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# Effect of Ascorbic acid supplementation on Glycosylated Hemoglobin, Salivary vitamin C and Periodontal health in Diabetes Mellitus: A clinical Trial

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## Abstract

**Introduction:** Diabetes mellitus (DM) is a multifactorial disease with no one specific drug available to treat. Oxidative damage to beta cells due to reactive oxygen species is one of the causes of DM2. Vitamin C is one of the most powerful dietary antioxidant available, closest in structure to carbohydrate. Vitamin C and glucose competitively inhibit each other to penetrate the cell membrane by the tunneling mechanism. Vitamin C in higher dose reduces the (glycosylated hemoglobin concentration) levels and low-density lipoproteins.

**Aim:** To assess the effect of mega dose of ascorbic acid (Vitamin C) on HbA1C in Diabetic patients and to concomitantly assess the salivary vitamin C levels and periodontal health.

**Material methods:** 50 subjects with Diabetes Mellitus type 2 were chosen and HbA1c, salivary vitamin C and Periodontal health was assessed in these subjects. Assessment was done at baseline, 3 months,6months and 9 months after supplementation with Oral Vitamin C 500 mg twice daily.

**Result:** There was significant reduction in the HbA1c values after supplementation with vitamin C at the end of 9 months. Besides, there was significant increase in salivary vitamin C levels along with improvement in periodontal health.

**Conclusion:** Vitamin C supplementation significantly reduces the HbA1c along with significant improvement in periodontal health in Diabetic subjects.

**Keywords:** Ascorbic acid, Diabetes Mellitus, HbA1C, Periodontal health,Vitamin C .

**Introduction**

Diabetes mellitus (DM) is clinically and genetically a heterogeneous metabolic disease. It is characterized by elevated blood glucose and dysregulation of carbohydrate, protein and lipid metabolism. Insulin is the principal regulatory hormone of glucose.(1) It primarily increases the facilitated transport of glucose into the cell, thus reducing blood glucose The primary cause of DM2 is either insulin resistance or decrease in insulin production. At a biochemical level, insulin has an inhibitory effect on the gluconeogenic enzyme phosphoenolpyruvate kinase and a stimulatory effect on glycolytic enzymes such as glucokinase, pyruvate kinase,phosphofructokinase, and fructose 2,6 bisphosphatase.(2)

One of the major causes of beta cell dysfunction in DM2 is the influence of oxidative stress. Oxidative stress is mediated by reactive oxygen species (ROS) produced due to advanced glycation end formation, glucose autoxidation, glucosamine production, and oxidative phosphorylation.(3)

Vitamin C is a water-soluble vitamin and a powerful dietary antioxidant. It is also a generous donor of electrons, thus involved in the scavenging mechanism. Mega doses of vitamin C in DM2 helps to reduce the blood glucose, reduces capillary fragility in diabetics, reduces glycosylation and decreased the production of Sorbitol.(4)

The role of vitamin C in DM2 is explained by the glucose ascorbate antagonism pathway. Glucose and ascorbate compete with each other to get entry into cell membrane. Their entry is mediated by insulin pumps present on the lymphocytes . Vitamin C and glucose helps in insulin-mediated tunneling mechanism into cells through the cell membrane. High levels of glucose obstruct vitamin C entry into the cell. This explains that vitamin C and glucose compete with each other. Vitamin C is structurally similar to glucose and may, therefore, compete with glucose for transportation into the cell. In the presence of hyperglycemia, the uptake of vitamin C into cells appears impaired.(5,6)

DM has been associated with periodontitis, and higher the blood glucose levels, the more likely that patients with DM develop periodontitis compared with individuals without DM .(7) On the other hand, the inflammatory process associated with periodontitis can interfere with glycemic control in DM 2 as patients with DM showed a decrease of 0.3% to 0.4% for glycated hemoglobin (HbA1c) levels after periodontal treatment.DM-associated hyperglycemia leads to alterations in hemostasis of alveolar bone and formation of advanced glycation end products, which play a pro inflammatory and pro oxidative role in cells. Periodontal infection potentiates the vicious cycle in DM, leading to faster periodontal destruction (8)

Improvement in the periodontal health in turn improves the health of diabetics. Studies have shown a significant decline of 0.4% in HbA1c after periodontal intervention therapy in type 2 diabetic patients. Hence, it is reasonable to consider that in type 2 diabetic patients effective periodontal intervention therapy improves glycemic control.(9)

Diabetes augmented the gingival inflammatory responses against plaque biofilm, and the diabetes prevalence was significantly higher in the gingivitis patients with high BOP (Bleeding on Probing) lesions compared with low BOP lesions. Thus, the amelioration of diabetes may improve the gingival inflammatory responses.(10)

Oxidative stress is known to cause Diabetes and periodontal destruction. Vitamin C is a powerful dietary antioxidant. Increase in the levels of salivary vitamin C are indicative of improved antioxidant potential of saliva.

This accounts for reduced periodontal destruction. Ascorbic acid is one of the best cellular indicators of oxidative stress .Plasma ascorbate concentrations are as low as 40% of normal in subjects with complications of Diabetes and thus increased oxidative stress. This deficit occurs even though these subjects have presumably adequate intakes of the vitamin (~150 mg/d). (11)

In further support of ascorbate as a marker for oxidative stress in diabetes, plasma ascorbate was decreased in elderly Diabetic subjects when other markers of oxidative stress were unaffected. Second, ascorbate may be difficult to fully replete in T2DM patients. In one study an oral dose of 1 g daily increased plasma ascorbate in 20 T2DM subjects with complications. These results suggest that the oxidative stress of diabetes causes a high turnover of ascorbate. These results suggest that the high oxidative stress of Diabetes causes increased turnover rate of Ascorbate. (12)

**Materials and methods**

50 Diabetic subjects were chosen for the study by consecutive sampling.The inclusion and exclusion criteria are as follows:

**Inclusion criteria**

1. Diagnosed DM2 subjects in the age group of 30 -60 years undergoing treatment no less than 6 months and greater than 3 years.
2. Subjects undergoing treatment for the same with only oral hypoglycemic agents like metformin and glibenclamide.
3. Subjects whose HbA1C is >=6.5%
4. Subjects having chronic form of generalized periodontitis .

**Exclusion criteria**

1. Subjects above the age of 60 years and less than 30 years were excluded.
2. Subjects whose are on insulin therapy were excluded.
3. Subjects with type 1 diabetes or Pregnancy induced diabetes were excluded.
4. Subjects with chronic complications of diabetes or any other endocrine disease were excluded
5. Subjects with history of supplementation with vitamins in the past 3 months were excluded.
6. Subjects with history of smoking and alcohol were excluded.

Subjects were chosen based on their Hba1c values. HbA1c > 6.5% were included in the group. The periodontal health was evaluated based on the gingival bleeding index and clinical attachment level (CAL). The past and present intake of vitamin C was assessed by a Food Frequency Questionnaire. Salivary vitamin c levels was estimated by DNPH (Di-Nitro Phenyl Hydrazine method) . Vitamin C was supplemented to the patients by oral route.500mg vitamin C cheweable tablets was given twice daily for 3 months

At the end of 3 months, all the baseline tests were repeated and supplementation with oral vitamin C 500mg twice daily was continued until next 3 months. At the end of 6 months, all baseline tests were repeated and scaling root planing procedure was performed. Dropout rate was checked. Since the dropout rate was less than 30%, remaining subjects were included and the study was further taken into the next phase. At the end of 9 months, the subjects were re evaluated.

A special Food Frequency Questionnaire was formulated for his study and was validated by a cross classification analysis. The purpose of this questionnaire was to assess if all the subjects are taking adequate amount of vitamin C through the diet. Otherwise, dietary intake will become a confounder during assessment of vitamin C.

HbA1c was tested by HPLC method .2ml Venous blood was drawn in EDTA coated vacationers and sent to the laboratory .Unstimulated saliva was collected in the

ependorff tubes .Subjects were seated on the dental chair and sufficient time was given to them to relax.Subjects were then asked to spit in the tubes provided without exerting much intraoral pressure .Later the sample was stored at 4 degree centigrade until it was transported to the lab. Optical density is read at 540nm against blank using spectrophotometry.

The Gingival Bleeding Index was assessed by the William's periodontal probe. Probing pocket depth was measured and Clinical Attachment Loss (CAL) was calculated.

**Results**

The statistical analysis was done using statistical package for social sciences (SPSS) for windows version 22.0.Released 2013.

The mean HBA1C in the Diabetic group was 7.21 % at baseline and 6.63 % after 9 months. Salivary vitamin C values in Diabetic subjects was 1.66mg/dl at baseline and 2.50 at the end of 9 months. FFQ measures the mean intake of vitamin C .The mean intake of vitamin C in the diabetic group at baseline was 80 mg/day.

Table 1: Mean values of HbA1c, Salivary vitamin C, FFQ and GBI.

| | Baseline | 3 months | 6 months | 9 months |
|---|---|---|---|---|
| HbA1C | 7.21 | 7.09 | 6.81 | 6.63 |
| Salivary Vitamin C | 1.66 | 1.77 | 2.14 | 2.50 |
| FFQ | 80.00 | 95.02 | 97.61 | 113.07 |
| GBI | 84.22 | | | 82.26 |

Table 2

| Comparison of mean HbA1c values between different time intervals in Pre diabetics group using Repeated Measures of ANOVA Test | | | | | | |
|---|---|---|---|---|---|---|
| Time | N | Mean | SD | Min | Max | P-Value |
| Baseline | 50 | 5.87 | 0.28 | 5.0 | 6.5 | 0.002* |
| 3 Months | 50 | 5.74 | 0.27 | 5.0 | 6.0 | |
| 6 Months | 50 | 5.70 | 0.24 | 5.0 | 6.0 | |
| 9 Months | 50 | 5.77 | 0.23 | 5.0 | 6.0 | |

The Hba1c values in the pre diabetic group at different time intervals were statistically significant. This shows that oral supplementation of vitamin C progressively reduced the HbA1C.

Table 3

| Comparison of mean HbA1c values between different time intervals in Diabetics group using Repeated Measures of ANOVA Test | | | | | | |
|---|---|---|---|---|---|---|
| Time | N | Mean | SD | Min | Max | P-Value |
| Baseline | 44 | 7.22 | 0.37 | 6.5 | 7.8 | <0.001* |
| 3 Months | 44 | 7.08 | 0.49 | 6.0 | 7.8 | |
| 6 Months | 44 | 6.81 | 0.39 | 6.0 | 7.3 | |
| 9 Months | 44 | 6.63 | 0.43 | 6.0 | 7.3 | |

The Hba1c values in the diabetic group at different time intervals were statistically significant. This shows that oral supplementation of vitamin C progressively reduced the HbA1C.

Table 4

| Multiple comparison of mean difference in HbA1c values b/w different time intervals in Diabetic group using Bonferroni's Post hoc Analysis | | | | | | |
|---|---|---|---|---|---|---|
| Time | BL vs 3M | BL vs 6M | BL vs 9M | 3M vs 6M | 3M vs 9M | 6M vs 9M |
| P-Value | 0.12 | <0.001* | <0.001* | <0.001* | <0.001* | 0.02* |

Bonferroni's Post hoc analysis was done to validate the difference in the HbA1c at baseline, 3 months, 6 months and 9 months.It was found significant thus proving that change in Hba1c was evident.

Table 5

| Comparison of mean Sal. Vit C values between different time intervals in Diabetics group using Repeated Measures of ANOVA Test | | | | | | |
|---|---|---|---|---|---|---|
| Time | N | Mean | SD | Min | Max | P-Value |
| Baseline | 44 | 1.71 | 0.55 | 0.8 | 3.2 | <0.001* |
| 3 Months | 44 | 1.81 | 0.55 | 0.8 | 2.8 | |
| 6 Months | 44 | 2.14 | 0.41 | 1.2 | 2.8 | |
| 9 Months | 44 | 2.55 | 0.70 | 1.3 | 3.5 | |

The salivary vitamin C values in the diabetic group at different time intervals were statistically significant. This shows that salivary vitamin C progressively increased.

Table 6

| Multiple comparison of mean difference in Sal. Vit C values b/w different time intervals in Diabetic group using Bonferroni's Post hoc Analysis | | | | | | |
|---|---|---|---|---|---|---|
| Time | BL vs 3M | BL vs 6M | BL vs 9M | 3M vs 6M | 3M vs 9M | 6M vs 9M |
| P-Value | 0.73 | <0.001 * | <0.001 * | <0.001 * | <0.001 * | <0.001 * |

Bonferroni's Post hoc analysis was done to validate the difference in the salivary vitamin C at baseline, 3 months, 6 months and 9 months. It was found significant thus proving that change in salivary vitamin C was evident.

**Discussion**

Oral supplementation of vitamin C had reduced the HbA1C values in the Diabetic group. Besides, a controlled environment was maintained wherein the subjects did not overdo the intake of vitamin C by means of diet.Thus in such a scenario, significant reduction in HbA1C proves the efficacy of oral supplementation of vitamin C. Previous studies have shown reduction in Post meal blood sugar and HbA1c after supplementation with oral vitamin C.(12) But the duration of those studies were less and limited. This is the only study of its kind where mega doses of vitamin C were supplemented for 9 months and then a reduction in HbA1c was documented. Vitamin C is a cost effective supplement with no adverse effects. Vitamin C reduces the chances of developing Diabetes as it is an effective antioxidant and a sucrose competitor. Both of these actions result in prevention of development of Diabetes Mellitus.

Diet is the main source of vitamin C. There is no internal synthesis of vitamin C. In order to show the efficacy of synthetic vitamin C, it is essential to have a controlled environment wherein the subjects do not over consume vitamin C due to its benefits .FFQ is an effective way to assess the dietary intake of vitamin C.(13) Significant results for FFQ imply that vitamin C alone from the synthetic source has been effective.

The periodontal status is altered in Diabetes mellitus. Studies have shown depleted periodontal health in Diabetic subjects.. In this study, statistically significant values were obtained for GBI scores thus proving that gingival health is more affected in diabetics.

Vitamin C is a powerful naturally occurring antioxidant. Studies have shown an alteration in the total salivary antioxidant capacity in Diabetic subjects. The salivary antioxidant capacity is inversely proportional to the inflammatory load. Diabetes along with chronic periodontitis increases the inflammatory load.(14) Statistically significant values were obtained for salivary vitamin C in pre diabetic subjects in this study thus proving that saliva can be used as a tool to monitor the state of oxidative stress.

**Conclusion**

There was considerable reduction in the levels of HbA1c after supplementation with vitamin C in the subjects with Diabetes. There was also considerable increase in the salivary vitamin C levels. The dietary levels of vitamin C remained stable over a period of 9 months. There was reduction on the gingival bleeding index scores after 9 months compared to baseline. Thus it can be concluded that oral supplementation of vitamin C has considerable therapeutic effects on reducing the hyperglycemia. It is proven that Vitamin C supplementation reduces HbA1C in Diabetic subjects .

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