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Effect Of Non-Surgical Periodontal Therapy on Serum and GCF Calcium Levels in Chronic Periodontitis Patients: A Clinico-Biochemical Study.

Dr Mallanagouda. B. Patil,[1]Dr Girija. Venkatesh. Arishinagodi,[2] Dr Soujanya[3],Dr Shobha Prakash[4]

[1]*Department of Periodontics, College of Dental Sciences Davangere, Rajiv GandhiUniversity of Health Sciences, India. (Professor)*

[2,3]*Department of Periodontics, College of Dental Sciences Davangere, Rajiv GandhiUniversity of Health SciencesIndia. (Postgraduate student)*

[4]*Department of Periodontics, College of Dental Sciences Davangere, Rajiv Gandhi University of Health Sciences India. (professor and Head of Department)*

ABSTRACT

Introduction: The elemental analysis of human blood serum and GCF is noteworthy in routine clinical practice as well as in medical research. Gingival Crevicular Fluid (GCF) and blood serum have been referred to as a promising medium for the detection of markers for periodontal disease activity. Analysis of GCF, blood serum shows minute changes in biomarker levels well before the onset of clinical signs and symptoms; which helps to even predict a person's predisposition towards periodontal disease occurrence.

Aim: To evaluate the effect of non-surgical periodontal therapy on Serum and GCF Calcium levels in chronic periodontitis patients.

Materials and Methods: A total of 24 subjects with age group of 25-60 years male and female subjects, divided into two groups, 12 each.Group I-Control-Healthy, Group II-Experimental-Chronic Periodontitis. All subjects underwent clinical examination for Plaque Index, Gingival Index,Gingival Bleeding Index,Probing Pocket Depth, and Clinical Attachment level using UNC 15 Probe.Blood and GCF samples were collected from each patient and Serum and GCF Calcium levels were estimated. All the parameters were evaluated at baseline and 3months after non-surgical periodontal therapy.

Results: Clinical parameters values at baseline were more than that of 3 months after therapy in Group I which was clinically significant within, GI, and PPD and was not significantin relation to GBI and CAL on comparison. In Group II(Chronic periodontitis) comparison at baseline values was more than that of 3 months and on comparison which was highly statistically significant ($p<0.01$)in all parameters with PI, GI,PPD, CAL, and GBI.On Intergroup comparison between two groups at baseline and 3 months, results were statistically significant ($p<0.05$). In relation to values of Serum and GCF, calcium was almost equal at baseline and after 3 months (did not show any variations) significant ($p>0.05$) in Group I, whereas in Group II the Serum and

GCFcalcium mean levels were less at baseline than after 3 months which was highly significant ($p<0.01$) on comparison.

Conclusion: Based on the observed relationship between calcium and the parameters, their 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, 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].

Nutrition plays an important role in overall health, including oral health, eating well and maintaining a healthy diet can help reduce the risk of developing problems in the oral cavity, including periodontal disease. Most people know that dairy products can help to build strong bones and can also help in reducing periodontal diseases. Animal, as well as the human status of calcium intake, bone mineral density, and tooth loss, provide a rationale for hypothesizing that low dietary intake of calcium is a risk factor for periodontal disease [2].

Calcium is the fifth most abundant element next to oxygen, carbon, hydrogen, and nitrogen [3].It is very essential for many activities in the body such as teeth and bone formation, cardiac activity, cell division, cell growth, and blood coagulation. It also acts as a second messenger affecting enzyme activity and secretion of hormones [4]. It plays an important role in building stronger, denser bones early in life keeping bones strong and healthy in life.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 is present in the plasma. The normal serum calcium level ranges between 8.5-10.5 mg/dl for a healthy individual [5].

Chronic periodontitis may be a sequel of chronic gingivitis, due to the accumulation of plaque and calculus. The gingiva detaches from the tooth, the periodontal membrane and alveolar bone are damaged, and pocket formation occurs which eventually leads to loss of the tooth [6]. 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 [7].

Previous scientific literature suggested that calcium deficiency results in bone loss and increased inflammation which are specific characteristic features of periodontitis. To maintain homeostasis, a negative calcium balance mechanism gets stimulated, thus the secretion of parathyroid hormone removes calcium from the bone including the alveolar bone, which in turn, leads to an increase in serum calcium levels. Such bone loss invariably contributes to the weakening of periodontal structures [8].

The removal of subgingival plaque and calculus constitutes the cornerstone of periodontal therapy. Mechanical therapy consisting of scaling and root planning (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 [9]. This study was carried out to assess the impact of calcium levels on the pathogenesis of periodontal disease and to evaluate the role of non-surgical periodontal therapy (NSPT) on Serum and GCFCalcium in periodontitis patients.

II. MATERIALS AND METHODS

This cross-sectional study conducted from April 2023 to February 2024 was performed in accordance with the Declaration of Helsinki, 2008, and was approved by an institutional ethical committee. The total sample size

was 24. Patients aged 25-60 years were selected from the Outpatient Department of Periodontics Davangere. Informed consent was taken from the subjects before the start of the study.

The patients were divided into two groups of 12 each:

Group I- Healthy subjects with no clinical signs of gingival and periodontal inflammation.

Group II -Chronic Periodontitis-Subjects who had clinical signs of gingival inflammation, presence of PD, and clinical attachment loss ≥5mm.

Inclusion criteria: Subjects with and without periodontitis were identified and selected according to the AAP World Workshop on Classification of Periodontal and Peri-Implant Diseases and Conditions. Periodontitis subjects of Group II were selected belonging to stage II, stage III, and stage IV Periodontitis.

Exclusion criteria: Any periodontal therapy in the past 6 months, Any antibiotic and steroids 6 months prior to sampling, Medically compromised patients (history of cardiovascular or renal disease, malignancies, multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, and fungal disease, bleeding disorders, calcium deficiency disorders including bone diseases.)Smokers and Tobacco chewers, patients with calcium supplementation, Pregnant, post-menopausal, and lactating females, and patients with PCOS.

Clinical examination

The PD and clinical attachment loss were measured at all sites by using the Williams Periodontal Probe. The Plaque Index by Silness P and Loe H,1964, Gingival Index by Loe H and Silness P,1963, Gingival Bleeding Index by Ainamo and Bay,1975 assessed for all patients according to the criteria given by the authors. Gingival units of each tooth (buccal, lingual, mesial, and distal) were given a score from 0-3 and the GI for each tooth was calculated. The GI for the patient was determined by adding the GI for each tooth and dividing it by the total number of teeth present in the oral cavity.

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 [10].

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 [10]. 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 [11].

Non-surgical periodontal treatment

Non-surgical periodontal therapy by SRP was completed. Clinical parameters, GCF, and serum were collected in Group I and Group II 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 and Group II. The results were obtained using the student's independent sample 't' test.

III. RESULTS:

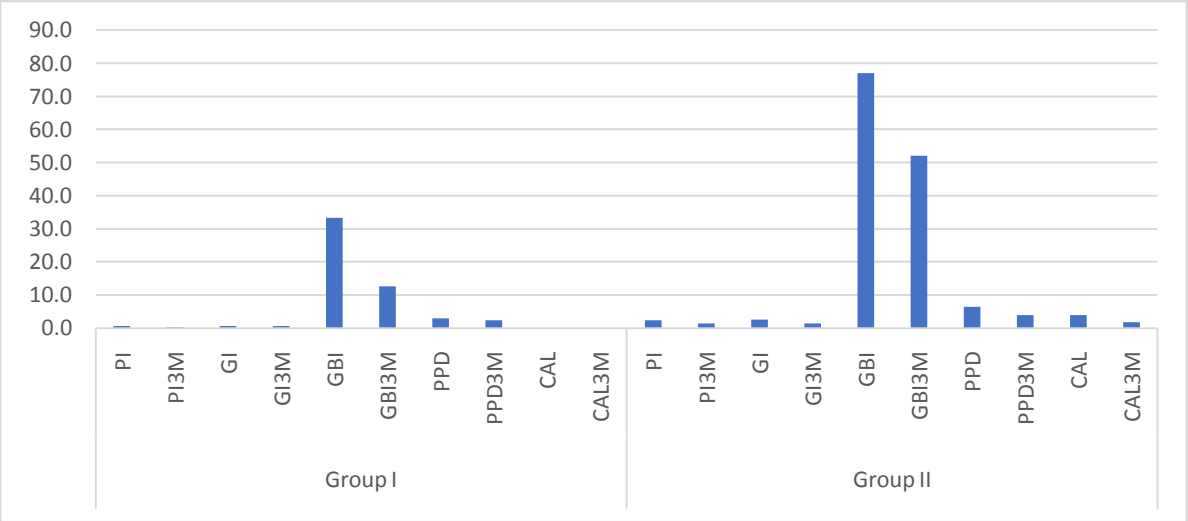
The study subjects were categorized into two groups of 12 patients each: those with healthy periodontium and those with chronic periodontitis**.**Group I exhibited improvement in clinical parameters (PI, GI, and PPD) 3 months after non-surgical periodontal therapy. Statistical highly significant differences 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 exhibited improvement in clinical periodontal parameters (PI, GBI, PPD, and CAL) Statistical highly significant differences were observed P value<0.01, respectively and a statistically significant difference was observed in GI. P value<0.05, respectively.

Table 1Intra-group comparison of the index scores in each of the two groups using paired t-test

Index	Time	Group I		Group II	
		Mean	SD	Mean	SD
Plaque index	Baseline	0.4	0.29	2.2	0.45
	3 months	0.1	0.09	1.2	0.41
	p-value	0.005<0.01		0.00<0.01	
Gingival bleeding index	Baseline	0.4	0.51	2.3	0.49
	3 months	0.5	0.52	1.1	0.38
	p-value	0.58>0.05		0.00<0.01	
Gingival index	Baseline	33.3	12.31	77.0	22.50
	3 months	12.5	13.06	52.0	24.90
	p-value	0.00<0.01		0.02<0.05	
Probing pocket depth	Baseline	2.9	0.51	6.3	0.65
	3 months	2.2	0.58	3.8	1.02
	p-value	0.005<0.01		0.00<0.01	
Clinical attachment loss	Baseline	0.1667	0.39	3.8	0.32
	3 months	0.1667	0.39	1.6	0.18
	p-value	0.00>0.05		0.00<0.01	

FIGURE 1

Comparison of the mean value of the clinical parameter in Group I and Group II



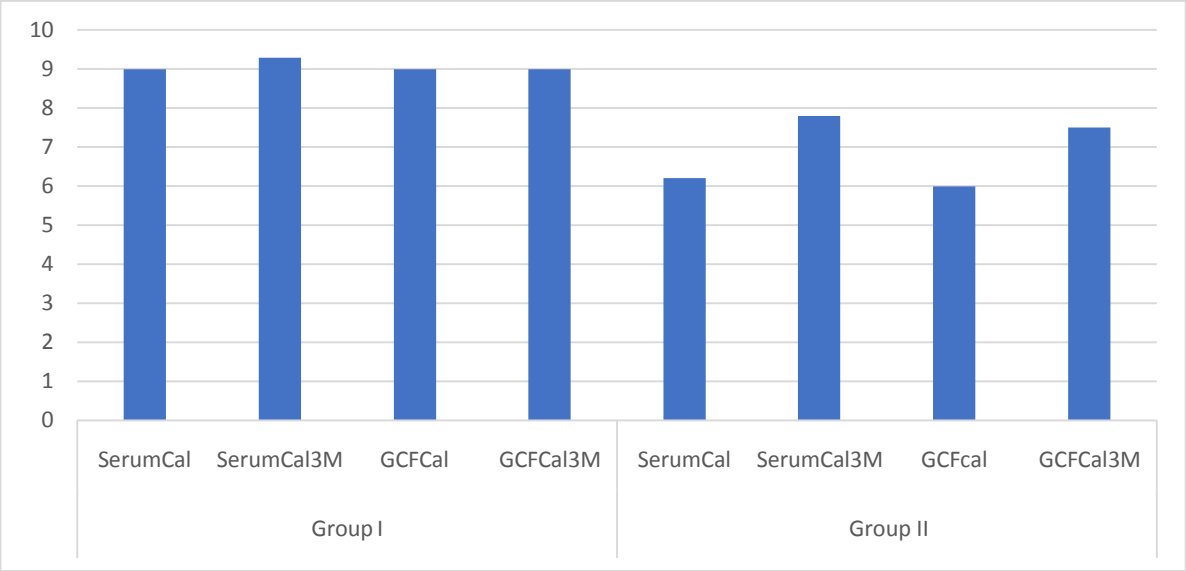
The Serum and GCF Calcium levels were analysed using the student's independent sample 't' test.Serum calcium levels were not significant in Group I subjects after 3 months of non-surgical periodontal therapy.(P value 0.077>0.05)GCF Calcium level did not show any variation in Group I subjects after 3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

Table 2

Intra-group comparison of the serum and gcf calcium levels in each of the two groups using paired t-test

Groups		Group I		Group II	
		Mean	SD	Mean	SD
Serum Calcium	Baseline	9.0	0.79	6.2	0.39
	3 months	9.3	0.81	7.8	0.95
	p-value	0.77>0.05		0.00>0.01	
GCF Calcium	Baseline	9.0	0.84	6.0	0.35
	3 months	9.0	0.79	7.5	0.93
	p-value	0.822>0.05		0.00>0.01	

Figure 2
Comparison of mean serum and gcf calcium levels in Group I and Group II



IV. DISCUSSION

Periodontal disease is a chronic inflammatory disease that affects the supporting structures of the tooth. While it is due to the interaction between a bacterial infection and host response, other factors such as genetics, diet, and lifestyle choices are thought to contribute to the disease progression. Calcium is the most abundant mineral in the body. It is found in food, dietary supplements, and some medications. There are many claims made as to calcium's health benefits. Both vitamin D and calcium are known to promote bone health and periodontal disease affects the alveolar bone.

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 [12]. Useful diagnostic indicators should indicate the presence or absence of periodontal disease, the response to treatment, and the need for supplementary treatment [13]. The discovery of predictive biomarkers is considerably difficult due to the episodic nature of the disease [14]. 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 blood cannot, its complexity in collecting a sample without contamination and time collection hinders its use during routine chair-side examinations [14]. 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 [14]. 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 [15]. The biochemical analysis of blood serum also provides a non-invasive means of estimating the host's systemic response in periodontal disease.

Erwin D Mandel et al, (1967) showed salivary calcium levels play an important role in calculus formation and suggested meancalcium concentration of saliva was significantly higher in heavy calculus formers than in lightcalculus formers.[16]

Sewon et al, (1990) showed that the mineralization potential of saliva plays an important role in periodontal destruction. The subjects who develop periodontitis have higher salivary calcium levels in comparison with subjects free from periodontitis.[17]

Meijer and Klassen et al, (1972) reported that patients with active periodontal involvement had increased production of ionized Ca in stimulated saliva compared with normal subjects.[18]

Many researchers have explored the role played by calcium in the etiology and/or progression of periodontal diseases. Several studies point to an association between dietary calcium and periodontitis. like Oliver W (1969) [19], Abe et al. (1989) [20]and Amano H (1989) [21] have observed a relationship between calcium-deficient diet and the progression of periodontitis in rats whereas Osborn et al. (1977) [22], Vogel et al. (1979) [23], Nishida et al. (2000)[24]and Krall et al. (2001)[25] have obtained similar findings in human studies.It has been hypothesized that low dietary intake of calcium may contribute to the progression of periodontitis.

Sejal A. Mehta et al, (2019) showed that the mean serum calcium level was observed to be 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 periodontitis.[26]

The present study attempted to evaluatethe effect of non-surgical periodontal therapy on Serum and GCF Calcium levels in chronic periodontitis patients. The study has elucidated that increased Serum and GCF Calcium levels were observed in patients with chronic periodontitis after 3 months of non-surgical periodontal therapy and hence that indicates that there was a direct relationship between the total Serum and GCF Calcium concentration and periodontal disease.

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 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.

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