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Low Plasma Ascorbate Levels in Type 2 Diabetic Patients With Adequate Dietary Vitamin C

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Abstract

Background Dietary intake of antioxidative vitamin C plays a protective role in the prevention of oxidative damage in diabetics, demanding increased requirement of vitamin C. Hyperglycemia results in impaired uptake of vitamin C in the cell. The present study was conducted to compare the plasma ascorbate levels in type 2 diabetic patients and controls consuming adequate dietary vitamin C.

Methodology Fifty consented type 2 diabetes mellitus (T2DM) patients who were on treatment with oral hypoglycemic drugs and consuming adequate vitamin C in diet were taken in the study and 50 healthy controls equitably matched for age, gender between 40 and 70 years, and dietary intake of vitamin C were compared. Dietary intake of vitamin C was estimated by a food frequency questionnaire. Subjects consuming more than 35 mg/d of vitamin C were included in the study. Fasting blood sugar was estimated by glucose oxidase and peroxidase method and estimation of ascorbic acid was done by using 2, 4 dinitro phenyl hydrazine method.

Result The mean ± standard deviation levels of plasma ascorbate levels in diabetic subjects were 0.22 ± 0.12 mg/dL, which were significantly lower as compared with controls with plasma ascorbate level of 0.47 ± 0.15 mg/dL. In diabetic subjects, insignificant positive correlation was observed between these parameters with *r*-value 0.168 and *p*-value 0.245.

Conclusion This study concludes that even with the recommended dietary intake of vitamin C low plasma ascorbate levels were found among T2DM patients, which necessitates increased demand and dietary advice to diabetic patients on consuming foods rich in vitamin C more than the recommended daily allowance.

Keywords
- type 2 diabetes mellitus
- dietary vitamin C
- plasma ascorbate
- FBS

Introduction

Diabetes is a clinical metabolic disorder with increased blood glucose levels because of total or comparative deficiency of insulin. Factors contributing to hyperglycemia are either reduced insulin secretion or decreased glucose utilization.[1] According to the International Diabetes Federation Atlas 2017, 72.9 million Indians are suffering from diabetes.[2] Normal metabolism of cells produces reactive oxygen species (ROS). They have a beneficial effect on the functioning of cells and, if they are increased, result in excessive lipid peroxidation, leading to the damage of proteins and DNA.[3]

The rate of ROS production depends on hyperglycemia, insulin resistance, and obesity, which are risk factors of

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metabolic syndrome.[4] Insulin resistance, β-cell dysfunction, and type II DM act as the root of increased oxidative stress and impaired antioxidant defense mechanism resulting in micro- and macrovascular dysfunction.[5-7]

Ascorbic acid is a water-soluble antioxidant and scavenge free radicals. It acts as a reducing agent in hydroxylation reactions and prevents from oxidative damage of biological macromolecules.[8] As per WHO (World Health Organization) recommendation, recommended daily allowance (RDA) of vitamin C for adults is 45 mg/d. People who consume low amount of fruits and vegetables have a higher risk of vitamin C deficiency. In general, fruits and vegetables consumption determines the vitamin C status.[9] With sodium-dependent vitamin C transporter 1, intestinal absorption of ingested vitamin C occurs as ascorbic acid and by facilitated diffusion via GLUT 2 (glucose transporter 2) and GLUT 3 transporters, dehydroascorbic acid (DHA) is transported. Further ascorbic acid is transported by GLUT1 and GLUT2, which facilitates uptake of DHA.[10] Hyperglycemia results in impaired uptake of vitamin C into the cell.[11]

In a study the possible interactions between glucose and vitamin C transport in the human intestine were evaluated. Ascorbate uptake was inhibited by increasing concentrations of glucose.[12] Several studies state that in diabetes there is increased oxidative stress and increase in requirement of vitamin C.[13-18] Some studies reported that there was no association with plasma ascorbate levels in diabetic patients.[19-21]

Although several mechanisms have been explained for low vitamin C levels among the type 2 diabetes mellitus (T2DM), dietary vitamin C intake was not considered. This present study was conducted to compare the plasma ascorbate levels in type 2 diabetic patients and controls with an adequate dietary intake of vitamin C, which was evaluated using a validated food frequency questionnaire (►**Supplementary Table S1** [online only]).

Materials and Methods

The present community-based cross-sectional study was conducted at KLE's Dr. Prabhakar Kore Charitable Hospital, Belagavi, India from January 2019 to December 2019. Seventy-five consented T2DM patients who were on treatment with oral hypoglycemic drugs and consuming adequate vitamin C in diet were taken in the study and 50 healthy controls equitably matched for age, gender between 40 and 70 years with dietary intake of vitamin C were compared. Subjects with type 1 DM, endocrine disorders, malabsorption syndrome, who have undergone previous gastrointestinal surgeries, smokers, and chronic alcoholics were excluded from the study. The study was approved by the Institutional Ethical and Research Committee.

Adequate dietary intake of vitamin C was estimated by food frequency questionnaire, which was validated with a cohort of individuals with T2DM subjects.[22,23] Prevalidation of the questionnaire was performed. Data regarding dietary recall of intake of food over a recent week's time was recorded with all the details. Dietary intake was marked by the subject and assembled through a structured interview. Average consumption of vitamin C was quantified from the questionnaire. As per WHO, intake of vitamin C more than 35 mg/d is sufficient for the Indian population, hence subjects consuming more than 35 mg/d of vitamin C were included. Fasting blood sugar was estimated by glucose oxidase and peroxidase method.[24] Estimation of ascorbic acid was done by using 2, 4 dinitro phenyl hydrazine method using Single Beam UV-visible spectrophotometer. In this procedure, ascorbic acid is first oxidized to DHA and 2,3-diketogulonic acid with the formation of a colored product that absorbs at 520 nm. This method, measures the total vitamin C content of the sample because ascorbic acid, DHA, and diketogulonic acid are also measured and is subject to interference from amino acids and thiosulfates.[25] As per guidelines of the National Institute of Nutrition, Hyderabad, normal level of plasma ascorbate is 0.4 to 2.0 mg/dL and deficiency is below than 0.2 mg/dL. The diagnosis of T2DM was established at fasting blood glucose value of more than or equal to 126 mg/dL.[8]

Statistical analysis was performed using SPSS software, version 20.0 for Windows; IBM Corporation, Software Group, Route 100, Somers, New York 10589. Continuous variables were expressed as mean ± standard deviation (SD). An independent *t*-test was used to compare the parameters in case and control population. $p < 0.05$ was considered statistically significant. The Karl Pearson's coefficient test was used to find out the correlation between the parameters.

Results

In this study, a total of 125 subjects were evaluated. In them 75 subjects were with T2DM and 50 were healthy controls. However, 25 diabetic subjects were excluded from this study as shown in ►**Fig. 1**. The overall distribution of subjects is shown in ►**Table 1**.

►**Table 2** shows the comparison of levels of dietary intake of vitamin C, fasting blood sugar (FBS), and plasma ascorbate levels among the type 2 diabetic patients and controls in the study population. The mean ± SD levels of dietary intake of vitamin C, was 57.28 ± 18.37 per day in diabetics, which was showing no statistical difference as compared with controls with a mean value of 57.28 ± 22.62 per day. The mean ± SD levels of FBS was 160.55 ± 57.63 mg/dL which was significantly increased in diabetic subjects as compared with controls with FBS 84.46 ± 11.156 mg/dL. The mean ± SD level of plasma ascorbate levels in diabetic subjects was 0.22 ± 0.12 mg/dL, which was significantly lower as compared with controls with plasma ascorbate level of 0.47 ± 0.15 mg/dL with $p < 0.05$.

Further correlation was done in diabetic and control group with FBS and plasma ascorbate levels by Karl Pearson's correlation coefficient method. In diabetic subjects insignificant positive correlation was observed between these parameters with *r*-value 0.168 and *p*-value 0.245, whereas in nondiabetic subjects there was no correlation between FBS and plasma ascorbate levels with *r*-value 0.007 and *p*-value 0.961 as shown in ►**Fig. 2**.

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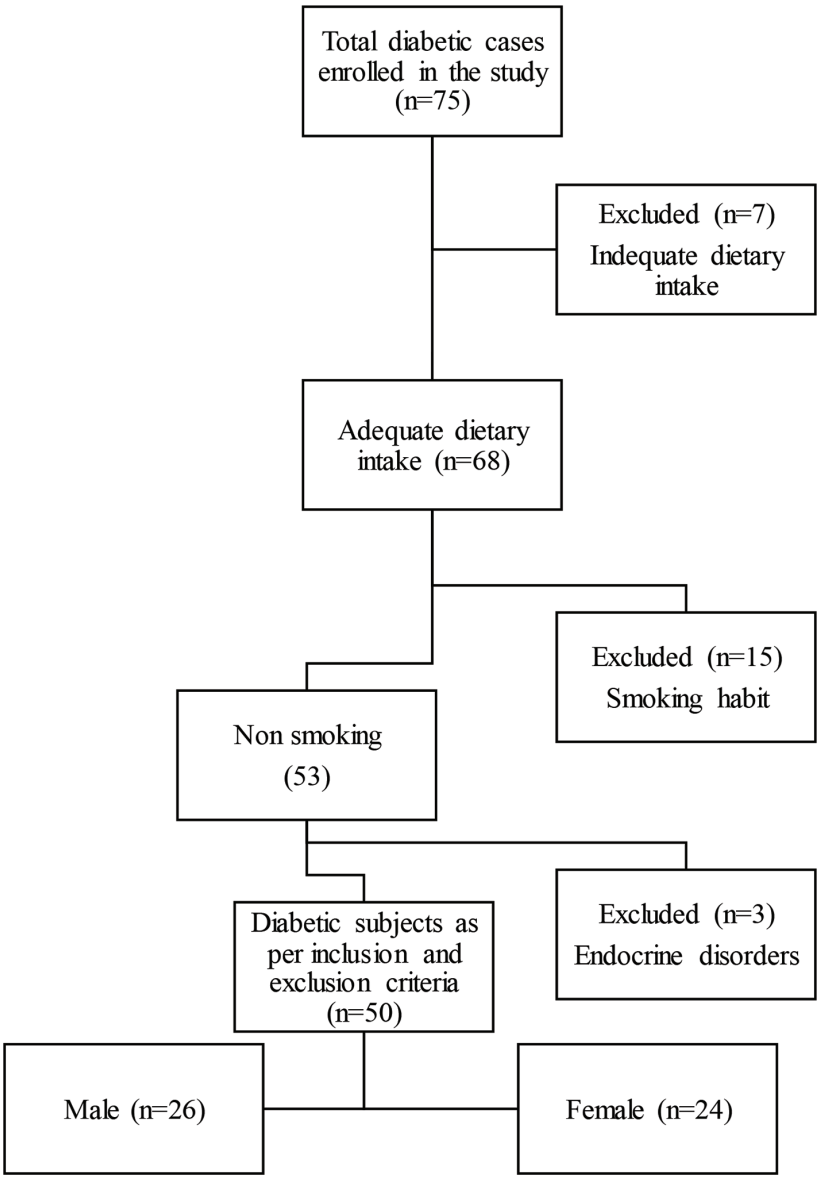

Fig. 1 Participants' flow diagram of T2DM patients. T2DM, type 2 diabetes mellitus.

Table 1 Distribution of type-2 diabetic subjects and controls by gender

Gender	Diabetic	Nondiabetic	Total
Female	24	24	48
Male	26	26	52
Total	50	50	100

Table 2 Comparison of dietary intake of vitamin C, FBS, and plasma ascorbate levels in diabetic and nondiabetic patients

	Diabetic		Nondiabetic		*t*-Value	*p*-Value
	Mean	Std. deviation	Mean	Std. deviation		
Dietary intake of vitamin C (RDA > 35 mg/d)	57.28	18.37	57.28	22.62	0.001	0.999
FBS(70–110 mg/dL)	160.55	57.63	84.46	11.156	9.166	<0.001[a]
Plasma ascorbate levels (0.4–2.0 mg/dL)	0.22	0.12	0.47	0.15	9.06	<0.001[a]

Abbreviations: FBS, fasting blood sugar; RDA, recommended daily allowance.
[a]Significant at the 0.01 level.

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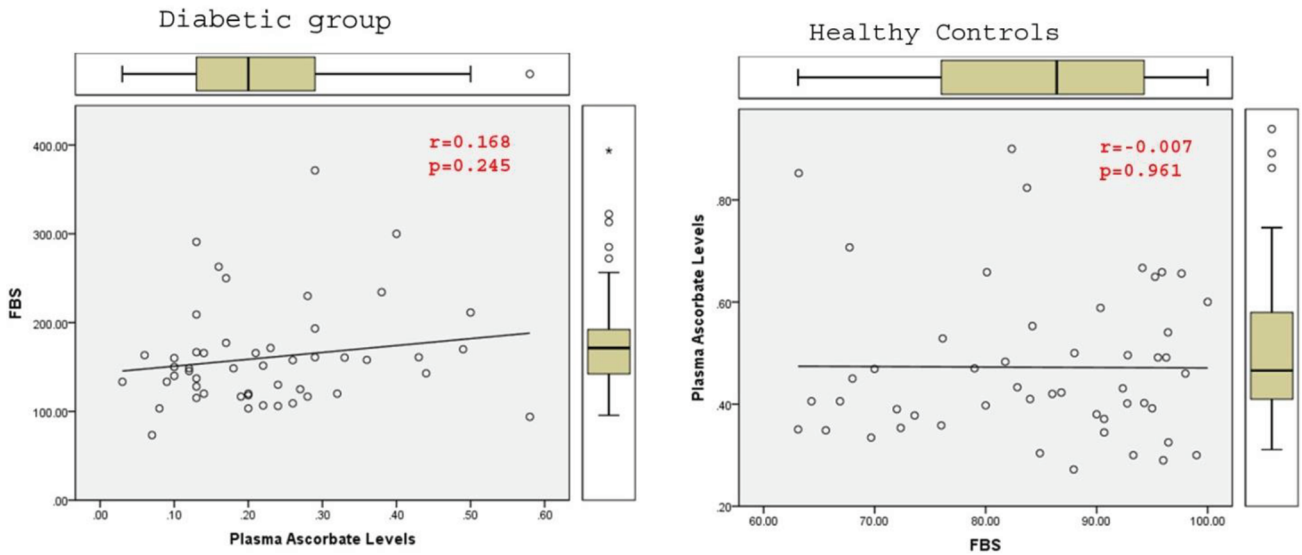

Fig. 2 Correlation between FBS and plasma ascorbate levels in diabetic subjects and healthy controls. FBS, fasting blood sugar.

Discussion

Dietary intake of antioxidative vitamin C plays a protective role in the prevention of oxidative damage in diabetics demanding increased requirement of vitamin C. In hyperglycemia cellular injury is due to oxidative stress induced by hyperglycemia. Weak defense system of the body fails to respond against ROS generation and causes imbalance between ROS and their protection that leads to oxidative stress.[6,11] Vitamin C protects organs from damage in diabetes by three mechanisms that are, it has functioned as an antioxidant, inhibits the intracellular accumulation of sorbitol, and reduces the glycosylation of protein.[11] Some studies suggested that supplementation of vitamin C could be helpful to improve better insulin action and helps to control secondary complication of T2DM.[26-31]

In this cross-sectional study plasma ascorbate levels were assessed in 50 T2DM patients (male = 26, female = 24) who were consuming adequate dietary vitamin C and compared with 50 healthy controls from local community, Belagavi. We found that plasma ascorbate levels were significantly lower in diabetic subjects, even with adequate RDA for vitamin C as compared with controls with p-value < 0.0001. The study showed vitamin C deficiency among diabetic patients compared with controls.

Similar to our results a study concluded that low ascorbate level in diabetes is a consequence of the disease itself and not due to inadequate dietary intake of vitamin C.[32] Similar results were reported from many studies which showed that in diabetics due to increased oxidative stress vitamin C levels were significantly low. This relationship was due to increased oxidative stress, which causes inflammation and dysglycemia and competitively inhibits ascorbic acid at the intestinal absorption level by high blood glucose levels.[13-18] In contradicting with our results some of the studies stated that there was no difference in plasma ascorbate levels between T2DM patients and controls.[19-21]

Although plasma ascorbate levels were lower among diabetic subjects, we found an insignificant positive correlation between FBS and plasma ascorbate levels in T2DM with r-value 0.168 and p-value 0.245. In contradicting to our results, some studies found significant negative association between FBS and plasma ascorbate levels.[31,33] Further, between FBS and plasma ascorbate levels, there was no correlation in control groups (r = 0.007; p = 0.961).

Conclusion

The present study demonstrates that even with an adequate dietary intake of vitamin C in T2DM patients, lesser plasma ascorbate levels are observed as compared with healthy controls. This necessitates increased demand and dietary advice to diabetic patients on the intake of vitamin C rich foods more than the RDA. Plasma ascorbate estimation in diabetic patients can be used as an additional investigation as a precaution to prevent vitamin C deficiency.

Limitations

The findings in the present study add to the limited sample size and only FBS levels were used to correlate with plasma ascorbate; further research with the inclusion of glycated hemoglobin value will indicate the degree of vitamin C deficiency with glycemic control.

Ethical Clearance
Approved by the Institutional Ethics Committee.

Funding Source
Not applicable.

Conflict of Interest
None.

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