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Estimation of salivary level of calcium in chronic periodontitis patients: A biochemical study

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Abstract

Background: Calcium is the most abundant mineral in the body. Ca is the widely studied inorganic constituent as a possible biomarker for periodontal disease. Hence, the aim of the present study was to evaluate the salivary levels of calcium in chronic periodontitis patients and healthy patients. **Methods:** A total of 40 patients were divided into 2 groups. Group 1 consisted of 20 individuals with healthy gingiva of probing depth ≤3mm, GI<1, PI<1 and CAL=0 and Group 2 consisted of 20 individuals with diagnosis of CP having PPD ≥ 5 mm, GI >1, PI> 1 and CAL≥ 3mm. Whole saliva samples were collected and subjected to estimation of salivary calcium levels. The results were analyzed by SPSS and Mann–Whitney analysis. **Results:** The level of salivary calcium increased as the disease progressed from health to periodontitis. The highest calcium levels from the Saliva were detected in group 2 while the lowest were detected in group 1. The results showed that the subjects in the periodontitis group had the higher levels of salivary calcium than the healthy group. **Conclusion:** The level of calcium was higher in the saliva of chronic periodontitis patients. Salivary calcium level can be used as biomarker for detection of periodontal disease.

Keywords: Calcium, Obesity, Periodontitis, Saliva.

INTRODUCTION

"Dental plaque" is a polymicrobial biofilm formed on tooth surfaces and is the primary etiological factor responsible for periodontal disease, if retained and not removed by frequent plaque removal methods.[1] Periodontitis is an infectious condition caused by periodontal pathogens, which affect the composition and integrity of periodontal structure and cause destruction of cells and connective tissue matrix, clinical attachment loss (CAL), alveolar bone resorption, periodontal pocket formation, and gingival inflammation.[2,3]

Saliva have a significant role in the establishment and progression of periodontal disease because of its importance in oral biofilm formation and host defense.[4] Saliva contains a large number of proteins that have metabolic, immune response, transporting, and several other cellular functions. When plaque mineralizes, it forms calculus. Saliva is the major source for mineralization of supragingival plaque.[5] Therefore, in saliva, Ca is the widely studied inorganic constituent as a possible biomarker for periodontal disease.

Calcium is the most abundant mineral in the body. It is found in food, dietary supplements and present in some medications. The high calcium level in saliva would result in a more rapid rate of plaque mineralization leading to periodontal diseases. An elevated level of salivary Calcium is related to a greater degree of bone loss and lower mineral density of bones which may contribute to weakening of the tooth attachment apparatus.[6] Calcium is the widely studied inorganic constituent as a possible biomarker for periodontal disease. In the light of the above facts, the current study was undertaken with an aim to evaluate the level of salivary calcium level in chronic periodontitis patients and healthy patients.

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MATERIALS AND METHODS

A total of 40 subjects of both sexes aged between 25 and 50 years participated in this case–control, cross-sectional study in the Department of Periodontology after obtaining ethical approval from the institutional review board of the college and a written informed consent was taken from all the participants before the start of the study.

Participants were categorized into two groups on the basis of Gingival index(GI), Plaque index (PI), pocket probing depths (PPD) and clinical attachment level (CAL).

• Group 1 consisted of 20 individuals with healthy gingiva of probing depth ≤3mm, GI =<1, PI=<1 and CAL=0.

• Group 2 consisted of 20 individuals with diagnosis of CP having PPD ≥ 5 mm, GI >1, PI> 1and CAL≥ 3mm.

Exclusion criteria

(1) cigarette smoking or tobacco use and alcoholism; (2) systemic diseases such as diabetes mellitus, hypertension, and rheumatoid arthritis; (3) pregnancy; (4) systemic bacterial, viral, or fungal infection; (5) history of antibiotic therapy or use of anti-inflammatory medications during the past 6 months; (6) periodontal therapy during the past 2 years; and (7) patients with aggressive periodontitis.

Sample collection

Subjects were requested to refrain from eating and drinking for at least 2 h before saliva collection. Using the spitting method, unstimulated saliva was collected between 11:00 am and 13:00 pm for 5 min (one spit per minute). The saliva was collected in sterile tubes and centrifuged at 3,000 rpm for 10 minutes and the supernatant was analyzed. The sample was stored at −70°C until the experiment. The estimation of salivary calcium was carried out by using Arsenazo reagent and calcium standard. The principal is dependent on the reaction of Arsenazo III, which reacts with calcium in a slightly acidic medium to form blue-purple complex. The measurement of optical density was carried out at 650 nm.

Data Analysis

Statistical analyses were performed using SPSS software version 21 (SPSS Inc., Chicago, IL, USA). The salivary level of calcium was compared between the two groups using the Mann–Whitney test and t-test. P < 0.05 was considered statistically significant. Data were reported as mean ± standard deviation.

RESULTS

The mean values of the clinical and biochemical parameters were expressed as mean ± SD (Table 1). The mean of calcium levels from the Saliva were2.02±0.52 mmol/l for group 1and 2.66 ± 0.89 mmol/l for group 2 (Graph 1).

Intergroup comparisons of the clinical and biochemical parameters are summarized in Table 2. A significant difference in calcium level in the saliva was found when group 1 was compared with group 2 (P<0.0001). Statistically significant differences were observed when comparison of gingival index, plaque index and pocket probing depth scores were made within groups 1 and 2.

Table 1: Values (mean ± SD) of Calcium, PPD, CAL, GI, PI in both Groups

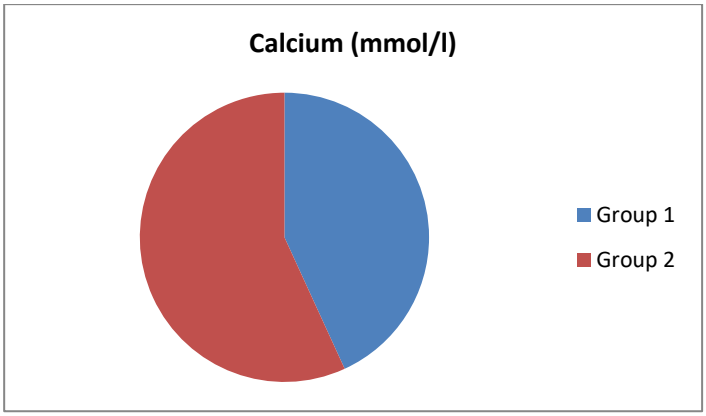
Variable	Group 1	Group 2
Calcium (mmol/l)	2.02±0.52	2.66 ± 0.89
PPD	1.47±0.57	6.80±1.21
CAL	0	4.13±1.31
GI	0.28±0.25	2.32±0.49
PI	0.22±0.19	2.13±0.41

‡ PPD – Pocket probing depth; CAL– clinical attachment level; GI – Gingival index; PI – Plaque index; SD - Standard deviation

Table 2: Consolidated Pairwise Comparison (p value) Among the Groups (p<0.05)

Variable	Mean difference (95% CI)	t	df	p
Calcium	-0.640 (−1.10-−0.17)	-2.777	38	0.0085*
PPD	-5.330 (−5.92-−4.72)	-17.821	38	< 0.0001*
CAL	--	--	--	--
GI	-2.040 (−2.28-−1.79)	-16.585	38	< 0.0001*
PI	-1.910 (−2.11-−1.70)	-18.90	38	< 0.0001*

*Statistically significant at P<0.05; p – Probability; Independent sample t-test; CI - Confidence interval, PPD – Pocket probing depth; CAL– clinical attachment level; GI – Gingival index; PI – Plaque index



Graph 1: Bar diagram shows the comparison of the salivary calcium level in both groups

DISCUSSION

The potential role of saliva in the diagnosis of oral and systemic health is evident in researches. Salivary biomarkers could be used to screen periodontal health status and disease progression.[7,8] Salivary Ca may be important with regard to dental and gingival health by way of its effect on mineralization of plaque. In the present study unstimulated whole saliva was used for the study as it predominantly bathes the oral cavity most of the time, and is a representative of pooled subgingival plaque samples.[9] In most of the previous studies stimulated saliva was used for analysis.[10]

The results showed that the subjects in the periodontitis group had the higher levels of salivary calcium than the healthy group. These results are consistent with the finding of Sewon *et al*.,[11,12] Fiyaz *et al*.,[13] Rajesh *et al*.[14], Kuraner *et al*[15]. The increase in salivary calcium level in periodontitis patients could be attributed to the fact that periodontitis affected subjects had a higher remineralisation potential than individuals with no signs of periodontal disease.

Patients in group 1 and group 2 showed statistically significant difference when the GI, PI and PPD were compared. Statistically significant difference was observed when group 1 and group 2 were

compared (p< 0.0001). A mean probing depth of 6.80±1.21mm and a GI score of 2.32±0.49mm in group 2 established the presence of an active inflammatory component along with a destructive component to the prevalent periodontal disease.

CONCLUSION

At present there are several biomarkers, studied in relation to periodontitis. In the current study, level of calcium was higher in the saliva of chronic periodontitis patients. Salivary calcium level can be used as biomarker for detection of periodontal disease. The limitation of this study was that we did not have information about the level of calcium after treatment of periodontitis. Further long-term and interventional studies with larger sample sizes are required to assess the efficacy of this biomarker for early detection of periodontal disease and prevention of its progression. Finally, for considering salivary calcium as biomarkers of inflammation in periodontitis, further studies are needed with more sample size in different populations.

Conflict of Interest

None.

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