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Influence of Serum Zinc on Calcium, Iron and Magnesium levels in Type 2 Diabetes Mellitus with Periodontitis

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Abstract: Calcium, iron, magnesium and zinc are essential micronutrients necessary for the growth and development. The disturbances in the micronutrient status will lead to the development of periodontitis and diabetic complications. Therefore, the study aimed to investigate the association between serum zinc and iron levels among the type 2 diabetes mellitus (T2DM) with and without periodontitis. This study was performed among the three groups as healthy individuals (group I, n=150), T2DM with periodontitis (group II, n=150), T2DM without periodontitis (group III, n=150), and Non-DM with periodontitis (group IV, n=150). Calcium, magnesium and zinc levels in the serum estimated using the commercial kits available in the market. Serum iron was determined, using Ramsay's dipyridyl method and the readings are measured using a SL 159-UV visible spectrophotometer (ELICO) at the wavelength of 520 nm. The mean serum calcium and iron level was significantly higher (p <0.0001) in T2DM with periodontitis (group III) when compared to control. The mean levels of serum magnesium in group III are lesser compared to other groups. The serum Zn level in T2DM with periodontitis (group II) was found to be significantly less when compared to other groups. High level of serum calcium and iron and low serum concentration of zinc and magnesium are major risk factors for T2DM with periodontitis, causing oxidative stress and increased cytokines production, all these might leads to insulin resistance and decreased insulin secretion.

Key Words: Calcium, Magnesium, Micronutrients, Iron, Type 2 diabetes mellitus, Periodontitis, Zinc.

Introduction

Type 2 diabetes mellitus (T2DM) and periodontitis are an important health concern worldwide. T2DM is characterized by significant losses of important micronutrients due to metabolic basis of the disease and its complications. The prevalence of this disease and its complications is increasing in a troublesome way. It is expected that by 2035, 592 million people will suffer from DM[1]. Control of blood glucose is crucial to prevent or delay diabetic complications that frequently result in increased morbidity and mortality. Most strategies include medical treatment and changes in lifestyle and diet[2]. However, recent studies have shown that changes in lifestyle and a better diet control are more effective than drugs[3]. Several clinical trials, have confirmed the role of micronutrients derived from functional foods for hyperglycemia management[4]. These compounds have antioxidant, anti-inflammatory and anti-carcinogenic activities. It also plays an important role in prevention of the development of DM and its complications.

Periodontal disease is a common chronic, which accounts for 80-85% of cases with diabetes, is an inflammatory response to bacteria that exist in the gum tissue (periodontal ligament) that causes an irreversible

loss of the supporting tooth structures, and may lead to eventual loss of teeth[5]. Hyperglycemia has been found to modify periodontal expression, by interfering with the host response and causing an excessive inflammatory response to infection, [6] as well as by the interaction of the receptor for advanced glycation end products (RAGE) with its ligands in gingival[7]. Inflammatory periodontal diseases are the most common chronic inflammatory conditions of humans worldwide. It is well established that genetic and environmental factors affect the severity and lifetime risk of developing periodontal disease. Furthermore, it is recognized that microbial factors cannot be solely responsible for periodontitis. Although the exact etiology is still unknown, the interaction between environmental factors and nutritional factors has been found to play a role in the pathogenesis of diabetes mellitus. Smoking is a well-known risk factor and periodontitis has emerged as another environmental risk factor.

Micronutrients are essential to fulfill a broad range of biochemical and physiological functions, and are tightly regulated by homeostatic processes. The micronutrients function as co-enzymes in key metabolic reactions, as an antioxidants in the control of damage caused by reactive oxygen species, as modulators of gene transcription, components of and co-factors for enzymes and structural components of tissues [8, 9]. In this century, it is hard to reconcile the concepts of the Western diet and overconsumption with the risk of micronutrient deficiencies. However, deficiencies can arise from poor dietary intake, alone or combined with physiological or metabolic injury.

The metabolic pool of calcium in the extracellular fluid (ECF) is very small compared with the large skeletal reserves, mobilization of which compensates for an inadequate intake of calcium. Conversely, there are no specific reserves for minerals such as zinc and magnesium, and the body is largely dependent on a regular supply in the diet. Interestingly, there is no physiological mechanism for iron excretion and iron balance is maintained through the regulation of its absorption from the diet. If iron is not required, it is stored in duodenal mucosal cells as ferritin and excreted in the faeces when mucosal cells are exfoliated.

Zinc has been shown to influence the synthesis, storage, and release of insulin. A 2009 study described a randomized, blinded, crossover trial involving 60 obese children. When given 20 mg zinc daily, the treatment groups experienced significant reductions in fasting plasma glucose levels and fasting insulin levels. These changes disappeared when the zinc was withdrawn. The authors concluded that, in addition to lifestyle modifications, zinc supplementation might be a useful and safe additional intervention for improvement of cardio-metabolic risk factors related to childhood obesity [10].

The role of micronutrients in the prevention or treatment of diseases including periodontitis and T2DM is of interest, a key driver for the micronutrients and supplement market is their advertised potential to optimize health and performance in healthy individuals. A nutritionally balanced diet is a safer way to achieve sufficiency. Therefore, the study aimed to assess the serum levels of calcium, iron, magnesium and zinc in T2DM with periodontitis, T2DM without periodontitis and Non-DM with periodontitis subjects.

Materials and Methods

The study consisted of a total of 600 subjects in the age group of 25 to 56 years among the three groups as healthy individuals (group I, n=150), T2DM with periodontitis (group II, n=150), T2DM without periodontitis (group III, n=150), and Non-DM without periodontitis (group III, n=150). Group I subjects were selected from a generalized population and group II subjects for the studies were enrolled from the SRM Speciality Hospital, India. Group III and group IV subjects were selected from the outpatients attending the 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.

Clinical Assessment of study subjects

Information about the age, gender, blood pressure, body mass index (BMI), duration of diabetes mellitus, current medications (insulin supplementation, and oral hypoglycemic agents), diet and diabetes mellitus complications, were obtained by a standardized questionnaire. For all the subjects, the basic clinical history and demographic data were recorded. The clinical assessment for periodontitis subjects included

examination of the gingiva, intra oral examination- number of teeth present and missing, pathological migration, and probing depth. The periodontal status was examined by a trained Periodontist of SRM Dental College, Department of Periodontology, Chennai -600 089. Patients with DM were under diabetic diet and did not take nutritional supplements and any drugs that are known to interfere with the serum levels of studied metals during the period of study. The healthy controls were not on any kind of prescribed medication or dietary restrictions.

Inclusion and Exclusion Criteria

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. Diabetic subjects should have T2DM, diagnosed by a physician by means of the oral glucose tolerance test, for at least the past 5 years. Type 2 diabetic patients having vascular complications as diabetic nephropathy, neuropathy and retinopathy were excluded from the study. 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, hypotensive diuretics, oral contraceptives, and pregnant 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. The 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. HbA1c, analyzed by the high-performance liquid chromatography method (Biosystems S.A, Costa Brava, Spain) was expressed in percentage, with a reference value of 5 to 7%. Serum glucose was measured by the glucose oxidase-peroxidase (GOD-POD) method, using the reagent kit purchased from Merck Specialities Private Limited, India.

Estimation of serum calcium, iron, magnesium, and zinc

Serum calcium determination was done using OCPC (o-Cresolphthalein Complexone) method, and the values are expressed in mg/dl. The absorbance was read at 570 nm. The kits were purchased from Crest Biosystems (a division of Coral Clinical Systems), Goa, India. Serum iron was determined, using Ramsay's [15] dipyridyl method. Equal volumes of serum, 0.1 M sodium sulphite and dipyridyl reagent were mixed in a glass stoppered tube and centrifuged. The supernatant is heated in boiling water for 5 minutes. It is cooled, 1ml. of chloroform added, stoppered and shaken vigorously for 30 seconds. It is then centrifuged for five minutes at 300 rpm to get a clear supernatant fluid. The readings were measured using a SL 159-UV visible spectrophotometer (ELICO) at the wavelength of 520 nm. Serum magnesium concentration was measured at the absorbance of 520 nm, with Xylidyl Blue dye reagent kit purchased from Agappe Diagnostics, Kerala, India. The value of magnesium concentration was expressed in mg/dl. Serum zinc was estimated, using the kits purchased from Crest Biosystems (a division of Coral Clinical Systems), Goa, India by Nitro-PAPS (pyridylazo-N-propyl-N-sulfopropylamino-Phenol) method, and the values 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 correlation equations. The statistical significance was taken as $p < 0.05$. All statistical analysis was performed, using the statistical software packages, Graphpad Prism 6 for windows (San Diego, California), and Winks SDA 7.0.5 (Windows Kwik Stat).

Results and Discussion

The demographic and the clinical characteristics within group I (healthy controls), group II (T2DM without periodontitis), group III (T2DM with periodontitis) and group IV (Non-DM with periodontitis) was shown in Table 1. There were no statistical differences in the mean of systolic blood pressure, and diastolic blood pressure among the four groups. The mean percentage of the HbA1c levels was found to be 7.74 ± 1.31 in

group II and 8.38 ± 1.17 in group III when compared to control. However, there were no differences in the HbA1c levels between the group I and group IV subjects. The mean FBG level was significantly elevated in the group II and group III subjects, when compared to group I and group IV. As expected the mean levels of periodontal probing depth (PPD) and clinical attachment level (CAL), were significantly greater than 4mm in T2DM with periodontitis and in Non-DM with periodontitis, when compared to healthy subjects.

Table 1 Demographic and clinical characteristics of the study population

Parameters	Control Group I	T2DM without periodontitis Group II	T2DM with periodontitis Group III	Non-DM with periodontitis Group IV
No of subjects	150	150	150	150
Gender (M/F)	80/70	78/72	77/73	75/75
Age, years	35.46 ± 10.74	46.26 ± 10.02***	44.42 ± 10.37***	41.66 ± 10.45***
BMI, kg/m^2	22.72 ± 1.5	23.32 ± 1.49 **	24.07 ± 1.51**	23.93 ± 1.12**
Systolic blood pressure(mm Hg)	119.5 ± 4.65	126.4 ± 5.70 ^{NS}	128.8 ± 5.09 ^{NS}	126.7 ± 8.39 ^{NS}
Diastolic blood pressure(mm Hg)	72.93 ± 2.10	75.14 ± 1.78 ^{NS}	79.05 ± 3.03 ^{NS}	76.47 ± 4.52 ^{NS}
HbA1c %	5.20 ± 0.51	7.74 ± 1.31***	8.38 ± 1.17***	5.14 ± 0.56 ^{NS}
Fasting blood sugar, mg/dl	95.28± 12.51	183.7 ± 57.16***	176.7 ± 59.12***	96.88 ± 12.67^{NS}

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

Serum calcium, iron, magnesium and zinc levels in the four groups

The mean calcium level in T2DM with periodontitis (group III) was significantly higher (p <0.0001) when compared to control. However, in the case of T2DM without periodontitis, the serum calcium levels were decreased non-significantly. The mean level of iron in group II is lesser than the means of all other groups. At the 0.05 significance level, the means of group II, group III, and group IV are significantly different. The level of iron in the group I and group IV subjects is greater than group II, but lesser than in group III subjects. Increased level of serum iron in T2DM with periodontitis can act as a strong pro-oxidant, which catalyze several reactions leading to the formation of reactive oxygen species. The mean levels of serum magnesium in group III are lesser compared to other groups. At 0.05 significance, the mean level of magnesium in group III is significantly different; however group II, group IV and group I are not. The means of magnesium in group II and group IV are greater than in group III but lesser than in group. The mean levels of serum zinc in group III was lesser than the means of all other groups. The data show that T2DM with periodontitis individuals have lower zinc than those T2DM without periodontitis. The serum zinc level in T2DM without periodontitis (group II) and Non-DM with periodontitis (group IV) was found to be significantly higher when compared to other groups, Table 2.

Table 2 Serum Calcium, Iron, Magnesium, and Zinc levels in the four groups

Parameters	Control Group I	T2DM without periodontitis Group II	T2DM with periodontitis Group III	Non-DM with periodontitis Group IV
No of subjects	150	150	150	150
Calcium, mg/dl	8.77 ± 1.08	8.59 ± 0.86 ^{NS}	11.79 ± 2.07***	10.56 ± 3.17***
Iron, µg/dl	82.46 ± 14.42	76.53 ± 20.23*	114.9 ± 40.91***	107.8 ± 52.66***
Magnesium, mg/dl	1.65 ± 0.32	1.56 ± 0.42 ^{NS}	1.45 ± 0.41***	1.57 ± 0.44 ^{NS}
Zinc, µg/dl	113.4±12.65	157.2±45.8***	106.8±31.83 ***	135.7±51.39***

Values are expressed as Mean ± SD; Differences were considered significant at
*** p <0.0001; *p<0.05 for parameters of group II, III, IV vs group I and NS, non-significant.

Correlation between serum zinc with calcium, iron and magnesium in the four groups

We observed a negative correlation between serum zinc and calcium among T2DM with periodontitis (group III) and a positive correlation among the non-DM with periodontitis (group IV). Zinc does not show any correlation with calcium among the T2DM without periodontitis (group II). Zinc correlated negatively with iron in the group II, group III and group IV. Zinc showed positive correlation with magnesium in the group II and group IV but it correlated negatively with magnesium in the group III subjects, Table 3

Table 3 Pearson's correlation between serum zinc with Calcium, Iron and Magnesium levels in the four groups

Parameters	Control Group I	T2DM without periodontitis Group II	T2DM with periodontitis Group III	Non-DM with periodontitis Group IV
ZINC				
CALCIUM				
R	-0.195	-0.074	-0.037	0.046
p	0.649	0.463	0.012*	0.052*
IRON				
R	-0.111	-0.049	-0.011	-0.013
p	0.271	0.002*	0.013*	0.894
MAGNESIUM				
R	0.108	0.209	-0.282	0.129
p	0.285	0.037*	0.004*	0.204

*significant *p* value; Pearson's correlation coefficient, r

Influence of zinc on calcium, iron, and magnesium in T2DM with periodontitis

Micronutrients 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. It has been reported that the urinary excretion of calcium, zinc and magnesium is increased in T2DM causing a decrease in the blood levels of these elements in these patients. Another study reported that the levels of zinc and magnesium were significantly lower in serum of patients with insulin dependent diabetes mellitus.

Zinc has a recognized action on the metalloenzymes, since it participates in their structure, and catalytic and regulatory action [11]. Zinc enzymes encompass all enzyme classes and many of them participate in a variety of metabolic processes, such as synthesis and/or degradation of lipids, carbohydrates, proteins and nucleic acids. Zinc is known to be present in appreciable amounts in cell nuclei, nucleoli, chromosomes, ribosomes, and secretory granules. It also plays a clear role in the synthesis, storage and secretion of insulin as well as its conformational integrity.

In addition to being a structural component of body tissues minerals are also involved in various physiological processes, such as proper metabolism and energy production. Antioxidants play a role as defenders against occurred damages by metal-mediated free radicals. Zinc acts as an antioxidant for the decrease of oxidative stress [12]. Usually the serum zinc concentration is used to determine the zinc status, but the serum zinc level is not only decreased in real zinc deficiency, but also in stress [13]. During stress, serum zinc is redistributed from the serum into the liver.

Zinc is an essential metal, necessary to the function of many enzymes participating in a wide variety of metabolic processes. It has been suggested that oxidative stress plays an important role in tissue damage associated with diabetes, and that peroxide formation is increased in parallel to elevated glucose oxidation. The protective effects of zinc against increased rates of lipid peroxidation could be due to its capability to combine and strengthen cellular membranes against lipid peroxidation and disintegration. It also suggests that changes in serum zinc levels are more indicative of some metabolic complications of T2DM than of periodontitis itself. High zinc concentrations are responsible for the induction of apoptosis in cells with deficiencies in uptake and complexing.

The lower concentration of serum zinc among those with T2DM with periodontitis may have resulted from lower intake, excessive loss or inherited disturbance in its metabolism. Thus, the results of the present study suggest that a low level of zinc is an additive factor for low dietary bioavailability among patients of T2DM with periodontitis. However, it is likely that the changes in serum zinc were caused not solely by periodontitis; i.e., the diabetic patients with periodontitis showed serum zinc values lower than that observed in diabetic patients without periodontitis and non-diabetes with periodontitis.

Calcium and iron play an essential role in regeneration, for coping with oxidative stress and for an adequate immune response. In this study it was shown that elevated calcium and iron levels may be a contributing factor in many inflammatory conditions[14]. The result of the study has shown a decreased serum zinc and higher level of calcium and iron in T2DM with periodontitis individuals. Among the T2DM without periodontitis, the serum level of zinc was elevated and serum calcium and iron levels were decreased. In non-diabetic with periodontitis subjects we found an increased serum zinc, calcium and iron level.

Zinc correlated negatively with calcium, iron and magnesium in T2DM with periodontitis (group III). Zinc correlated positively with calcium in Non-DM with periodontitis (group IV). It is of interest to note that very high serum calcium concentration in T2DM with periodontitis and non-diabetes with periodontitis indicates that a strong correlation found between the pocket depth and alveolar bone loss. Zinc causes the release of calcium from mitochondria possibly by the inhibition of respiration rather than a direct effect on a calcium transporter.

Excess levels of calcium and iron in the serum may promote the development and progression of oxidative stress, altered immunity and altered insulin secretion or its action. Zinc and iron are trace elements required for the function of various enzymes and other cellular proteins, and become toxic in the case of excessive intracellular accumulation [15]. In addition, zinc also plays a role in iron homeostasis.

In particular, it has been shown that zinc supplementation in hepatoma cells can alter the iron transporter expression, and functionally decrease iron absorption, reflecting a homeostatic control in response to high iron accumulation [16]. The ingestion of excess zinc depresses the apparent absorption of iron. Supplements containing Fe and multiple trace elements and minerals are used by millions of people world-wide. It is also a common practice to take iron supplements during pregnancy in the developing countries. Iron was shown to inhibit zinc absorption in humans in a dose dependent manner, when given in a water solution but not when given with the meal[17].

Magnesium is a crucial metal ion that catalyzes the enzymatic reactions in all phosphate transfer reactions through the formation of Mg–ATP complexes. Magnesium and zinc are reported to possess antioxidant property[18,19]. Several mineral deficiencies have been associated with some of the complications of diabetes. Magnesium deficiency has been frequently described in both adults and children with diabetes [20] and has been implicated as a risk factor in the development and progression of periodontitis in adult diabetics. This was supported by our finding that a highly significant decrease in the serum magnesium levels among the group II, group III and group IV subjects. Decreased magnesium levels may be extremely important for the long-term outcome of diabetes with periodontitis. In the correlation, magnesium correlated positively with zinc in T2DM without periodontitis (group II) whereas it correlated negatively with zinc in T2DM with periodontitis (group III).

Potential mechanisms of Zinc's antioxidant function may be related to several factors. First, zinc is an essential component of the cell's first lines of defense against reactive oxygen species (ROS), which functions to remove the superoxide anion. The second potential mechanism for zinc's antioxidant effects is the antagonism of redox-active transition metals, such as iron or copper, and the prevention of oxidation of sulfhydryl groups within proteins. A third mechanism by which zinc acts as an antioxidant is through its regulation of metallothionein metabolism[21].

In chemically defined systems, zinc can prevent hydroxyl radical formation by transition metals. Most biological molecules cannot be damaged at a significant rate by direct reactions with oxygen, superoxide or hydrogen peroxide. However, they can be oxidized by hydroxyl radicals. This species is formed when a single electron is transferred to H_2O_2. Transition metals, such as iron and copper, can donate electrons to hydrogen

peroxide via Fenton reactions [22]. By competing for binding sites for pro-oxidant transition metals, such as iron and copper, zinc can decrease their ability to transfer electrons and their availability Fenton-like reactions.

Excess amounts of iron or reactive oxygen species can initiate lipid peroxidation[23], which may lower the serum concentration of zinc and magnesium ions. Lipid peroxidation can cause deleterious effects on membranes in the cell including the lysosomal membrane. Lipid peroxidation facilitated by lipofuscin-associated iron is indeed considered to be one of the main mechanisms underlying the impairment of the lysosomal membrane[24]. Zinc can directly bind and activate metal transcription factor 1, which then bind to metal-responsive elements to induce gene expression of proteins, such as metallothionein. Metallothionein itself acts as a potent scavenger of hydroxyl radical. Thus, loss of this protein can impair cellular antioxidant defenses and contribute to the cell's sensitivity to oxidative stress[25].

Zinc has been demonstrated to have a wide variety of roles in mammalian systems. Zinc may play a physiologica1 role in the growth and calcification of bone tissue. Recently, Yamaguchi et al. [26] have demonstrated that the oral administration of a comparatively high dose of zinc stimulates bone resorption in rats, although at low dosages zinc stimulates bone growth in weanling rats. Thus Zinc-induced bone resorption might be mediated by the parathyroid hormone secreted to maintain calcium homeostatis, because of the hypocalcemia produced by zinc administration. This further supports our study that the alveolar bone loss in periodontitis subjects might be due to decreased serum zinc related to calcium homeostasis in humans.

Conclusions

High level of serum calcium and iron and low serum concentration of zinc and magnesium are major risk factors for T2DM with periodontitis, causing oxidative stress and increased cytokines production. The human body is highly adaptable with efficient homeostatic mechanisms, often under hormonal control, that balance the absorption, transport, storage, utilization and excretion of micronutrients. Vitamin C will increase iron absorption, as will the presence of haem iron in the duodenum and the iron deficient status of the individual. Conversely phytates, and iron-binding phenolic compounds deplete iron stores will decrease absorption. Several minerals, such as calcium, iron, zinc and a number micronutrients display such interactions. A nutritionally balanced diet provides all micronutrient levels in the body which are tightly regulated by homeostatic processes.

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