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Evaluation of Serum and GCF Calcium Levels in Healthy and Periodontal Disease Patients: A Clinico-Biochemical Study

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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:*The aim of this study was to evaluate the correlation between Serum and GCF Calcium levels in Healthy and Periodontal disease patients.*

Materials &Methods:*The present study was conducted to evaluate the Serum and GCF Calcium levels in 24 sampleswith age groups of 25-60 years including males and females, which were divided into two groups of 12 each. Group I-Control-Healthy, Group II-Experimental -Chronic Periodontitis. All subjects underwent for clinical examination including Plaque Index, Gingival Index, Gingival Bleeding Index, Probing Pocket Depth, and Clinical Attachment Level using a UNC 15 Probe. Blood and GCF samples were collected from each patient and Serum and GCF Calcium levels were estimated.*

Results:*All samples were screened for the levels of Serum and GCF Calcium from both groups. The experimental chronic periodontitis group showed a mean of 6.25mg/dl Serum Calcium and 6.01mg/dl GCF Calcium levels respectively, while the healthy group showed a mean of 8.97 mg/dl Serum Calcium and 9.00 mg/dl GCF Calcium levels respectively.The Serum and GCF Calcium levels were highly significant in healthy subjects compared to chronic periodontitis patients, $P<0.01$, respectively.*

Conclusion:*This study concludes that there is a decrease in Serum and GCF Calcium levels in periodontitis patients.Serum and GCF Calcium levels may be a useful tool to assess the periodontal host response and disease progression.*

Keywords: *Serum Calcium, GCF Calcium, Periodontitis, Healthy patients.*

I. INTRODUCTION

Specific microorganisms in dental plaque can lead to an inflammatory disease Chronic periodontitis, resulting in loss of periodontium which can be seen clinically as periodontal pocket formation and gingival recession[1].The combined activity of the pathogenic bacteria and the host's immune and inflammatory responses result in tissue destruction in periodontal disease[2].The host's immune system acts against the local microbial attack and prevents the spread of their damaging products. However, this defense mechanism may destroy the surrounding cells and connective tissue structures of the host[3].

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 human status of calcium intake, bone mineral density, and tooth loss, provide rationale for hypothesizing that low dietary intake ofcalcium is a risk factor for periodontal disease[4].

Calcium is the fifth most abundant element next to oxygen, carbon, hydrogen, and nitrogen[5].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[6].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[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].There are a limited number of studies available in the literature hence the present study is carried out to evaluate the correlation between Serum and GCF Calcium levels in Healthy and Periodontal disease patients.

II. MATERIALS AND METHODS

Study population

This cross-sectional study conducted from April 2023 to February 2024 was performed in accordance to the Declaration of Helsinki, 2008, and was approved by an institutional ethical committee.The totalsamplesizewas 24. Patientsaged 25-60 years were selected from the Outpatient Department of PeriodonticsDavangere.Informed consent was taken from thesubjects prior to the start of the study.Subjects were randomly recruited for this study based on the exclusion and inclusion criteria mentioned below.

INCLUSION CRITERIA

1)Systemically and periodontally healthy subjects within the age group of 25 to 60years.(Both the genders)

2) Patients who fulfill the criteria of chronic periodontitis according to AAP classification 2017 (Stage II, Stage III, and Stage IV Periodontitis)

3) Minimum of 20 natural teeth in the oral cavity.

4) Radiographic bone loss (moderate rate- rapid rate progression according to AAP 2017 classification)

EXCLUSION CRITERIA

1) Any periodontal therapy in the past 6 months.
2) Any antibiotic and steroids 6 months prior to sampling.

3) Medically compromised patients (history of cardiovascular or renal disease,malignancies, multiple sclerosis, rheumatoid arthritis,inflammatory bowel diseaseViral and fungal disease, bleeding disorders,calcium deficiency disorders including bone diseases.)
4) Smokers and Tobacco chewers.
5) Patients on calcium supplementation.
6) Pregnant, post-menopausal, and lactating females and patients with PCOS.

The patients were divided into two groups of 12 each:
GroupI-Healthy subjects with no clinical signs of gingival and periodontal inflammation.
GroupII-Chronic periodontitis-Subjects who had clinical signs of gingival inflammation, presence of PD,and clinical attachment loss ≥5mm.
All selected subjects were explained about the study in their regional language and written informed consent was obtained from the participants.

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,1975assessed for all patients according to the criteria given by the authors.Gingivalunitsof 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 dividingitby the total number of teeth present in the oral cavity.

Collection of Blood

Blood samples (5 ml) weredrawnby patients of both groups.Samples were collected from the antecubital fossa by venipuncture by using a 5 ml disposable syringe and a 23 gauge needle. The blood was collected in sterile vacuum tubes with no added anticoagulant and kept at room temperature for 2 hrs, where it was allowed to clot, as this is designated for serum separation.

Collection of GCF

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

Calcium Estimation

The samples collected were pretreated with double distilled deionised water. The estimation of Calciumin GCF and Serum was done by using Dual-Viewing (DV) ICP-OES (Perkin Elmer Optima 5300). Qualitative information ontheelement 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[9].

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[9].In ICP-OES, liquid samples were introduced into a radiofrequency (RF)-induced argon plasma and instantly dried, vaporised, and energised 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[10].

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 A and Group B. The results were obtained using the student's independent sample 't'test.Ap-value of <0.01 was consideredhighlysignificant for all analyses.

III. RESULTS

Thestudy subjects were categorised into two groups of 12 patients each: those with healthy periodontium and those with chronic periodontitis**.**The plaque index, gingival index, and gingival bleeding index were significantly increased in periodontitis patients compared to the control groups indicating the presence of gingival inflammationp<0.01,respectively. PD and CAL were highly significantly increasedin periodontitis patients compared to the control groups indicating the severity of periodontal disease p<0.01,respectively.

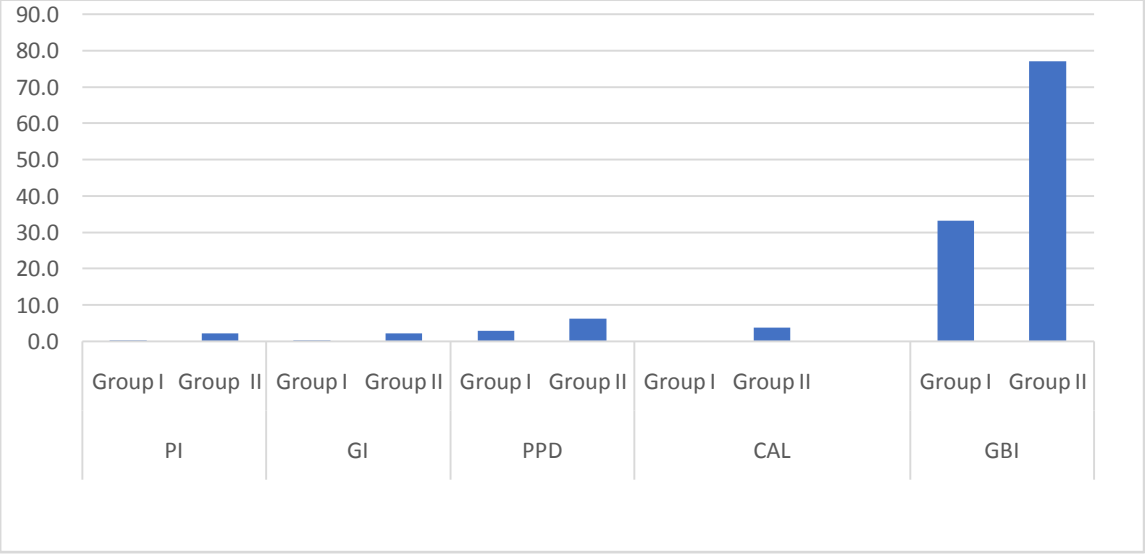
Table1

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

Parameter	Group I		Group II		P-value
	Mean	SD	Mean	SD	
PI	0.4	0.28	2.3	0.45	P<0.01
GI	0.4	0.51	2.3	0.49	P<0.01
GBI	33.33	12.30	77.08	22.50	P<0.01
PPD	2.9	0.51	6.3	0.65	P<0.01
CAL	0.0	0.00	3.8	1.14	P<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 analyzed using the student's independent sample 't' test. The Serum and GCF Calcium levels were significantly increased in healthy patients compared to chronic periodontitis patients, p<0.01, respectively.

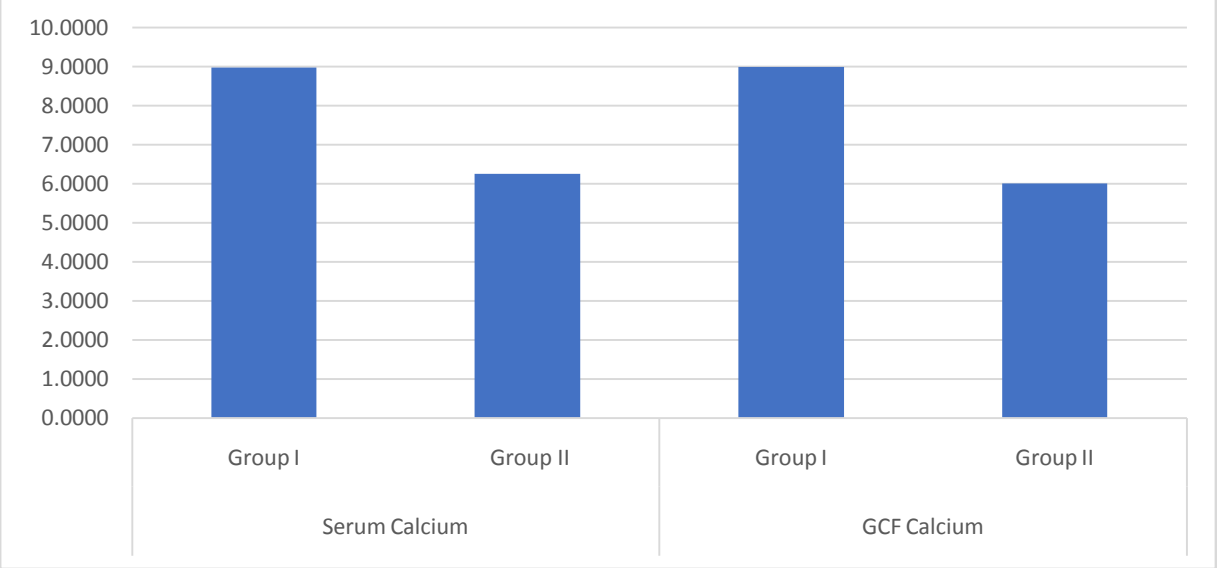
Table 2

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

Groups		Mean	SD	P-Value
Serum Calcium	Group I	8.97	0.78	P<0.01
	Group II	6.25	0.39	
GCF Calcium	Group I	9.00	0.83	P<0.01
	Group II	6.01	0.35	

Figure 2

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



IV. DISCUSSION

Risk factors play an important role in the periodontal disease progression[11].The onset and rate of progression ofperiodontitisis unique and is determined by various factors like microbiological, environmental, immune, and inflammatory[12].Therefore, it is necessary that the periodontal evaluation of a patient should focus on a comprehensive valuation of the clinical condition and estimation of risk for disease[13].

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, andpocket formation occurs which eventually leads to loss of tooth[14]. 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[15].

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[16].Useful diagnostic indicators should indicate the presence or absence of periodontal disease, the response to treatment, and the need for supplementary treatment[17].The discovery of predictive biomarkers is considerably difficult due to the episodic nature of the disease[18].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[18]. 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[18].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[19].The biochemical analysis of blood serum also provides a non-invasive means of estimating the host's systemic response in periodontal disease.

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[20].

Sridevi Sivarama Krishnan et al,(2021)showed that iron and calcium are present in GCF and Serum samples of healthy persons and patients with chronic periodontitis. A significant difference in serum iron levels between health and disease could indicate a patient's predisposition toward developing periodontitis. Calcium levels in Serum and GCF do not point towards periodontal disease activity[21].

Calcium is the most abundant mineral in humans,99% of the total calcium in the human body exists in the bones and teeth and provides a structural function, while the remaining 1% found in tissues and fluids is crucial for the maintenance of cell metabolism, nerve 2 transmissions, and muscle contraction[22].

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)[23], Abe et al.(1989)[24]and Amano H (1989)[25]have observed a relationship between calcium-deficient diet and the progression of periodontitis in rats whereas Osborn et al. (1977)[26],Vogel et al.(1979)[27], Nishida et al. (2000)[28]and Krall et al. (2001)[29] have obtained similar findings in human studies.It has been hypothesized that low dietary intake of calcium may contribute to the progression of periodontitis.

The present study attempted to evaluate and co-relate Serum and GCFCalcium levels in healthy subjects and patients with chronic periodontitis. The study has elucidated that decreasedSerum and GCFCalcium levels were observed in patients with periodontitis and hence that indicates that there was a direct relationship between the total Serum and GCFCalcium concentration and periodontal disease.

V. CONCLUSION

Within the limitations of our study,it can be said that theSerum and GCFCalcium levels may aid in predicting periodontitis.Thestudy suggests that there is highly significant decrease in Serum and GCF Calcium levels in periodontitis patients compared tohealthy subjects. 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 size are required to confirm the findings of this study.Calciumanalysis of Serum and GCF is essential for the diagnosis and assessment of periodontal disease.

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