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Estimation of serum zinc level in type 2 diabetic individuals with and without periodontitis: A comparative study

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Abstract

Diabetes mellitus is a systemic disease which has been increasing rapidly worldwide, creating huge health pressure on the society in both developed and developing countries. Periodontitis was considered as one of the main, oral health problems encountered in patients with diabetes mellitus. American Dental Association has recognized periodontitis as its sixth complication. Micronutrients play a vital role in redressing the balance between microbial challenge & host response. Specific nutrients such as Zinc can modulate immune and inflammatory responses.

Aim: The aim of the study was to access & compare the serum Zinc level in Type2 diabetes mellitus subjects with and without chronic periodontitis to that of periodontally healthy subjects.

Materials and methods: 5ml of blood was collected from each study participant through venipuncture and collected in a Z serum sep clot activator tube. The blood was then centrifuged and the serum Zinc level was estimated using flame atomic absorption spectroscopy.

Statistical Analysis: It was carried out using Scheffe Multiple Comparisons.

Results: The mean serum zinc levels were significantly higher in healthy controls as compared to diabetic individuals with and Periodontitis.

Conclusion: Results of present study demonstrated lower levels of zinc in type 2 diabetes mellitus patients with & without periodontitis compared to healthy individuals.

Keywords: Type 2 Diabetes Mellitus, Periodontitis, Serum Zinc

Introduction

Diabetes mellitus is a systemic disease which has been increasing rapidly worldwide, creating huge health pressure on the society in both developed and developing countries. Diabetes mellitus is mainly classified into type 1 (Insulin Dependent Diabetes Mellitus) and type 2 (Non insulin Dependent Diabetes Mellitus). Type 1 diabetes mellitus, is because of cell-mediated autoimmune destruction of insulin producing beta cells of the islets of Langerhans in the pancreas, which inturn results in insulin deficiency. Type 2 diabetes mellitus, resulted by peripheral resistance to insulin action, impaired insulin secretion, and increased glucose production in the liver. Type 2 diabetes accounts for 90% to 95% of all diabetes cases. A bidirectional relationship is present between diabetes and periodontal disease Diabetes influences the progression of periodontitis, whereas periodontitis considered as the sixth major complication of diabetes. (1) Periodontitis is an immuno-inflammatory disease of multifactorial etiology characterized by progressive attachment loss, bone loss and eventually tooth loss. Variety of risk factors for periodontitis have been identified which includes diabetes, smoking, host response, diet etc. (1) Although microbes are the initiation factor for periodontitis, most of the periodontal tissue destruction is caused by an inappropriate host response to those microorganisms. Vitality of the periodontal tissues, in both health and disease, depends strongly upon an adequate source of essential nutrients being available to the host. (2)

Micronutrients play a vital role in diabetes and periodontal disease. Periodontitis may occur as a result of variation in concentration of various plasma nutrients. Micronutrients including vitamins and minerals are required in an adequate quantity for human health and for functioning of many enzymes. Zinc which is a micronutrient is important for several different intracellular processes and is part of more than 3000 Zn-dependent gene transcription factors and other protein domains. (3) A direct relation exists between type 2 diabetes and Zn-dependent gene. The zinc transporter gene (SLC30A8, coding for ZnT8) is important in insulin storage and release. (4) A polymorphism in this specific gene can lead to alterations in insulin storage and release leading to type 2 diabetes. The present study evaluates the serum Zinc level in type 2 diabetic individuals with and without periodontitis.

Materials and Methods

The study consisted of total of 48 subjects with the age group of 25-60 years. The subjects were divided into three groups consisting of 16 participants in each group as follows: periodontally healthy subjects (Group A), type 2 diabetes without chronic periodontitis subjects (Group B), type 2 diabetes with chronic periodontitis subjects (Group C). The subjects were randomly selected from outpatient clinic of the Department of Periodontics, PMS college of dental science and research, Trivandrum. The study protocol was approved by the Institutional Ethical Committee (PMS/IEC/2018-19/18). A written consent was taken from each subject and all participants completed the study. Biochemical evaluation done at a Laboratory in Trivandrum, Kerala

Criteria for subject selection

Inclusion criteria

- Periodontitis and periodontal health are diagnosed according, to 1999 World Workshop on the Classification of Periodontal Diseases and Conditions.
- Type 2 diabetes mellitus, diagnosed according to 2017 Classification of American Diabetic Association.
- Group A - Consist of periodontally healthy subjects with probing pocket depth ≤3mm, no attachment loss, bleeding on probing scores <20%, no past history of glucose intolerance, absence of family history of

diabetes in the close relatives, fasting plasma glucose (FPG) less than 100 mg/dL.

- Group B - Consist of Type 2 diabetes with chronic periodontitis subjects with probing pocket depth ≥5mm,in 30%sites, clinical attachment level(CAL) ≥3mm, bleeding on probing ≥50%, type 2 diabetes mellitus asessment using HbA1c ≥7.5%, RBS ≥200mg/dl
- Group C - Consist of Type 2 diabetes without chronic periodontitis subjects with probing pocket depth ≤3mm, no attachment loss, bleeding on probing scores <20%, type 2 diabetes mellitus asessment using HbA1c ≥7.5%, RBS ≥200mg/dl
- Presence of minimum of 20 teeth
- Subjects who have not received periodontal therapy within the last 6 months.
- Those willing to participate in the study

Exclusion criteria

- Diagnosis of any current major illness other than type 2 diabetes mellitus
- Pregnant and lactating females
- Current smokers
- Immunocompromised patient

A detailed medical and dental history along with other readings were taken before the collection of blood sample. The samples were coded before being sent for laboratory investigations

Evaluation of Zinc: 5ml of blood sample was collected from the subject through venipuncture [Fig:1] using a disposable syringe and transferred into a Z serum separator clot activator tube [Fig:2]. The sample was centrifuged at 3000 rpm for 15 min [Fig:3] and the supernatant serum was collected. The serum Zinc estimation was done using flame atomic absorption spectroscopy. [Fig:4]

Fig.1:Collection of venous blood

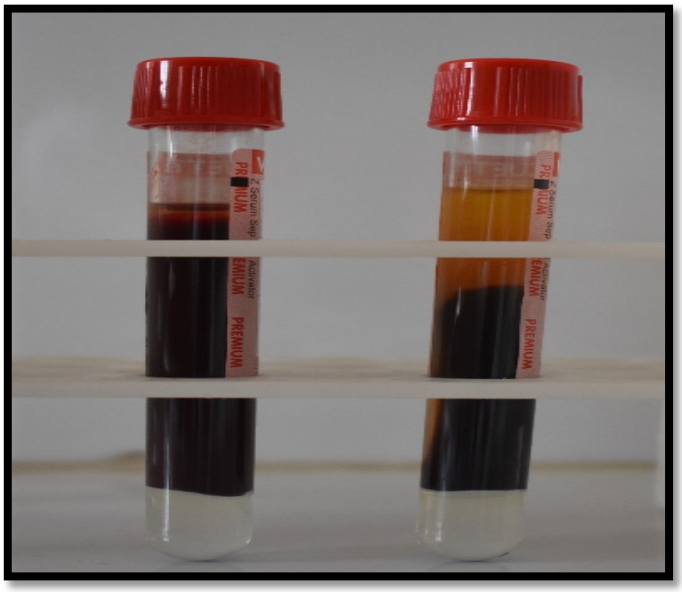

Fig.2: Sample collected into Z serum seperator clot activator tube

Fig.3:Centrifuge

Fig.4: Atomic spectrometer;Courtesy:Von Talos, Germany

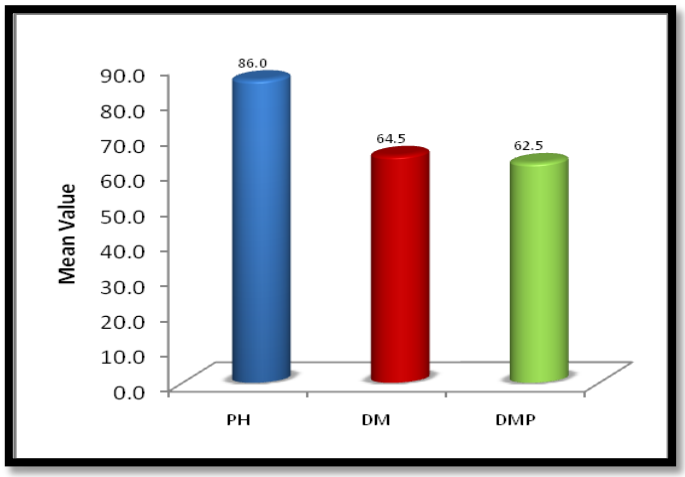

Fig.5: PH – Periodontally Healthy, DM – Diabetic individuals without periodontitis, DMP - Diabetic individuals with periodontitis

Statistical analysis

The results obtained from the study were tabulated and subjected to statistical analysis. The data were expressed as mean and standard deviation. Inter group comparison was done using Scheffe Multiple Comparisons.

Results

Comparison of mean values of results obtained from the three groups were obtained [Fig: 5] and the inter group comparison was done using the mean and standard deviation values from the three groups. [Table 1] The serum levels of zinc in Group A, B and C showed mean and standard deviation of 86.0 ± 7.6, 64.5 ± 2.3 and 62.5 ± 1.8 respectively. The mean value comparing all the three groups was statistically significant ($p<0.01$). The intergroup comparison results showed high statistical significance between Group A and Group B, between Group A and Group C and less statistical significance between Group B and Group C.

Discussion

Periodontal disease, the second most common oral disease which is initiated by specific pathogenic microorganisms in the oral biofilm. If not treated, it results in apical migration of the junctional epithelium, and destruction of the periodontal attachment apparatus including the alveolar bone. (5)

Micronutrients like Zinc play a major role in maintaining adequate immune response. Along with the specific and nonspecific immune responses occurring in malnourished individuals exposed to infections, certain series of metabolic adjustments also occurs. Inflammatory stimuli from dental plaque promotes the release of reactive free radicals and exhibit metabolic changes modulated by soluble mediators known as cytokines. (1) Zinc is an essential trace element responsible for the function of many cellular enzymes and proteins. An excess of free Zn ions could lead to oxidative damage in the mitochondria, which explains for early disruption of mitochondrial potential and for the decrease in anti-apoptotic membrane-bound protein, located predominantly in the mitochondria and endoplasmic reticulum. This promotes cell survival by inhibiting the adapters needed for the activation of

proteases that dismantle the cell.(6) Zinc also play an important role in type 2 diabetes mellitus. There is a direct relationship between type 2 diabetes mellitus and zinc dependent gene. For type 2 diabetes mellitus, genetic susceptibility is a critical factor and these genome wide association studies were able to identify a genetic susceptibility locus for type 2 diabetes mellitus which include single nucleotide polymorphism in a beta cell specific zinc transporter gene. (4) SL30A8, coding for zinc transporter gene is important in insulin storage and release. A study conducted in rat model evaluated the effects of ZnT8 gene and beta cell function and concluded that ZnT8 gene is required for normal insulin crystallization and insulin release in vivo.(7)

Diabetes mellitus is a systemic disease associated with increased formation of free radicals and decrease in antioxidant potential, which results in disturbed balance between radical formation and antioxidant protection in normal cell. (8) Hyperglycemia can stimulate ROS formation from a variety of sources like oxidative phosphorylation, glucose oxidation, NAD(P)H oxidase, lipooxygenase, cytochrome P450 monooxygenases, and nitric oxide synthase (NOS). (9) Experimental evidence in diabetes mellitus patients has suggested that micronutrient deficiency leads to glucose intolerance. Many studies have assessed the levels of micronutrients in diabetic patients and also in diabetic patients with periodontitis. The present study is done to estimate the serum Zinc level in Type 2 diabetic individuals with and without Periodontitis.

In the present study, the mean value of serum Zinc level in type 2 diabetic individuals seemed to be decreased compared to healthy individuals. Comparing the three groups, lower levels of zinc was detected in type 2 diabetes mellitus patients with & without periodontitis (62.5 and 64.5) compared to healthy individuals (86.0) and the difference was statistically significant. This is in comparison to previous study by Thomas et al (2010) who found decreased serum zinc levels in type 2 diabetes mellitus patients with periodontitis when compared to healthy individuals.(2) Study by Pushparani et al (2019) concluded that patients with DM and periodontitis had altered metabolism of Zinc.(6)

Table 1: Comparison of Serum Zinc Level among three groups

Group	Mean	SD	N	F	p	Scheffe Multiple Comparisons		
						Pair	F`	p
PH (A)	86.0	7.6	16	123.17	p<0.01	A &B	83.7	p<0.01
DM (B)	64.5	2.3	16			A & C	100.3	p<0.01
DMP (C)	62.5	1.8	16			B & C	0.7	0.480

Conclusion

The results of the study underline the importance of investigation of Zn metabolism in diabetes pathogenesis. Assessment of its level in body may indicate a risk of disease as well as demonstrate the potential of Zinc containing food additives as a preventive measure. Supplementation of Zinc might have utility in the treatment of type 2 diabetes mellitus with periodontitis, thus may help in the control of blood glucose preventing or delaying serious clinical events in these patients.

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