either sex, with or without co-morbidities, minimum of 6 months ongoing oral hypoglycaemic treatment (metformin and/or glibenclamide) and ≥ 3 years duration of the disease. None of the patients were on antioxidant supplementation. Patients with type 1 diabetes, juvenile diabetes, pregnant women and lactating mothers, voluntary withdrawals and patients with any significant hepatic and renal dysfunction were excluded.

Informed consent and ethics committee approval

The experimental protocol was approved by Institutional Human Ethical Committee, JSS College of Pharmacy, Ootacamund, and Tamilnadu, India (JSSCP/DPP/IRB/020/2009-2010). Informed

written consent was obtained from each subject. The study was carried out at the outpatient department of secondary care of Government Headquarters Hospital, Ootacamund, The Nilgiris, Tamil Nadu, India, during the period of July 2010 to June 2011.

Procedure

Enrolled patients were randomized by using computer assisted randomization procedure and assigned to control group and intervention group. Patients in the intervention group received 500 mg/OD Vitamin C supplementation along with oral hypoglycemic agents (OHA's) like glibenclamide and/or metformin for a period of three months, whereas patients in control group received only OHAs for a period of 3 months.

Demographic data and general health characteristics including education, occupation, height, social habit and food habits were collected on standard structured data collection form by face to face interview during the baseline visit. The primary objective of the study was assessment of HBA_{1C} and fasting blood sugar. Secondary objective includes assessment of body weight, BMI, systolic and diastolic blood pressure, lipid profile, urea nitrogen, creatinine protein and oxidative stress. Measurements were performed at both baseline and after 3 months follow up.

Fasting blood glucose was measured by using Dr. Morepen Gluco-One blood glucose monitor system. HbA_{1C} were determined by using A1C Now+ (Bayer Healthcare LLC, USA). Blood samples were collected by venous puncture in heparinised sample collection tubes and the plasma was separated by centrifugation at 4000 rpm for 20 min. The isolated plasma was used for biochemical analysis. Measurement of lipid profile, urea nitrogen creatinine and total protein was performed by using enzymatic and colorimetric method at the main laboratory Government Headquarter Hospital, Ootacamund.

Statistical analysis

Statistical analysis was performed by using GNU PSPP version 0.7.5-g70514b software. Data are presented as mean± SD or geometric mean (95% CI). Differences between baseline and 3 months values within the groups were checked by paired Student's *t* test. A p value of less than 0.05 was taken as statistically significant.

Table 1: Baseline characteristic of participants

Variables	Control group	Intervention group
Age	63.17±9.07	57.53±7.44
Sex (Female/male)	20/9	22/8
Education[a]	I=10,P=6,M=6,S=2,HS=3, G=2	I=8,P=10,M=3,S=5,HS=4
Occupation[b]	H=12, FT=4, PT=5, R=6, D=2	H=15,FT=4, PT=6, R=4, D=1
Duration of disease	6.68±4.70	8.2±6.22
Family history	9	15
Smoking	6	2
Alcoholic	6	1
Non-vegetarian/ Vegetarians	28/1	25/5
Co morbidity	18	23

[a] *I* Illiterate, *P* Primary, *M* Middle, *S* Secondary, *HS* Higher Secondary, *G* Graduate

[b] *H* House wife, *FT* Full Time, *PT* Part Time, *R* Retired, *D* Disable to work

Results are expressed as means ± 1 SD, median and (range) or absolute numbers, as applicable

RESULTS

Baseline characteristics of the patients enrolled in the study are summarised in Table 1. Out of the 32 patients in the control group, 2 relocated to other places for job and 1 discontinued for personal reasons. Of the 33 intervention subjects, 2 were lost during the follow up and 1 discontinued for personal reasons. In total, data for 29 patients on control group and 30 patients for intervention group were used for the analysis at the end of three months. In the control group 3 patients were managing their diabetes with only metformin, 3 with glibenclamide and 23 with the combination of these two drugs. Among the control group 18 patients had hypertension co-morbidity. In the intervention group 8 patients were on metformin, 3 on glibenclamide and 18 on the combination of these two drugs. In the intervention group 23 patients had hypertension co-morbidity.

Table 2 shows the biochemical and clinical variables at baseline and after three months for control and intervention groups. The parameters include body weight, BMI, fasting blood glucose, HbA_{1C}, systolic and diastolic blood pressure, lipid profile, urea nitrogen, creatinine and total protein. Significant changes were observed in the group supplemented with vitamin C with respect to total cholesterol and LDL cholesterol. The change was not quite significant in the case of urea nitrogen. However, 3 months of vitamin C supplementation decrease the BMI, fasting blood glucose, blood pressure and triglyceride levels though not significantly.

DISCUSSION

In the present study most of patients were non-vegetarian and overweight. The Nilgiris District is situated above 2400 m and the atmospheric temperature is very low. This is an important factor which may affect the basal metabolic rate of the population. They are forced to have carbohydrate rich non-vegetarian diet which perhaps is one of the epidemic causes for the prevalence of high population of diabetes in Ootacamund. In a fast catching urban lifestyle in Ootacamund, fast foods, changing diet pattern, lack of exercise, obesity, smoking etc. also contribute to this epidemic. Research has shown that these factors greatly undermine the body's ability to remove toxic free radicals which are constantly being produced in the body.[13]

Studies reported earlier show that reactive oxygen species play a major role in the pathogenesis of complication in type 2 diabetes mellitus. An epidemiologic study has reported that HbA_{1C} level is inversely correlates with plasma levels of vitamin C.[14] However, in our study the change is partial and statistically not significant. This might be due to the worsening of the pathological condition with age due to altered defence system and elevated free radical production.[15] Long term of supplementation may possibly show beneficial effect.

The results reveal that significant changes in the group supplemented with vitamin C with respect to total cholesterol and LDL cholesterol. It has been suggested LDL level from subjects with type 2 DM are more prone to oxidation.[16] Supplementation of vitamin E has been shown to protect their LDL against *in vitro* peroxidation in normal subjects and patients with DM,.[17,18] It has been proposed that recycling of vitamin E is dependent on vitamin C.[19] *In vitro* studies have shown that addition of 40-60 µM of vitamin C can prevent initiation and termination lipid peroxidation in LDL by recycling vitamin E.[20,21] It has also been proposed that the hydrophilic property of vitamin C facilitate their localisation at the interface of the lipid bilayers in membranes thereby suggesting two advantages, namely, effective inhibition of attack by free radical in the aqueous phase and effective repair of lipophilic antioxidant.[22-24] This protection preserves the ability of LDL to be recognized by LDL receptors in the liver and, therefore, expedite its removal from the blood by LDL cholesterol catabolic pathway.[25] Vitamin C has been also shown to protect HDL cholesterol from lipid oxidation thus allowing it to be involved in the process known as reverse cholesterol transport.[26] However, in our study no significant change was observed with respect to HDL. Change in the level of urea nitrogen was also not quite significant. Our findings are contrary to the earlier reported study.[27]

The role of oxidative stress in the causation of chronic tissue damage is being increasingly recognized if antioxidant deficiency occurs. The low antioxidant level in diabetes may be due to lower intake of antioxidant or increased oxidative stress. Supplementation of antioxidants is, therefore, expected to be beneficial.

In our study most of the enrolled patients come from economically deprived background. Thus lower consumption of fruits and vegetables may have contributed to the reduced consumption of vitamin C and carotenes.

Although our results may have some clinical implications, measurement of antioxidants is not routinely performed in clinical practice. Reviewing the intake of food rich in antioxidants particularly citrus fruits and green leafy vegetables and the appropriate antioxidant supplements among patients with the metabolic syndrome may be instructive for the smooth control of diabetes. If dietary intake of vitamins A, C and E fails to meet the recommended daily allowance, healthcare professionals should encourage people with the metabolic syndrome to increase their intake of these vitamins, preferably through the consumption of healthy food sources rich in these vitamins or otherwise through the use of appropriate vitamin supplements. The future studies on the effect of vitamin C supplementation may be directed towards extended duration of treatment and large number of patients.

Table 2: Biochemical and clinical variables at baseline and after three months for control and intervention groups

Variables	Control group		Difference	Intervention group		Difference
	Baseline	After 3 months		Baseline	After 3 months	
Body weight (kg)	63.10±9.02	63.31±8.92	- 0.21	62.63±7.28	61.68±6.22	0.95
BMI (kg/m^2)	24.92±3.05	24.86±2.89	0.05	25.82±4.05	25.05±3.48	0.77
Fasting blood glucose (mg/dl)	182±46.22	195.03±52.56	-13.07	201.03±82.69	189.77±68.26	11.26
HbA_{1C} (% Hb) [3.9-5.1]	8.75±1.56	8.92±1.65	0.17	9.38±1.75	9.01±1.57	0.37
Systolic blood pressure (mmHg)	134.51±14.61	142.27±12.99	7.76[a]	139.71±16.54	136.92±16.74	2.79
Diastolic blood pressure (mmHg)	78.62±10.86	85.72±9.14	7.10[a]	81.13±7.58	80.46±6.20	0.67
Total cholesterol (mg/dl) [100-240]	191.03±34.91	197.93±35.22	-6.90	196.66±35.54	178.16±26.61	18.50[a]
Triglyceride (mg/dl) [50-175]	168.62±53.21	171.42±50.25	+2.80	170.46±72.96	160.46±53.34	10.00
LDL cholesterol (mg/dl) [<100]	108.34±31.01	115.43±30.66	-7.09[a]	113.67±27.64	96.42±27.80	17.25[a]
HDL cholesterol (mg/dl) [35-65]	48.96±6.92	46.96±9.51	-2	48.90±7.94	51.93±8.35	-3.03
Urea nitrogen (mg/dl) [10-50]	29.27±3.99	29.44±3.73	0.17	33±5.92	29.71±7.27	3.29[c]
Creatinine (mg/dl)[0.5-1.3]	0.95±0.11	0.99±0.09	-0.04	1.01±0.22	0.97±0.12	0.04
Total Protein (g/dl) [6.3-8.4]	7.65±0.35	7.73±0.29	0.08	7.64±0.64	7.59±0.47	0.05

Figures in square brackets refer to normal reference range in the laboratory

a $p<0.05$, b $p<0.001$, c not quite significant

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