Original Article

Evaluation of Micronutrient (Zinc, Magnesium, and Copper) Levels in Serum and Glycemic Status after Nonsurgical Periodontal Therapy in Type 2 Diabetic Patients with Chronic Periodontitis

Abstract

Aims and Objectives: To find out the effect of nonsurgical periodontal therapy on serum zinc (Zn), magnesium (Mg), and copper (Cu) concentration and glycemic status in type 2 diabetes with chronic periodontitis (CP). **Materials and Methods:** One hundred and twenty patients were included in this study, which was further divided into three groups. Group 1 consisted of forty patients with CP, Group 2 consisted of forty patients of CP with controlled diabetes, and Group 3 consisted of forty patients of CP with uncontrolled diabetes. Periodontal parameters such as plaque index, gingival index, bleeding on probing, pocket depth, and clinical attachment levels (CALs) were evaluated. Blood samples were collected to assess the levels of fasting blood sugar, glycosylated hemoglobin, Zn, Mg, and Cu. All parameters were evaluated at baseline and 3 months after nonsurgical periodontal therapy. **Results:** The results showed statistically significant reduction in all the clinical parameters within the groups except for the CAL in group 1 patients ($P = 0.05$). The glycemic status also showed a statistically significant reduction after treatment ($P < 0.001$). The intragroup comparison was taken between the values of micronutrients, showed substantial increase in the levels of both Zn and Mg and decrease in the level of Cu after nonsurgical periodontal treatment ($P < 0.001$). **Conclusion:** Patients with diabetes and periodontitis had altered metabolism of Zn, Mg, and Cu contributing to the progression and complication of diabetes mellitus and periodontitis. Nonsurgical periodontal treatment improved the variation and concentration of plasma micronutrients and also the periodontal status and glycemic level.

Keywords: *Chronic generalized periodontitis, glycosylated hemoglobin, micronutrients, type 2 diabetes mellitus*

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Introduction

Periodontitis is a multifactorial disease caused by Gram-negative anaerobic bacteria along with systemic and environmental factors. Additional factors contributing to this multifaceted local disease process include a number of systemic diseases, especially diabetes that can exaggerate the host response to the local microbial factors.[1] Diabetes and periodontal disease share a bidirectional relationship. Diabetes influences the progression of periodontitis, and it is considered as the sixth major complication of diabetes.[2] Poor glycemic control leads to worsened periodontal condition and vice versa. Nonsurgical therapy of periodontal disease has shown to improve the glycemic status of diabetic individuals.

Micronutrients play a role in both diabetes and periodontal disease. The vitality of the periodontal tissues in both health and disease depends on the adequate source of essential nutrients being available to the host. The effect of nutrition on the immune system and its role in periodontal disease have been reviewed by many authors.[3] Periodontitis can attribute to the variation and concentration of plasma micronutrients.[4] Similarly, numerous studies have found alterations in micronutrient status of patients with diabetes mellitus (DM) and the deficiency of certain minerals or vitamins has been correlated with the presence of diabetic complications. The daily diet consists of both micronutrients and macronutrients. The micronutrients are required in milligram (mg) to microgram (μg) quantity and include vitamins and minerals. Recommended daily requirement of micronutrient such as iron, copper (Cu), and zinc (Zn) is <100 (μg). These

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micronutrients are essential for human health and also necessary for functioning of many enzymes systems such as deoxyribonucleic acid polymerase, ribonucleic acid polymerase, superoxide dismutase, catalases, and alkaline phosphatase.[5]

Several studies suggest that diabetes can disrupt the homeostasis of several trace elements. Zn is an essential trace element which is required for the successful growth of many internal organs, stabilizing cell membranes, and modulating bound enzymes and insulin action.[6,7] Zn is stored in liver cells by binding to the cytosolic proteins and also accumulates in the mitochondria and markedly increases succinate dehydrogenase activity in that organelle. Zn is taken up by the liver binds to the subcellular organelles and affects the cellular metabolic system. Zn absorption decreases in diabetic patients, which causes intercellular depletion. Altered metabolism of Zn would also lead to some diabetic complications.[8]

Magnesium (Mg) is an integral part of the structure of cellular and subcellular membranes and helps in stability of the membrane.[9,10] Mg also plays an important role in the activities of various enzymes involved in glucose oxidation and release of insulin.[11,12] Insulin is a major hormone involved in the regulation of Mg metabolism. Mg is involved in the metabolism of carbohydrate, lipid, and protein. It activates over 300 cellular enzymes and is important for the synthesis of proton and electron transporters in the energy cycle of the cell. Diabetic patients have lower serum Mg levels than those without diabetes.

Cu is one of the essential trace elements and has an important role in cytochrome oxidase functions at the terminal end of the mitochondrial electron transport chain. The loss of this activity can contribute to the characteristic swelling and distortion of mitochondria which can be observed in Cu deficiency, particularly in metabolically active pancreatic acinar cells, enterocytes, and hepatocytes.[13] Elevation of serum Cu level is observed in periodontitis patients which can cause certain alterations in collagen metabolism. Cu is essential for proper connective tissue development and the elevation in serum Cu may reflect the changes in periodontal collagen metabolism.[14]

Since the micronutrients levels are altered in diabetes and also in periodontitis, it is possible that nonsurgical periodontal therapy can improve the micronutrient levels in diabetic patients along with improvement in glycemic status. Therefore, in this study, we evaluate the levels of these micronutrients and also the glycemic status in type 2 diabetic patients with chronic periodontitis (CP) after nonsurgical periodontal treatment.

Materials and Methods

The subjects were randomly selected from the outpatient clinic of the Department of Periodontics, Thai Moogambigai Dental College and Hospital, Maduravoyal, Chennai. Written consent was taken from each subject. All participants completed the study. The study protocol was approved by the Ethical Committee of Dr. M. G. R. University, Maduravoyal, Chennai, India, in accordance with the Declaration of Helsinki, as revised in 2000.

This study consists of 120 subjects which are divided into:

- Group 1: 40 subjects with CP
- Group 2: 40 subjects with CP patients with controlled type 2 DM
- Group 3: 40 subjects with CP patients with uncontrolled type 2 DM.

In the selected patients, detailed medical history was recorded. The treating physician's consent and details of the patients, regarding diabetes control were also obtained. The uncontrolled DM was defined based on glycosylated hemoglobin (HBA1C) values more than 8 mg/dl. The history of these diabetic patients selected for the study was more than 5 years. All the clinical parameters, blood samples were obtained from these subjects at baseline and 3 months after nonsurgical periodontal therapy. The duration of the study to procure 120 patients was 3 months.

Inclusion criteria

Subjects who were included in this study should have CP, with or without Type 2 DM. They presented at least four teeth with one or more sites with probing depth ≥5 mm, clinical attachment level (CAL) ≥4 mm, and bleeding on probing (BOP).

Exclusion criteria

Patients who had undergone periodontal treatment in the past 6 months, those with a history of antibiotic administration within the last 3 months, those with <20 remaining natural teeth, subjects who are pregnant, and subjects with a history of smoking and tobacco consumption were excluded from the study.

Periodontal treatment and clinical measurements

All patients were subjected to a periodontal examination performed in six sites per tooth excluding third molar. Periodontal parameters such as plaque index (Silness and Loe 1964), gingival index (Loe and Silness 1963), BOP (Muhlemann and Son 1971), pocket depth, and CAL were evaluated. Blood samples were collected after a minimum of 8 h of overnight fasting for all individuals at baseline and 3 months after treatment. After recording the periodontal status, patients received oral hygiene instructions and underwent full mouth nonsurgical periodontal treatment comprising scaling and root planing under local anesthesia. After the periodontal treatment, a professional plaque control program was performed twice a month for 3 months consisting of supragingival plaque removal and reinstruction of oral hygiene procedures.[15] During this experimental period, patients were questioned about changes in medications related to diabetes therapy, use of anti-inflammatory or antibiotic and alteration of lifestyle, including exercise and diet.

Sample collection

Venous blood samples were collected in the morning after an overnight fast. Samples were analyzed for fasting blood sugar (FBS), HbA1C, Zn, Mg, and Cu. The samples were centrifuged at 3000 rpm for 15 min to separate the plasma for estimation of Zn, Mg, and Cu [Figure 1]. The supernatant was aspirated and collected in separate test tubes to measure the levels of Zn, Mg, and Cu using atomic absorption spectrophotometry [Figure 2]. Fasting plasma glucose level was measured by glucose oxidase-peroxidase method. The HbA1c concentration was measured by column method.

Statistical analysis

Data are presented as mean ± standard deviation. Statistical analyses were performed using a software program (SPSS Version 16, IBM, Chicago, Illinois, USA) Comparison of variables within the groups was calculated by paired *t*-test and between the groups was analyzed using one-way analysis of variance.

Results

When intragroup comparison was done for all the clinical parameters, there is statistical significance in all the three groups except for CAL in Group 1 [Table 1]. The glycemic status was measured using FBS and HbA1C values. When intragroup comparison was done for FBS and HbA1c at baseline and after nonsurgical periodontal treatment, there was statistically significant reduction seen. When intergroup comparison was done for FBS and HbA1c individually, there was statistical significance observed, except between Groups 1 and 2 [Tables 2 and 3]. When intragroup comparison was done for Zn, Mg, and Cu, there was statistical significance observed in all the three groups. When intergroup comparison was done for Zn, there is statistical significance seen after treatment between Groups 1 and 2 and between Groups 1 and 3 ($P = 0.04$) [Table 4]. When intergroup comparison was done for Mg, there is no significance at baseline and after treatment [Table 5]. When intergroup comparison was done individually for Cu, there was statistical significance in the baseline between Groups 1 and 2 and between Groups 1 and 3 ($P = 0.041$ and 0.042), respectively [Table 6].

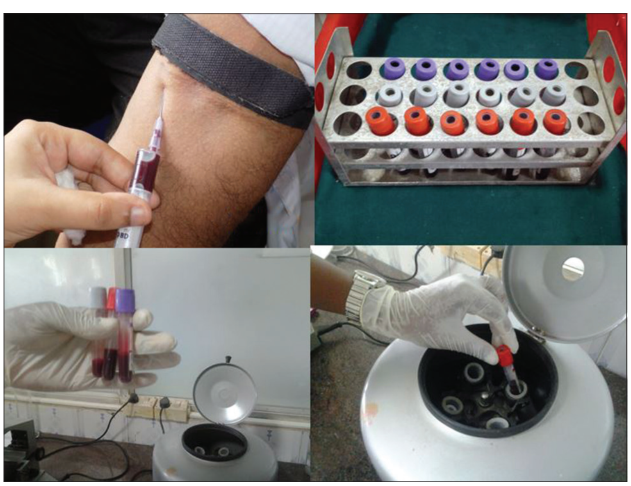

Figure 1: Collection of venous blood samples for measuring fasting blood sugar, glycosylated hemoglobin, and micronutrients and centrifugation

Discussion

Micronutrients such as Zn, Cu, and Mg play an important role to maintain adequate immune response as well as to combat oxidative stress. When malnourished individuals are exposed to infections, the host responds not only by mounting appropriate specific and nonspecific immune responses but also by initiating a well-characterized series of metabolic adjustments. Inflammatory stimuli from dental plaque promote release of reactive free radicals and also exhibit metabolic changes that are modulated by potent soluble mediators known as cytokines. Many studies have assessed the levels of micronutrients in diabetic patients and also in diabetic patients with periodontitis. However, ours is the first study to explore the effect of nonsurgical periodontal therapy on the level of micronutrients in diabetes with CP.

In our study, there was a statistically significant change observed within the groups in all the clinical parameters, except for the CAL in Group 1 after 3 months of nonsurgical periodontal treatment when compared to the baseline values. The subjects with DM had significantly more clinical attachment loss than nondiabetic subjects. Cross-sectional studies done by Grossi *et al*. have shown that diabetic subjects were twice as likely as nondiabetic subjects to have clinical attachment loss.[16] In another cross-sectional study done by Bridges *et al*., it was found that DM affected all periodontal parameters. The improvement in all the clinical parameters in this study was similar to a study done by Rodrigues *et al*.[17]

In our study, there was a reduction in the FBS and HbA1C values after nonsurgical periodontal therapy when compared

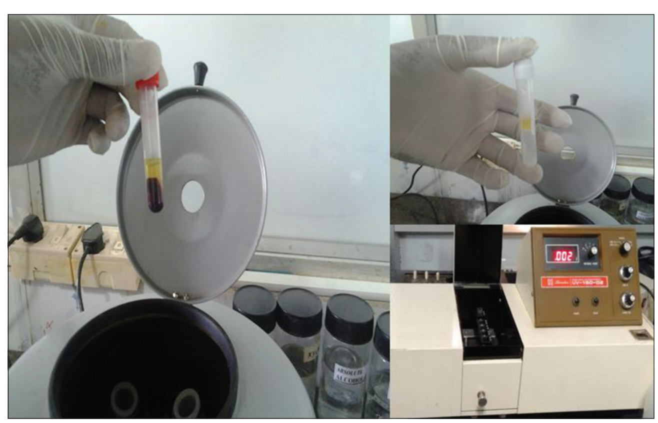

Figure 2: Supernatant serum analyzed using spectrophotometer

Table 1: Intragroup comparison of mean±standard deviation of clinical parameters at baseline and 3 months after treatment using paired *t*-test

	Mean±SD				
	Plaque index	Gingival index	Bleeding on probing	Pocket depth	Clinical attachment level
Group 1					
Mean value at baseline	2.13±0.69	2.43±0.62	2.16±0.68	3.84±1.33	4.13±0.79
Mean value after 3 months	0.80±0.24	0.69±0.26	0.92±0.38	1.87±1.06	2.97±0.99
P	0.003 (significance)	0.0016 (significance)	0.0089 (significance)	0.0031 (significance)	0.05 (NS)
Group 2					
Mean value at baseline	2.11±0.44	2.66±0.37	2.16±0.53	4.04±1.02	4.36±0.67
Mean value after 3 months	0.94±0.08	0.67±0.21	1.02±0.31	1.88±0.54	3.06±1.05
P	0.0039 (significance)	0.0001 (significance)	0.0075 (significance)	0.0121 (significance)	0.018 (significance)
Group 3					
Mean value at baseline	1.66±0.55	2.46±0.77	2.11±0.59	4.80±0.57	4.03±0.47
Mean value after 3 months	0.60±0.20	0.94±0.34	0.79±0.29	1.99±0.71	2.73±1.12
P	0.0052 (significance)	0.0046 (significance)	0.0039 (significance)	0.0003 (significance)	0.0363 (significance)

P<0.05 is considered statistically significant. SD: Standard deviation; NS: Nonsignificance

Table 2: Intergroup comparison of fasting blood sugar at baseline and after treatment in the three groups

Groups	Baseline		After treatment	
	Mean±SD	*P*	Mean±SD	*P*
CP (Group 1)	82.8±7.5	0.114	79.9±7	0.052
CP with controlled diabetes (Group 2)	100±9.7		91.5±6.9	
CP (Group 1)	82.8±7.5	<0.001*	79.9±7	<0.001*
CP with uncontrolled diabetes (Group 3)	174.1±56		132.5	
CP with controlled diabetes (Group 2)	100±9.7	<0.001*	91.5±6.9	<0.001*
CP with uncontrolled diabetes (Group 3)	174.1±56		132.15	

**P* value is significant <0.001. *P*<0.05 is considered statistically significant. SD: Standard deviation; CP: Chronic periodontitis

Table 3: Intergroup comparison of glycosylated hemoglobin at baseline and after treatment in the three groups

Groups	Baseline		After treatment	
	Mean±SD	*P*	Mean±SD	*P*
CP (Group 1)	5.8±1.1	0.83	5.5±0.7	0.63
CP with controlled diabetes (Group 2)	6.0±0.7		5.7±0.6	
CP (Group 1)	5.8±1.1	<0.001*	5.5±0.7	<0.001*
CP with uncontrolled diabetes (Group 3)	9.0±2.1		7.6±1.4	
CP with controlled diabetes (Group 2)	6.0±0.7	<0.001*	5.7±0.6	<0.001*
CP with uncontrolled diabetes (Group 3)	9.0±2.1		7.6±1.4	

**P* value is significant <0.001. *P*<0.05 is considered statistically significant. SD: Standard deviation; CP: Chronic periodontitis

with the baseline values in all the three groups. When intergroup comparison of FBS and HbA1C values after therapy was done, a statistically significant reduction was observed between Groups 1 and 3 and between Groups 2 and 3. Periodontal treatment that reduces periodontal inflammation may help to restore insulin sensitivity, thereby improving glycemic control.[18] Studies done by Janket *et al*. and Wang *et al*. suggested that an improvement in a subject's periodontal health can positively affect his/her glycemic metabolic control.[19,20]

Deficiency of Zn, Mg, and Cu increases the susceptibility to infection, impairs the function of neutrophils and macrophages, and reduces the antibody-mediated, cell-mediated, phagocytic and delayed type of hypersensitivity reactions and depletion of antioxidants. Diabetes can exaggerate the host response to the local

Table 4: Intergroup comparison of zinc at baseline and after treatment in the three groups

Groups	Baseline		After treatment	
	Mean±SD	*P*	Mean±SD	*P*
CP (Group 1)	67.7±14.3	NS	77±12.8	0.04 (significance)
CP with controlled diabetes (Group 2)	76.6±19.5		85±14.5	
CP (Group 1)	67.7±14.3	NS	77±12.8	0.04 (significance)
CP with uncontrolled diabetes (Group 3)	74.2±19.5		85±10.6	
CP with controlled diabetes (Group 2)	76.6±19.5	NS	85±14.5	NS
CP with uncontrolled diabetes (Group 3)	74.2±19.5		85±10.6	

SD: Standard deviation; NS: Nonsignificance; CP: Chronic periodontitis

Table 5: Intergroup comparison of magnesium at baseline and after treatment in the three groups

Groups	Baseline		After Treatment	
	Mean±SD	*P*	Mean±SD	*P*
CP (Group 1)	1.6±0.2	NS	1.7±0.1	NS
CP with controlled diabetes (Group 2)	1.7±0.2		1.8±0.1	
CP (Group 1)	1.6±0.2	NS	1.7±0.1	NS
CP with uncontrolled diabetes (Group 3)	1.5±0.2		1.8±0.2	
CP with controlled diabetes (Group 2)	1.7±0.2	NS	1.8±0.1	NS
CP with uncontrolled diabetes (Group 3)	1.5±0.2		1.8±0.2	

SD: Standard deviation; NS: Nonsignificance; CP: Chronic periodontitis

Table 6: Intergroup comparison of copper at baseline and after treatment in the three groups

Groups	Baseline		After treatment	
	Mean±SD	*P*	Mean±SD	*P*
CP (Group 1)	99.3±20.9	0.041 (significance)	92.3±16.7	NS
CP with controlled diabetes (Group 2)	115.8±31.8		102.3±23	
CP (Group 1)	99.3±20.9	0.042 (significance)	92.3±16.7	NS
CP with uncontrolled diabetes (Group 3)	99.5±32.2		93±21.4	
CP with controlled diabetes (Group 2)	115.8±31.8	NS	102.3±23	NS
CP with uncontrolled diabetes (Group 3)	99.5±32.2		93±21.4	

SD: Standard deviation; NS: Nonsignificance; CP: Chronic periodontitis

factors, resulting in unusually destructive periodontal breakdown. Many studies have revealed that the complication of diabetes and periodontal disease has revealed that a hyperactive innate immune response may be the antecedent of both diseases. In type 2 diabetic patients with periodontitis, there is alteration of serum Zn, Mg, and Cu status. Several studies in diabetic patients has suggested that micronutrient deficiency leads to glucose intolerance.[21] Serum content of Zn and Mg was decreased and Cu levels were increased in diabetics than nondiabetics.[22] These alterations may contribute to some of the complications of diabetes. Improvement in Zn and Mg status in patients with type 2 diabetes may counteract the deleterious effects of oxidative stress, helping to prevent complications associated with diabetes.[23]

Similarly, in our study, there is a decrease in the Zn and Mg levels and increase in Cu content before treatment in all the subjects in the three groups. Studies have suggested that DM is associated with increased urinary excretion of Zn which can lead to its lower level in serum.[24] However, after nonsurgical periodontal management, there is an increase in the Zn and Mg content and decrease in the Cu content. Zn plays a key pathophysiological role in major neurological disorders as well as diabetes and also has a highly complex role in the physiology of islets of Langerhans. Stimulation of lipogenesis in the adipocyte explains the synergism between insulin and Zn.

The results of this study indicate that low serum Zn level may probably be a reason for poor insulin resistance in type 2 diabetic patients with periodontitis. Further, the low level of Mg could also aggravate insulin resistance and predisposes diabetic patients to pathogenesis of atherosclerosis and it is observed in experimental studies done by Barbagallo *et al.*[25] Zn and Mg are reported to possess antioxidant property.[26] Zn along with Mg helps trigger insulin action and entry of glucose within the cells.

In the present study, there was increased serum Cu level in all the three groups at the baseline similar to study done by Freeland *et al.*,[27] where they have shown that there is an increased serum Cu level in individuals with periodontitis. The increase in serum Cu concentration cannot be influenced by dietary intake. Elevation of serum Cu can occur during a variety of stress conditions including inflammation.[28] It is suggested that a leukocyte endogenous mediator acts as a feedback signal to mobilize hepatic Cu, primarily as ceruloplasmin.[29] Since periodontal disease involves inflammation of gingival tissue, this mechanism could account for elevated serum Cu found in periodontitis patients. This was similar in our studies showing high level of Cu content at baseline in all the three groups and statistically significant reduction observed after nonsurgical periodontal treatment within the groups. Studies done by Turnlund reported that increased serum Cu can modulate immune function and antioxidant status. Several studies have shown that increased Cu levels can have an impact on the immune system, including neutrophil numbers, lymphocyte proliferation, and antigen-specific antibody production.[30] Elevated serum Cu level can alter collagen metabolism and hence can promote periodontitis. In our study, there is a reduction of serum Cu level and increase in Zn and Mg level, showing that there is an improvement in the periodontal status and also in the diabetic level after nonsurgical periodontal therapy.

Conclusion

The results obtained in our study collectively provide possible link of Cu, Zn, and Mg to periodontal disease and DM. Imbalance in these micronutrients can predispose an individual to the risk of developing periodontitis due to decreased regenerative capacity and impaired immune function along with the development of excessive oxidative stress. Conversely, the improvement in periodontal status by nonsurgical treatment can also maintain the homeostasis of Zn, Mg, and Cu level and also improvement in the glycemic status, thereby preventing its various complications.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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