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Effects of Non-Surgical Periodontal Therapy on Serum And Gcf Calcium Levels in Periodontitis Patients With and Without Type 2 Diabetes Mellitus

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ABSTRACT

Background: Evidence has shown a bi-directional link between diabetes mellitus and periodontitis. Calcium and Insulin are important for the formation of bone collagen and depletion of bone calcium leads to alveolar bone loss, and tooth loss in periodontitis patients with diabetes. Non-surgical periodontal therapy (NSPT) for patients with periodontitis and diabetes mellitus improves the periodontal condition and regulates glycemic level.

Aims and Objectives: To assess the impact of calcium on periodontal disease and diabetes mellitus and to analyze impact of NSPT on Serum and GCF calcium levels in periodontitis with and without type 2 diabetes mellitus (T2DM).

Materials and Methods: A total of 36 Subjects were divided into three groups: Group I: Systemically healthy subjects(n=12), Group II: Systemically healthy subjects with periodontitis (n=12), Group III: Subjects with periodontitis and T2DM (n=12). Indices like plaque index (PI), gingival index (GI), gingival bleeding index (GBI), periodontal probing depth (PPD), and Clinical attachment level (CAL) were assessed at baseline and three months after NSPT in all subjects. Random blood sugar (RBS) level was taken at baseline and 3 months after NSPT in Group III subjects. Serum and GCF Calcium levels were assessed at baseline and 3 months after NSPT in all subjects.

Results: After NSPT, there was a statistically significant difference was observed in comparing clinical parameters, Serum, and GCF calcium levels in Group II and Group III subjects at baseline and after 3 months (P<0.001). A positive correlation was observed between serum and GCF calcium levels and RBS in Group III subjects and A negative correlation was observed between GCF and Serum calcium levels and GI in Group I subjects (p>0.001).

Conclusion: Based on the observed relationship between calcium and the clinical parameters, Calcium utility as a biomarker for diagnosis and prognosis in periodontal disease seems promising. However, further studies with a larger sample size on the role of calcium in health and various states of diseases are required to substantiate the result of the study.

Keywords: Chronic Periodontitis, Diabetes Mellitus, GCF Calcium, Periodontal Therapy, Serum Calcium

I.   INTRODUCTION

Periodontitis is a disease of inflammatory origin characterized by gingival enlargement, bleeding on probing, loss of alveolar bone, and anchorage between the tooth and periodontium or the supporting structures in its anatomical and functional position. It is often described as a state of hard tissue imbalance between the anabolic and catabolic processes resulting in the loss of alveolar bone and supporting hard structures.[1]

Diabetes, a systemic disorder with significant periodontal disease manifestation, is also viewed as an inflammatory condition and its development is preceded by a low-grade systemic inflammation.[2] Periodontitis, the sixth most common and frequently manifested oral complication in diabetic patients has been directly correlated with the levels of glycemic control.[3]

Calcium (Ca) is the fifth most abundant element next to oxygen, carbon, hydrogen, and nitrogen.[4] It is an intracellular "second messengers" affecting enzyme activity and secretion of many hormones including insulin, aldosterone, vasopressin, and rennin. It is crucial for the maintenance of cell metabolism, nerve transmission, and muscle contraction.[5] In addition, it plays many important roles in the synthesis, release, and receptor responsiveness to neurotransmitters.[6] It is an important element present in the bones and teeth, providing a structural function.[4]

In a normal young healthy adult, there is about 1200g of calcium in the body. 98% of calcium is present in bones, and teeth and the rest are present in the plasma. The normal serum calcium level ranges between 8.5-10.5 mg/dl for a healthy individual [7]. It is stated that chronically low intakes of calcium may lead to a negative calcium balance, thus causing a secondary increase in calcium removal from the bone, including the alveolar bone. Such bone loss may contribute to the weakening of the tooth attachment apparatus [8].

Researchers have been exploring the role played by calcium in the etiology and/or progression of periodontal diseases for well over four decades. Several animal studies point to an association between dietary calcium and periodontitis. For instance, Oliver W (1969) [9], Abe et al. (1989) [10] and Amano H (1989) [11] have observed a relationship between calcium-deficient diet and the progression of periodontitis in rats whereas Osborn et al. (1977) [12], Vogel et al. (1979) [13], Nishida et al. (2000) [14] and Krall et al. (2001) [15] have obtained similar findings in human studies. Accordingly, it has been hypothesized that low dietary intake of calcium may contribute to the progression of periodontitis [9-15].

The removal of subgingival plaque and calculus constitutes the cornerstone of periodontal therapy. Mechanical therapy consisting of scaling and root planing (SRP) is the gold standard for periodontal therapy. The efficacy of SRP as a part of non-surgical periodontal disease management is established through several longitudinal studies [16]. The present study was carried out to assess the impact of calcium on the pathogenesis of periodontal disease and diabetes mellitus and to evaluate the role of non-surgical periodontal therapy (NSPT) on GCF and Serum calcium levels in periodontitis patients with and without type 2 diabetes mellitus.

II.   Materials And Methods

This randomized controlled trial was conducted at the Department of Periodontics and Implantology, College of Dental Science Davangere. A total of 36 subjects of both genders were randomly selected from the outpatient clinic of the Department of periodontics and recruited for the study based on the eligibility criteria established for each group. Subjects were divided into three groups with 12 subjects in each group as follows:

 Group I: Systemically and periodontally healthy subjects

Group II: Systemically healthy subjects with generalized periodontitis

Group III: Subjects with generalized periodontitis and type 2 diabetes mellitus

All patients were scheduled for a sampling of GCF, Serum, and Clinical parameters assessment at baseline and after 3 months. Subjects with and without periodontitis were identified and selected according to the 2017 AAP World Workshop on Classification of Periodontal and Peri-Implant Diseases and Conditions. The subjects with and without type 2 diabetes mellitus were diagnosed based on the criteria of the American Diabetes Association. Periodontitis subjects of Group II were selected belonging to Stage II and Grade B of the disease. Periodontitis subjects of Group III were selected belonging to Stage II and Grade C of the disease.

Systemically compromised patients other than those with diabetes mellitus, pregnant or lactating women, and calcium deficiency disorders including bone diseases. antibiotic and/or anti-inflammatory drug regimen six months before the study, history of any therapy six months before the preliminary visit, smokers or any form of tobacco chewers, alcoholics, patients with poorly controlled diabetes (HbA1c values < 8%), or any history of diabetic complications, and patients under insulin therapy.

The following clinical parameters were evaluated for the subjects: PI by Silness J and Loe H (1964), GBI by Ainamo and Bay (1975), GI by Loe H and Silness J (1963), PPD (Carranza FA et al. 2013), and clinical attachment level (CAL) (Carranza FA et al. 2013).

Collection of Blood

Blood samples (5 ml) will be collected by venipuncture of the cubital vein in the antecubital fossa by using a 5 ml disposable syringe and a 23-gauge needle. The blood will be collected in sterile vacuum tubes with no added anticoagulant and kept at room temperature for 2 hrs, where it will be allowed to clot, as this is designated for serum separation.

Collection of GCF

Each sample site will be carefully isolated using cotton rolls to avoid saliva contamination. A paper strip will be placed in the pocket until mild resistance is felt and then left in place for 30 seconds. In the case of visible contamination with blood, the strip will be discarded. On the day of the assay,180 ml phosphate buffered solution will be added to the tubes containing the sample strips. The tubes will be gently shaken at 4^0 C for 20 minutes and centrifuged at 13,000 rpm (r=5.5 cm) for 10 minutes. The GCF eluates and plasma will be used for the measurement.

Calcium Estimation

The samples collected were pretreated with double distilled deionized water. The estimation of Calcium in GCF and Serum was done by using Dual-Viewing (DV) ICP-OES (Perkin Elmer Optima 5300). Qualitative information on the element present in the sample was involved in identifying the presence of emission at wavelength, characteristic of the selected element: calcium-wavelength 317.933 nm. Quantitative measurement of the element in the sample can be obtained using calibration curves [17].

ICP-OES is used for multielement determination over a wide range of concentrations. The precision, and accuracy of ICP-OES are considered sufficient for most trace elemental analyses. The ICP-OES technique experiences the fewest interferences of the commonly used analytical atomic spectrometry techniques [17]. In ICP-OES, liquid samples were introduced into a radiofrequency (RF)-induced argon plasma and instantly dried, vaporized, and energized through collisional excitation at a higher temperature. The resulting atomic emission was observed in either a radial or axial configuration and imaged onto the entrance slit of a wavelength selection device [18].

Non-surgical periodontal treatment

Non-surgical periodontal therapy by SRP was completed. Clinical parameters, GCF, and serum were collected in Group I, Group II, and Group III subjects at baseline and after 3 months to assess the changes.

STATISTICAL ANALYSIS

The data obtained was suitably tabulated using SPSS version 25.0. All the values were expressed in the form of mean, and standard deviation. The parameters were compared between Group I, Group II, and Group III. The results were obtained using the student's independent sample 't' test.

III. RESULTS

Group I exhibited improvement in clinical parameters (PI, GI, and PPD) 3 months after non-surgical periodontal therapy. Statistical highly significant differences were observed in P value<0.01, respectively and the insignificant difference was observed in clinical parameters (GBI and CAL) after 3 months after non-surgical periodontal therapy p>0.05, respectively.3 months after non-surgical periodontal therapy group II and group III exhibited improvement in clinical periodontal parameters (PI, GBI, PPD, and CAL) Statistical highly significant differences were observed with P value<0.01, respectively and a statistically significant difference was observed in GI in group II. P value<0.05, respectively.

Table 1

Intra-group comparison of the index scores in each of the three groups using paired t-test.

Index	Time	Group I		Group II		Group III	
		Mean	SD	Mean	SD	Mean	SD
Plaque index	Baseline	0.4	0.29	2.2	0.45	2.6	0.3
	3 months	0.1	0.09	1.2	0.41	1.6	0.3
	p-value	0.00<0.01		0.00<0.01		0.00<0.01	
Gingival bleeding index	Baseline	0.4	0.51	2.3	0.49	2.2	0.4
	3 months	0.5	0.52	1.1	0.38	1.6	0.3
	p-value	0.58>0.05		0.00<0.01		0.00<0.01	
Gingival index	Baseline	33.3	12.31	77.0	22.50	87.5	13.0
	3 months	12.5	13.06	52.0	24.90	37.5	13.0
	p-value	0.00<0.01		0.02<0.05		0.00<0.01	
Probing pocket depth	Baseline	2.9	0.51	6.3	0.65	8.0	1.1
	3 months	2.2	0.58	3.8	1.02	5.1	0.5
	p-value	0.00<0.01		0.00<0.01		0.00<0.01	
Clinical attachment loss	Baseline	0.1667	0.39	3.8	0.32	5.5	1.1
	3 months	0.1667	0.39	1.6	0.18	3.4	0.6
	p-value	0.00>0.05		0.00<0.01		0.00<0.01	

Table 2

The Serum and GCF Calcium levels were analyzed using the student's independent sample 't' test. Serum calcium levels were not significant in Group I subjects after 3months of non-surgical periodontal therapy. (P value 0.077>0.05) GCF Calcium level did not show any variation in Group I subjects after 3 months of periodontal therapy. (P value 0.822>0.05) The difference is not significant. The Serum and GCF Calcium levels were highly significant in Group II and Group III Subjects after non-surgical periodontal therapy, p<0.01, respectively. RBS levels were highly significant after 3months of non-surgical periodontal therapy in Group III subjects, p<0.01, respectively.

Intra-group comparison of the Serum and GCF calcium levels in each of the three groups using paired t-test.

Groups		Group I		Group II		Group III	
		Mean	SD	Mean	SD	Mean	SD

Serum Calcium	Baseline	9.0	0.79	6.2	0.39	4.8	0.5
	3 months	9.3	0.81	7.8	0.95	7.7	1.3
	p-value	0.77>0.05		0.00>0.01		0.00>0.01	
GCF Calcium	Baseline	9.0	0.84	6.0	0.35	4.7	0.6
	3 months	9.0	0.79	7.5	0.93	7.0	1.1
	p-value	0.822>0.05		0.00>0.01		0.00>0.01	
RBS	Baseline					327.5	47.0
	3 months					154.4	31.3
	p-value					0.00>0.01	

Figure 1

Comparison of mean Serum and Gcf calcium levels in Group I, Group II, and Group III
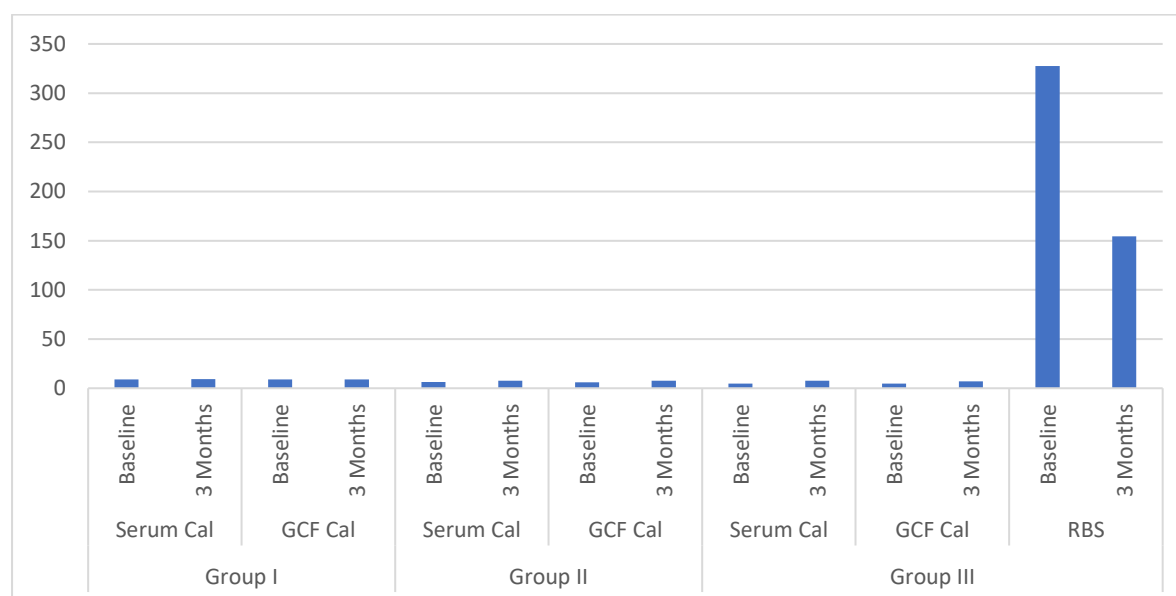
IV.   DISCUSSION

Inflammation causes progressive destruction of the periodontal ligament and alveolar bone, resulting in pocket formation, gingival recession, or both [19]. Diabetes has been found to be an important host risk factor in periodontal diseases in large epidemiological studies [20].

The prompt diagnosis of periodontal disease is extremely challenging because the bone loss and soft tissue loss are progressive and it is also difficult as the initial phase of the disease is painless and patients seldom seek prophylactic care [21]. Useful diagnostic indicators should indicate the presence or absence of periodontal disease, the response to treatment, and the need for supplementary treatment [22]. The discovery of predictive biomarkers is considerably difficult due to the episodic nature of the disease [23]. Thus, an idealistic and objective diagnostic method is still being sought to assess the active disease status of periodontitis.

Though GCF provides site-specific information, its complexity in collecting a sample without contamination and time collection hinders its use during routine chair-side examinations [23]. Likewise, blood cannot provide site-specific information; however, it is simple, rapid, and can be carried out as part of a routine general diagnostic check-up [23]. The GCF is constituted by various indicators and markers of connective tissue and bone

destruction, providing a window for non-invasive analysis of periodontitis and ascertaining the severity of gum disease [24]. The biochemical analysis of blood serum also provides a non-invasive means of estimating the host's systemic response in periodontal disease.

Sewon et al with their series of studies have shown that oral mineralization potential of saliva plays an important role in periodontal destruction[25]. They have shown that the subjects who develop periodontitis have higher salivary calcium level in comparison with subjects free from periodontitis and they have suggested that an elevated levels of salivary calcium is a characteristic feature of periodontitis affected subjects.

Erwin D. Mandell has shown earlier that the salivary calcium level plays an important role in calculus formation. He suggested that the mean calcium concentration of saliva was significantly higher in heavy calculus formers in comparison to light calculus formers [26].

The results of this investigation indicated that non-periodontitis patients had lower salivary calcium levels when compared to the group of patients with periodontitis, suggesting that increased calcium level is a characteristic feature of periodontal disease and these results are in accordance with the results of Sewon et all [25,26] who studied the relationship of salivary calcium level in periodontitis.

Sejal A. Mehta et al, (2019) showed that the mean serum calcium level was observed significantly higher in patients with periodontitis compared to patients with healthy periodontium. A decreased intake of calcium has adverse effects on the oral cavity leading to periodontitis. Hence, Serum calcium may be considered a risk factor for periodontal disease [27].

The present study showed that Serum and GCF calcium levels were significantly higher in healthy subjects compared to periodontitis and diabetes patients at baseline.3 months after non-surgical periodontal therapy Serum and GCF calcium levels were increased in periodontitis and diabetes patients. Hence, Serum and GCF calcium may be considered a risk factor for periodontal disease.

Non-surgical periodontal therapy not only reduces clinically evident inflammation but also has been associated with decreasing pro-inflammatory cytokines and RBS levels, indicating that periodontal diseases have systemic effects extending beyond the local periodontal environment. This study provides a definitive substantial association of Serum and GCF Calcium in patients with periodontitis and type 2 diabetes mellitus.

The observations of the present study could be significant in understanding their role in the changing dynamics of periodontal disease progression, thereby enhancing its capacity as a diagnostic and prognostic marker of disease activity. Increase in the levels of calcium in serum and GCF after non-surgical periodontal therapy could prove the effect of SRP in reducing the inflammatory component during periodontitis. It can be observed that calcium can serve as one of the potential markers for periodontitis with diabetes mellitus.

V.   CONCLUSION

Within the limitations of our study, it can be said that the Serum and GCF Calcium levels may aid in predicting periodontitis. The study suggests that there is a highly significant increase in Serum and GCF Calcium levels in periodontitis and T2DM patients after 3 months of non-surgical periodontal therapy. Alteration in Serum and GCF Calcium levels could be considered as a useful factor to assess the periodontal host disease progression. Further studies with larger sample sizes are required to confirm the findings of this study. Calcium analysis of Serum and GCF is essential for the diagnosis and assessment of periodontal disease. Non-surgical periodontal therapy (NSPT) for patients with periodontitis and diabetes mellitus improves the periodontal condition and regulates glycemic level.

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