Research Article

Low Serum Vitamin C and Zinc is Associated With the Development of Oxidative Stress in Type 2 Diabetes Mellitus with Periodontitis

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ABSTRACT

The aim of this study was to assess the serum level of vitamin C and zinc in type 2 diabetes mellitus with and without periodontitis, and to elucidate whether increased or decreased serum vitamin C and zinc could be related to a risk factor for developing oxidative stress in type 2 diabetes mellitus with periodontitis. In the study, 150 healthy controls, 150 type 2 diabetes mellitus without periodontitis, and 150 type 2 diabetes mellitus with periodontitis subjects were included. Serum vitamin C level was determined using 2, 4- dinitrophenylhydrazine method. Zinc was estimated using Nitro-PAPS (pyridylazo-N-propyl-N-sulfopropylamino-Phenol) method by UV Spectrophotometer. The results showed that mean serum vitamin C and zinc levels were lowered significantly in type 2 diabetes mellitus with periodontitis ($p< 0.0001$) when compared to type 2 diabetes mellitus without periodontitis and healthy controls. The present findings support that decreased level of antioxidants vitamin C and zinc are associated with an increased risk for the development of oxidative stress in type 2 diabetes mellitus with periodontitis.

Keywords: Antioxidants, Periodontitis, Oxidative stress, Type 2 diabetes mellitus, Vitamin C, Zinc.

INTRODUCTION

Periodontal disease is one of the most widespread oral diseases, characterized by destruction of soft tissues, alveolar bone, and other supporting structures of the dentition eventually leading to tooth loss.[1,2] It resulted in the formation of soft tissue pockets or deepened crevices between the gingiva and tooth root. Periodontitis is postulated to place individuals at an increased risk of type 2 diabetes mellitus and is globally under estimated.[3] Diabetes is associated with impaired wound healing, overstated monocyte response to dental plaque antigens[4] and impaired neutrophil chemotactic responses all of which can lead to augmented local tissue destruction. It has been estimated that diabetes mellitus afflicts over 552 million people worldwide by the year 2030.[5]

Vitamin C is an important water-soluble vitamin that has antioxidant, anti-carcinogenic and anti-inflammatory effects.[6-8] Epidemiological studies point out a negative relationship between plasma vitamin C level and severity of periodontitis.[9,10] Vitamin C deficiency leads to scurvy with decreased formation and maintenance of collagen, increased periodontal inflammation, haemorrhage, and tooth loss. However, Staudte et al[11] reported that the intake of grape fruit leads to an increase in plasma vitamin C levels and reduces gingival bleeding. It is most likely that these biochemical functions of vitamin C[12, 13] are resultant largely from its chemical properties as a reducing and chelating agent.

Zinc is a trace element essential to all forms of life because of its fundamental role in gene expression, cell development and replication. The involvement of zinc in diabetes mellitus is not surprising because zinc is a component of a wide variety of metalloproteins, including zinc finger proteins and more than 300 different enzymes. It can stabilize protein domains, acting as an essential requirement for the successful growth of many internal organs, stabilizing the structural and functional integrity of eukaryotic cells and tissues.[14, 15] Disturbances of zinc homeostasis seem to be associated with diabetes, cirrhosis of the liver, tumors, bowel disease, and impaired function of the immune system, taste acuity and also with in vitro effectiveness of insulin.[16]

All these observations suggest that zinc and vitamin C are more intimately linked to human pathophysiology than previously thought. This study was performed to evaluate the serum vitamin C and zinc status in patients of type 2 diabetes mellitus with and without periodontitis.

MATERIALS AND METHODS

Study Subjects and Ethical Approval

The study consisted of a total of 450 subjects between the age group 25 to 56 years. The subjects were divided into three groups, consisting of 150 participants in each group as:

Group I – Control healthy subjects

Group II- Type 2 diabetes mellitus without periodontitis

Group III - Type 2 diabetes mellitus with periodontitis

Group I subjects were selected from a general population. Group II subjects for the studies were enrolled from SRM Speciality Hospital, India and group III subjects were selected from the outpatients attending Department of Periodontology & Oral Implantology, SRM Dental College, India. The study plan was approved by the Institutional

Ethical Committee of Medical and Health Sciences, SRM University, India and an informed written consent was obtained from all the participants. Patient records were used to retrieve data regarding the diabetic state and general health status of the subjects.

Clinical Assessment of Study Subjects

Information about the age, gender, blood pressure, body mass index (BMI), duration of diabetes mellitus, current medications (insulin supplementation, oral hypoglycemic agents, combination of oral hypoglycaemic drug and insulin), diet and diabetes mellitus complications were obtained by a standardized questionnaire. For all subjects, the basic clinical history and demographic data were recorded. The clinical assessment for periodontitis subjects included examination of gingiva, intra oral examination- number of teeth present and missing, pathological migration, and probing depth. Mean pocket probing depth (PPD), and clinical attachment loss (CAL) were measured using mouth mirror and William's periodontal probe to assess the periodontal status. PPD was measured as the distance from the gingival margin to the bottom of the probed pocket. Probing depths were recorded at six sites per tooth, rounded up to the nearest millimetre. Periodontitis was confirmed by bone loss evident on radiographic examination. The periodontal status was examined by a trained Periodontist of SRM Dental College, Department of Periodontology, Chennai - 600 089. The healthy controls were not on any kind of prescribed medication or dietary restrictions.

Inclusion and Exclusion Criteria

Diabetic subjects should have type 2 diabetes mellitus, diagnosed by a physician by means of oral glucose tolerance test, for at least the past 5 years. Diabetes was treated with sulphonyluureas, biguanide, combinations of these two drugs or diet alone. All periodontitis individuals included under the category of periodontitis should have more than 30% of the sites with Clinical attachment level (CAL) ≥ 3mm and pocket depth (PD) ≥ 5 mm, at least 2 teeth in each quadrant with the condition of 20 teeth in all the subjects. Smokers, alcoholics, drug abused, patients who had periodontal therapy six months prior to the study, patients under antibiotics and having systemic disease other than diabetics, taking hormone drugs, lipid lowering drugs, oral contraceptives, pregnant and lactating women were excluded from the study.

Basic Measurements

BMI was calculated based on measures of body weight and height as weight in kilograms divided by height in meters squared. Systolic and diastolic blood pressure was determined as the mean of two measurements. Blood samples were collected after an overnight fast for each subject. Serum was obtained by centrifuging the blood at 1500 rpm for 10 minutes. Serum glucose concentration were measured with the glucose oxidase - peroxidase method, was expressed in milligrams per decilitre and blood glycated hemoglobin (HbA1c), analyzed by high-performance liquid chromatography method (Biosystems S.A, Costa Brava, Spain) was expressed in percentage with a reference value of 5 to 7%.

Determination of Serum Vitamin C and Zinc

Vitamin C level was determined using a 2, 4-dinitrophenylhydrazine[17] (DNPH) method. Serum was deproteinised and 1ml of it was treated with 2 ml of 10% TCA and was allowed to stand for 5 minutes. The mixture was centrifuged in a cooling centrifuge at 2500 X g for 15 minutes and 2 ml of the supernatant was used for vitamin C assay. The final readings were performed using a SL 159-UV visible spectrophotometer (ELICO) at the wavelength of 520 nm. Serum zinc was estimated using Nitro-PAPS (pyridylazo-N-propyl-N-sulfopropylamino-Phenol) method, and the value is expressed in µg/dl.

Statistical Analysis

Data were presented as mean ± SD (standard deviation). An unpaired Student's t test and Newman-Keuls multiple comparison test were used to evaluate the differences between groups. Correlations between various variables are done using Pearson's moment correlation equations. Statistical significance was taken as $P < 0.05$. All statistical analysis was performed using Winks SDA 7.0.5 (Windows Kwik Stat) statistical software package.

RESULTS AND DISCUSSION

Baseline demographic characteristics features are given in Table 1. The level of vitamin C and zinc in serum of studied population were shown in Fig. 1 and Fig. 2 respectively. Pearson Correlation coefficients between zinc and vitamin C in all the three groups were represented in Fig 3.

The mean serum vitamin C level in type 2 diabetes mellitus with periodontitis (group III) was significantly lowered ($p < 0.0001$) when compared with those from diabetes mellitus without periodontitis (group II) and control subjects (group I). At the 0.05 significance level, the mean value of group III (type 2 diabetes with periodontitis) was found to be significantly different and the means of group II (type 2 diabetes without periodontitis) and I (Control) do not differ significantly. The mean level of vitamin C in group II is greater than the means of group III but lesser than group I, Fig 1.

The serum concentration of zinc in group I, II, and III were shown in Fig.2. According to Newman-Keuls Multiple Comparison test, the means levels of serum zinc of group III was lesser than the mean value of all other groups. At the 0.05 significance level, the means of group III was significantly different when compared to other groups. The level of serum zinc in control subjects is greater than type 2 diabetes mellitus with periodontitis (group III) but lesser than type 2 diabetes mellitus without periodontitis (group II) subjects.

Table 1: Demographic and descriptive statistics of each parameter within the three groups

Parameters	Control (Group I)	Type 2 diabetes without Periodontitis (Group II)	Type 2 diabetes mellitus with periodontitis (Group III)
No of samples	150	150	150
Gender (M/F)	80/70	78/72	77/73
Age, years	35.46 ± 10.74	46.26 ± 10.02***	44.42 ± 10.37***
BMI, kg/m^2	22.72 ± 1.5	23.32 ± 1.49 **	24.07 ± 1.51**
Systolic blood pressure (mm Hg)	119.5 ± 4.65	126.4 ± 5.70**	128.8 ± 5.09**
Diastolic blood pressure(mm Hg)	72.93 ± 2.10	75.14 ± 1.78**	79.05 ± 3.03**
FBG, mg/dl	95.28 ± 12.51	183.7 ± 57.16***	176.7 ± 59.12***
PD (mm)	1.45 ± 0.13	1.42 ± 0.17 ^{NS}	4.61 ± 0.51***
CAL (mm)	0.70 ± 0.27	0.64 ± 0.15**	4.91 ± 0.37***

Values are expressed as Mean ± SD; except for gender (Male, M / Female, F). Glycosylated hemoglobin, HbA1c; Body mass index, BMI; Fasting blood glucose, FBG; Probing depth, PD; Clinical attachment level, CAL. Differences were considered significant level at *** p <0.0001; ** p <0.001 for parameters of group II, III vs group I and NS, non-significant.

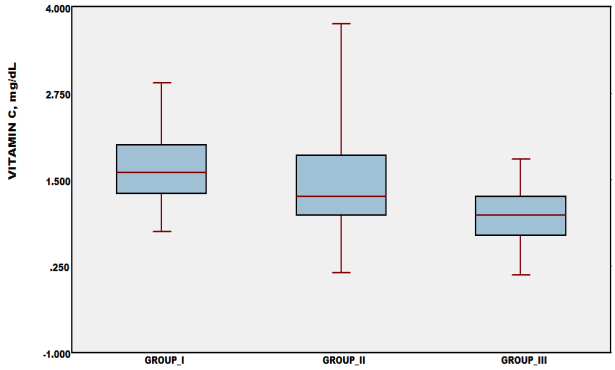

Figure 1: Mean ± SD of Vitamin C level in Group I: Control healthy individuals; Group II: Type 2 diabetes mellitus without periodontitis; Group III: Type 2 diabetes mellitus with periodontitis.

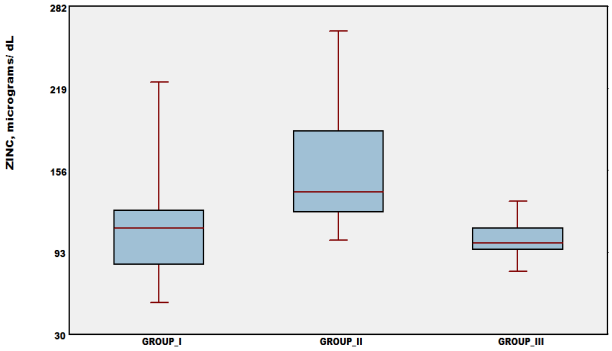

Figure 2: Serum zinc level in the three groups. The box represents the mean, mean ± SD and the range. Group I: Control healthy individuals; Group II: Type 2 diabetes mellitus without periodontitis; Group III: Type 2 diabetes mellitus with periodontitis.

We found a negative correlation between serum zinc and vitamin C in the control, type 2 diabetes mellitus without periodontitis and type 2 diabetes mellitus with periodontitis subjects, Fig 3.

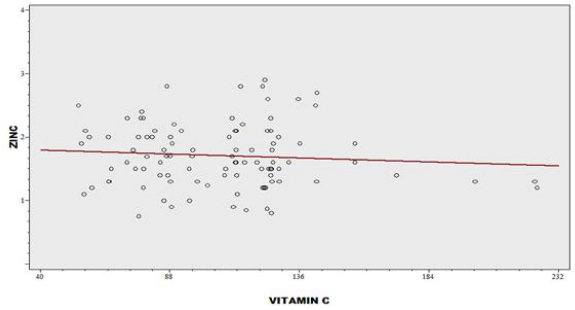

Fig a

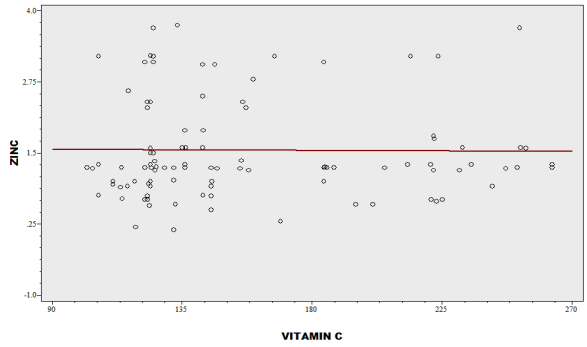

Fig b

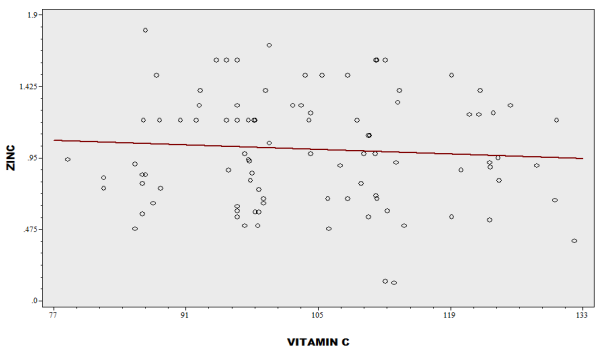

Fig c

Figure 3: Pearson Correlation between zinc and vitamin C in a) control subjects b) type 2 diabetes mellitus without periodontitis c) type 2 diabetes mellitus with periodontitis

Many human diseases are associated with an increased oxidative stress resulting either from the altered production of free radicals or from the altered antioxidant content or activity. Metal ions are known to play an essential role in living systems, both in growth and in metabolism. Impaired metabolism of trace elements is observed in diabetic patients with periodontitis. Among the antioxidants normally consumed by humans, the antioxidant zinc and vitamin C are of particular importance. Our study revealed that the serum level of vitamin C and zinc was altered during type 2 diabetes mellitus with periodontitis. We found a low serum vitamin C and zinc level in type 2 diabetes mellitus with periodontitis (group III) when compared to type 2 diabetes mellitus without periodontitis (group II), and healthy control subjects (group I).

One key link between periodontitis and the chronic systemic inflammatory condition is that they are all strongly associated with the presence of oxidative stress. Oxidative stress underpins the pathogenesis of periodontitis[18] and type 2 diabetes mellitus.[19] Oxidative stress may result from mitochondrial electron leakage at complex III of the hydrogen-electron transfer chain on the inner mitochondrial membrane during normal metabolism. This gives rise to the single electron reduction of molecular oxygen forming the superoxide radical. Another mechanism by which dietary refined carbohydrate and saturated fat intake generate oxidative stress is by receptor binding of advanced glycation end products (AGE) to their complimentary receptor (RAGE), or oxidized LDL to Toll-like receptor-4, on the neutrophil membrane. Another endogenous source for oxygen radical formation is its functional generation by host defense cells (phagocytic lymphocytes) during an inflammatory response following stimulation by e.g. opsonized particles, bacterial DNA or peptides and activation of the hexose monophosphate shunt which utilizes molecular oxygen and NADPH as electron donor.[20]

Studies have observed that grown-up and elderly patients with type 2 diabetes mellitus have lower levels of vitamin C and higher levels of oxidative stress markers[21] compared to control population. It has been proposed that the hydrophilic properties of Vitamin C aid their localization at the boundary of the lipid bilayers in membranes thereby leading to the effective inhibition of attack by free radicals in the aqueous phase, and effective repair of lipophilic antioxidants.[22] Hyperglycemia evoked oxidative stress plays a crucial role in the development of diabetic complications, which is considered to result from augmented reactive oxygen species generation and decreased antioxidant defenses. An increase in blood sugar levels in diabetic rats was observed after the induction of diabetes by streptozotocin.[23]

The relationship between periodontitis and vitamin C is also supported by a Finish study[24] which demonstrated an inverse relationship between plasma vitamin C values and serum antibody levels to P. gingivalis in a random subsample of Finnish and Russian men. Furthermore, in a non-scorbutic experimental periodontitis rat model it was shown that vitamin C supplements reduced the polymorphonuclear leukocyte infiltration in the gingival.[25] In a more recent study, Amaliya et al.[26] also found in an Indonesian population deprived of dental care a significant inverse relationship between plasma vitamin C levels and attachment loss.

Vitamin C is a powerful antioxidant radical scavenger within the aqueous phase. Antioxidants serve to stabilize these highly reactive free radicals, thereby maintain the structural and functional integrity of cells. Association with periodontal-pathogenic bacteria disturbs this sensitive balance resulting in collagen degradation and consequently in pocket formation. An increase in serum vitamin C level would have an advantageous effect on periodontitis. As a water-soluble vitamin it protects particularly the aqueous environments against oxidative attacks, the same as leucocytes accumulate vitamin C to protect themselves during phagocytosis against oxidative stress.

Specific mechanisms of transport and metabolism concentrate vitamin C intracellularly to enhance its function as an enzyme cofactor and a scavenging antioxidant.[27] However it is unclear how vitamin C intake affects gingival oxidative stress, gene expression encoding inflammation, and cell behavior in periodontal lesions. Although some studies suggest that vitamin C increases the number of collagen bundles in the regenerating periodontal tissue and detoxifies histamine[28] in gingival inflammation. Experiments show that the antioxidant activity of ascorbic acid involves transfer of hydrogen rather than an electron.

Deficiency of zinc may play an important role in the development of diabetes with periodontitis through several potential mechanisms. Zinc could protect cells against oxidative damage by binding to vicinal sulfhydryl groups, therefore preventing intramolecular disulfide formation.[29] It could also protect cells by inhibition of the production of reactive oxygen species (ROS) such as hydroxyl (•OH) or superoxide (O2•) radicals by transition metals. This occurs certainly by competition between zinc and other transition metals, e.g., iron or copper, for binding sites.[30] Zinc also plays a different role in mitochondria, because it prevents H_2O_2-induced apoptosis of cells through regulation of the Bcl-2/Bax proteins ratio. We observed a high serum zinc level in type 2 diabetes mellitus without periodontitis (group II). Excess of zinc is cytotoxic and induces cell death. [31] In addition, micromolar concentrations of zinc inhibit caspase-3, a proteolytic enzyme considered as an executioner of apoptosis[32] while intracellular zinc depletion induces direct caspase-3 activation.

Oxidative stress may release zinc from internal stores into cytosol, thereby inhibiting various cellular functions. In some cases, zinc can even act as a pro-oxidant metal, by enhancing production of reactive oxygen species[33] and therefore proceeds directly as a pro-oxidant on signal

transduction. High zinc levels can also decrease the transmembrane mitochondrial potential and cause the degradation of the antiapoptotic protein Bcl-2. An excess of zinc is well known to inhibit various zinc-dependent enzymes such as copper-zinc superoxide dismutase or thioredoxin reductase, or enzymes of DNA repair like glycosylases or endonucleases.[34] The sensitivity of cells to oxidative stress was paradoxical, as zinc is usually considered to be an antioxidant.[35]

The inhibition of multiple biological processes by zinc demonstrates that its cellular concentration must be controlled carefully and kept well below the micromolar range to preserve the activity of zinc metalloenzymes and other enzymes critical to metabolism. The lower concentration of serum zinc among the type 2 diabetes mellitus with periodontitis subjects may have resulted from lower intake, excessive loss or inherited disturbance in its metabolism. Thus, the results of the present study suggest that low level of zinc is an additive factor to low dietary bioavailability among patients of diabetes mellitus with periodontitis.

It has previously been proposed that a continued photochemical and ambient generation of active oxygen species may constitute a significant risk factor in the etiology of periodontitis. However, under conditions of elevated radical production and/or decreased antioxidant defences, oxidative tissue damage may occur. During type 2 diabetes mellitus with periodontitis, ROS produced by zinc and vitamin C may not only play a role in the host defence against the bacteria but also may cause oxidative damage to host tissues.

CONCLUSION

We conclude that lower vitamin C and zinc concentration are associated to an increased risk for type 2 diabetes mellitus with periodontitis when compared to healthy subjects. Vitamin C acts as an efficient scavenger of aqueous radicals and oxidants, thereby protecting other biomolecules from oxidative damage. More controlled clinical trials are required to evaluate the effects of decreased levels of vitamin C and zinc on the risk of developing disease pathogenesis and progression of type 2 diabetes mellitus with periodontitis before making widespread public health recommendations.

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