

Comparison of salivary calcium level in smokers and non-smokers with chronic periodontitis, aggressive periodontitis, and healthy controls

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Abstract

Objective: The purpose of this study was to compare salivary calcium (Ca) level in smokers and non-smokers with chronic periodontitis, aggressive periodontitis, and healthy controls. **Materials and Methods:** 56 subjects were included in the study and were grouped as follows: 12 subjects who were periodontally healthy (Group I), 12 subjects having chronic periodontitis who were non-smokers (Group II), 12 non-smokers having aggressive periodontitis (Group III), 12 smokers with chronic periodontitis (Group IV), and 8 smokers with aggressive periodontitis (Group V). Clinical measurements and non-stimulated whole saliva samples were obtained and analyzed for Ca levels by ion-selective electrolyte analyzer. **Results:** When salivary Ca values were compared between the groups, they showed statistically significant values ($P < 0.001$) with the highest mean Ca level in Group IV and Group V, which include smokers with chronic periodontitis and smokers with aggressive periodontitis, respectively, than in other groups. Between groups II and III also, the mean salivary Ca level was statistically significant ($P < 0.001$) with higher mean salivary Ca in non-smokers having chronic periodontitis than in non-smokers having aggressive periodontitis. **Conclusions:** The present study showed that smokers having chronic periodontitis as well as smokers having aggressive periodontitis have higher salivary calcium levels. Also, patients with aggressive periodontitis were found to have lesser salivary calcium level than chronic periodontitis patients by ion-selective electrolyte analyzer.

Key words: Aggressive periodontitis, calcium, calculus, chronic periodontitis, saliva, smokers

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] Calculus plays a major contributing factor in retention of plaque in close contact with the periodontal tissue and creates an area where plaque removal is impossible.^[2] Saliva plays a major role in

plaque initiation and maturation. The main inorganic constituents of supragingival plaque are calcium (Ca), phosphorus (P), and small amount of other minerals which are mainly derived from saliva. When plaque mineralizes, it forms calculus. Saliva is the major source for mineralization of supragingival plaque.^[3] Therefore, in saliva, Ca is the widely studied inorganic constituent as a possible biomarker for periodontal disease. It has

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been known that precipitation of Ca and P salts occurs with rise in pH of the saliva.^[2]

Evidence has proved that smoking is a major risk factor for the prevalence and severity of periodontal disease. The amount of plaque and calculus seen in smokers is more than in non-smokers. Also, Ca concentration in early plaque of smokers is higher, which may be because of their smoking habit.^[4] An elevated level of salivary Ca in smokers is related to a greater degree of bone loss and lower mineral density of bones than in non-smokers.^[5]

Aggressive periodontitis is a severe and rapidly progressing form of periodontal disease seen in systemically healthy individuals at their early age, although it may be seen in older patients. There is a tendency of cases to aggregate in families. Clinically, patients with aggressive periodontitis show less amount of plaque and calculus, which seems inconsistent with the amount of periodontal destruction present. The plaque that is present forms a thin biofilm on the teeth and rarely mineralizes to form calculus.^[2]

As salivary Ca influences mineralization of plaque, in periodontitis patients^[3] and increased salivary Ca level is seen in smokers due to decreased bone mineral density,^[3] while individuals affected with aggressive periodontitis show minimum amount of calculus,^[2] this study was carried out to investigate and compare salivary Ca levels in patients with aggressive periodontitis and smokers having aggressive periodontitis.

MATERIALS AND METHODS

Source of data

A total of 56 subjects of both sexes (age range 25–55 years) were selected from the outpatient Department of Periodontology of Darshan Dental College, Udaipur, Rajasthan, India, after obtaining prior approval from the ethical committee of the college.

Out of 56 selected patients 12 subjects who were periodontally healthy were selected and included in Group I as a control group, 12 non-smokers with chronic generalized periodontitis were included in Group II, 12 patients with aggressive periodontitis who were non-smokers were included in Group III, 12 patients who were smokers and having chronic generalized periodontitis were included in Group IV, and 8 patients who were smokers and having aggressive periodontitis were included in Group V. A detailed systemic and family history was recorded. Only

those voluntary subjects who agreed to give a written informed consent were included in the study.

Inclusion criteria

- Patient's age between 25 and 55 years
- No history of any periodontal therapy in last 6 months
- Patients who were current smokers, i.e. those who had smoked 100 cigarettes and were smoking at the time of interview were included in groups IV and V^[6]
- Subjects with at least 20 permanent teeth present.

Exclusion criteria

- Patients with systemic diseases that have effects on the course of periodontal diseases
- Intake of antibiotics or anti-inflammatory drugs 1 month before the study
- Pregnant or lactating women
- Patients suffering from xerostomia due to any systemic or local conditions or as a result of any form of therapy like radiation therapy or any drug therapy
- Any history of periodontal therapy in last 6 months.

Clinical assessment

Periodontally healthy individuals with clinically evident probing depth (PD) ≤ 3 mm, no clinical attachment loss (CAL), and no radiographic bone loss were included in the study as the control group. Clinical diagnosis of chronic periodontitis was made with evident bone loss on radiograph, PD of ≥ 4 mm, and CAL of ≥ 1 mm at more than 30% of sites. PD and CAL were recorded using Williams's calibrated probe. Clinical diagnosis of aggressive periodontitis was made with evident vertical loss of alveolar bone around the first molars and incisors, severe periodontal tissue destruction which was inconsistent with the amount of microbial deposits, and interproximal attachment loss that should involve incisors and molars in individuals with non-contributory medical history. Only current smokers were included in the study.^[6]

Collection of saliva sample and analysis

After periodontal recordings, saliva samples were collected between 10.30 a.m. and 2.30 p.m. After the subjects were made to rinse their oral cavity with water, 2 ml of unstimulated whole saliva was collected by having them instructed to expectorate for 1–2 min into a sterile container, which was then stored on ice.

Subjects were asked to avoid intake of food 1 h before collection of the sample. Samples were then assessed by ion-selective electrolyte analyzer for Ca ion.

Estimation of ionized salivary Ca level by ion-selective electrode method

Specimen collection

To assay the specimen, 2 ml of the collected saliva sample was centrifuged at 3500 rpm and the clear solution was taken for analyzing ionized Ca. The AVL9180 Electrolyte Analyzer (Roche Diagnostics GmbH, Mannheim, Germany) accepts the samples directly from the sample cup and, with the use of an adaptor, from capillary tubes or the AVL micro-sampler. After selecting the measuring mode, the analyzer automatically processes the sample through the necessary steps, then prints and displays the result.

Ion-selective electrolyte analyzer

This analyzer makes potentiometric measurements of ionized Ca (Ca^{2+}) which is reliable and precise. The analyzer uses ion-selective electrodes that respond selectively to ions in the presence of others. It has one electrode which acts as a reference having a constant potential, while the other electrode measures the potential of a specific ion in the solution. The concentration of a specific ion in the solution is related to its activity that causes potential difference between the two electrodes, thereby giving analytical measurement of that specific ion.

Statistical analysis

Data were expressed as means and standard deviations. Statistical significance of differences between the groups (groups I–IV) was tested according to analysis of variance (ANOVA) using Tukey–Kramer multiple comparison test. Mean values for age and sex were calculated. The level of statistical significance was set at $P < 0.05$.

RESULTS

A total of 56 subjects were enrolled in this study, with a mean age of 30.25 (± 9.03) years in Group I, 39 (± 3.46) years in Group II, 30.91 (± 5.29) years in Group III, 42.33 (± 6.23) years in Group IV, and 31.37 (± 3.87) years in Group V [Table 1 and Figure 1].

ANOVA test results for salivary Ca using Tukey–Kramer multiple comparisons test between the groups are shown in Table 2. Overall, the P values for salivary Ca level showed statistically significant correlations. Salivary

Table 1: Mean values of age difference between the study groups

Groups	Age, years Mean \pm SD	Sex	
		Male	Female
I	30.25 \pm 9.03	4	8
II	39 \pm 3.46	6	6
III	30.91 \pm 5.29	7	5
IV	42.33 \pm 6.23	12	0
V	31.37 \pm 3.87	8	0

SD=Standard deviation

Table 2: One-way ANOVA for groups I-V (Tukey-Kramer multiple comparison test) for salivary Ca levels

Comparison of salivary Ca levels between groups	Mean difference	q	P
Group I vs Group II	-0.2270	17.740	<0.001***
Group I vs Group III	-0.09700	7.581	<0.001***
Group I vs Group IV	-0.6470	50.563	<0.001***
Group I vs Group V	-0.6070	42.429	<0.001***
Group II vs Group III	0.1300	10.159	<0.001***
Group II vs Group IV	-0.4200	32.823	<0.001***
Group II vs Group V	-0.3800	26.562	<0.001***
Group III vs Group IV	-0.5500	42.982	<0.001***
Group III vs Group V	-0.5100	35.649	<0.001***
Group IV vs Group V	0.04000	2.796	>0.05*ns

***Statistically highly significant at $P < 0.001$, *ns=Not significant at $P > 0.05$, ANOVA=Analysis of variance

Ca levels varied considerably between the groups. Highest values of salivary Ca were seen in smokers with chronic generalized periodontitis (Group IV) with the mean salivary Ca value being 2.66 ± 0.02 ($n = 12$; males = 12, females = 0), followed by smokers having aggressive periodontitis (Group V) with the salivary Ca value being 2.62 ± 0.01 ($n = 8$; males = 8, females = 0), patients having chronic periodontitis (Group II) with the salivary Ca value being 2.24 ± 0.02 ($n = 12$; males = 6, females = 6), patients with aggressive periodontitis (Group III) having salivary Ca value 2.11 ± 0.01 ($n = 12$; males = 7, females = 5), and then healthy controls (Group I) with the salivary Ca value being 2.01 ± 0.08 ($n = 12$; males = 4, females = 8), as shown in Table 3 and Figures 2 and 3.

When groups II–V were compared with group I for salivary Ca, all the groups showed statistically significant values ($P < 0.01$). When non-smokers with aggressive periodontitis and smokers with aggressive and chronic periodontitis were compared with non-smokers having chronic periodontitis for mean salivary Ca, they showed statistically significant values ($P < 0.001$) with the highest mean Ca level found in smokers having chronic generalized periodontitis. When smokers having

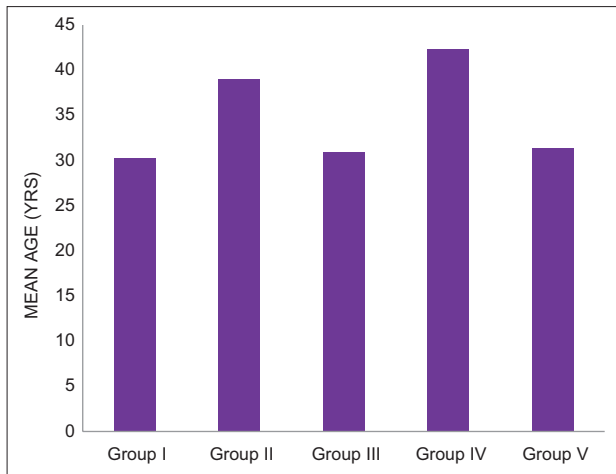


Figure 1: Mean age difference between group I, II, III, IV, V

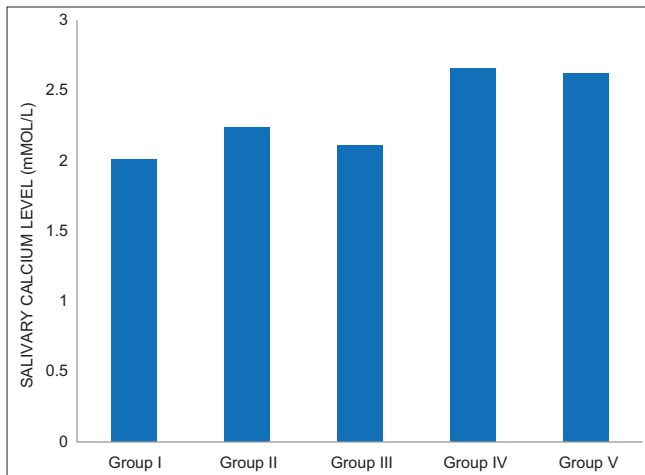


Figure 2: Variations in salivary calcium level between group I, II, III, IV, V

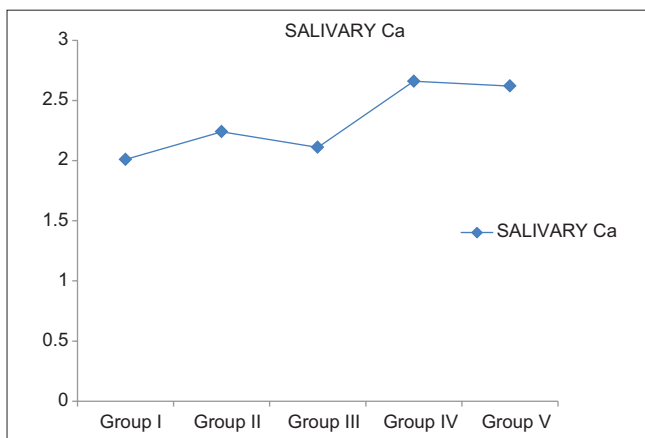


Figure 3: Variations in salivary calcium between the groups

chronic and aggressive periodontitis were compared with chronic generalized periodontitis patients who are non-smokers for mean salivary Ca, they showed statistically significant values ($P < 0.001$). Salivary Ca

Table 3: Mean and SD values for salivary Ca level

Groups	Mean	SD	Standard error of mean (E)
I	2.013	0.08900	0.02569
II	2.240	0.02000	0.005774
III	2.110	0.01000	0.002887
IV	2.660	0.02500	0.007217
V	2.620	0.01000	0.003536

SD=Standard deviation

of smokers having chronic periodontitis and aggressive periodontitis was statistically not significant ($P > 0.05$).

DISCUSSION

Salivary composition for Ca has been studied several times and different techniques have been used, such as atomic absorption spectrophotometry, flame photometry, and by inductively coupled plasma-atomic emission spectrophotometry technique. To our knowledge, this is the first time where salivary Ca has been studied for reference purposes in aggressive periodontitis patients to this extent using ion-selective electrolyte analyzer.

Salivary Ca may be important with regard to dental and gingival health by way of its effect on mineralization of plaque. An elevated level of salivary Ca has been reported as a characteristic of periodontally diseased patients.^[7-9]

Patients with chronic generalized periodontitis who were non-smokers showed higher mean salivary Ca values (2.24 ± 0.08) than healthy controls whose mean salivary Ca level was 2.01 ± 0.02 , with a statistically significant $P < 0.001$. The significantly higher level of salivary Ca has also been reported in a previous study, where salivary Ca level in whole saliva of subjects with chronic periodontitis was studied by the same method of ion-selective electrolyte analyzer.^[10] However, our result was in contrast with the study where higher salivary Ca level was not correlated with periodontal bone destruction.^[11] This is likely to be, as there is difference in age of the subjects evaluated. One study also reported that there is no age wise difference in Ca level and periodontal status.^[12] In our study, average age of subjects was 35 years; therefore, change in Ca level with age was not determined.^[13,14]

Patients with aggressive periodontitis showed higher mean salivary Ca values (2.11 ± 0.01) with a significant $P < 0.001$ when compared with healthy controls, while patients having aggressive periodontitis showed lower mean salivary Ca level, which was statistically significant

(with $P < 0.001$) when compared with patients having chronic periodontitis. Sewon and Söderling,^[15] in one of their studies, have shown that Ca concentration of supragingival plaque was higher in adult periodontitis patients when compared with juvenile periodontitis patients. In aggressive periodontitis, generally, there is little amount of supragingival plaque or calculus. Although the quantity of calculus and plaque is limited, it seems that the quality of plaque, that is the bacteria present, is of etiologic importance in aggressive periodontitis, which includes highly virulent strains of *Aggregatibacter actinomycetemcomitans* in combination with *Bacteroid*-like species.^[16]

It has been demonstrated that a positive association exists between smoking and both supragingival and subgingival calculus formation as a result of calcification of dental plaque biofilm.^[17] Smoking is reported to independently increase salivary Ca levels by decreasing skeletal bone density.^[5] Moreover, the constant exposure of taste receptors to tobacco presumably affects the salivary reflex and, in turn, salivary Ca levels.^[18]

Smokers having chronic generalized periodontitis showed higher mean salivary Ca values (2.66 ± 0.02) than chronic generalized periodontitis patients whose mean salivary Ca value was 2.24 ± 0.08 , with a statistically significant $P < 0.001$. Whereas the healthy controls showed lower mean salivary Ca level (2.01 ± 0.02), as compared to smokers having chronic generalized periodontitis, with a statistically significant $P < 0.001$. These results are in accordance with the study where higher salivary Ca level was seen in smokers having periodontitis as compared to non-smokers;^[19] however, by inductively coupled plasma-atomic emission spectrophotometry method, no significant difference was found between the mineral content of smokers and non-smokers.^[20] This can be due to the different techniques used for detection of salivary Ca. Also, the present study measured ionized Ca levels in saliva, which could vary from the total Ca levels.

When smokers having chronic periodontitis were compared with smokers having aggressive periodontitis for mean salivary Ca level, the mean salivary Ca value was statistically not significant ($P > 0.05$). But smokers having aggressive periodontitis showed higher mean salivary Ca value (2.62 ± 0.01) than aggressive periodontitis patients who were non-smokers whose mean salivary Ca value was 2.11 ± 0.01 , with a statistically significant $P < 0.001$. This increase in salivary Ca level of smokers having aggressive

periodontitis is because of the effect of smoking on the composition of saliva.

In the present study, unstimulated whole saliva was used as it predominantly bathes the oral cavity most of the time, as opposed to stimulated saliva.^[21]

CONCLUSION

This study supports the view that higher salivary Ca level could be associated with the development of periodontal diseases, possibly by increasing the mineralization potential of dental plaque. In subjects with high salivary Ca, rapidly hardening plaque is more difficult to clean and, usually, prolonged microbial irritation leads to periodontal disease. In aggressive periodontitis patients, salivary Ca is less; so, they form little supragingival calculus. The quality of plaque rather than quantity, with highly virulent bacteria, is of main etiologic importance, along with disturbance of the immune system. Smoking independently increases the salivary Ca levels by decreasing skeletal bone density.

Large-scale prospective studies including other salivary parameters are essential to further assess this relationship.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Bowen WH. Nature of plaque. *Oral Sci Rev* 1976;9:3-21.
2. Newman MG, Takei HH, Klokkevold PR, Carranza FA. Carranza's Clinical Periodontology. 10th ed. Philadelphia: WB Saunders 2010. p. 172-6.
3. Miller CS, King CP Jr, Langub MC, Kryscio RJ, Thomas MV. Salivary biomarkers of existing periodontal disease: A cross-sectional study. *J Am Dent Ass* 2006;137:322-9.
4. Pejčić A, Obradović R, Kesić L, Kojović D. Smoking and periodontal disease - A review. *Med Biol* 2007;14:53-9.
5. Sewón L, Laaine M, Karjalainen S, Doroguinakalaa A, Lehtonen-Veromaac M. Salivary calcium reflects skeletal bone density of heavy smokers. *Arch Oral Biol* 2004;49:355-8.
6. Centre for Disease Control Classification of Smokers. Available from: http://www.cdc.gov/tobacco/data_statistics/fact_sheets/. [Last accessed on 2013 Jun 12].
7. Sewon LA, Karjalainen SM, Sainio M, Seppä O. Calcium and other salivary factors in periodontitis-affected subjects prior to treatment. *J Clin Periodontol* 1995;22:267-70.
8. Sewon LA, Karjalainen SM, Söderling E, Lapinlaimu H, Simell O. Association between salivary calcium and oral health. *J*

- Clin Periodontol 1998;25:915-9.
9. Sewón L, Söderling E, Karjalainen S. Comparative study on mineralization-related intraoral parameters in periodontitis-affected and periodontitis-free adults. *Scand J Dent Res* 1990;98:305-12.
 10. Acharya A, Kharadi MD, Dhavale R, Deshmukh VL, Sontakke AN. High salivary calcium level associated with periodontal disease in Indian subjects--a pilot study. *Oral Health Prev Dent* 2011;9:195-200.
 11. Sewón L, Mäkelä M. A study of the possible correlation of high salivary calcium levels with periodontal and dental conditions in young adults. *Arch Oral Biol* 1990;35(Suppl):211S-2S.
 12. Nupur S, Shobha PM, Hemant B. Estimation and comparison of salivary calcium levels in healthy subjects and patients with gingivitis and periodontitis: A cross-sectional biochemical study. *AOSR* 2012;2:13-6.
 13. Grossi SG, Zambon JJ, Ho AW, Koch G, Dunford RG, Machtei EE, *et al.* Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. *J Periodontol* 1994;65:260-7.
 14. Al-Shammari KF, Al-Khabbaz AK, Al-Ansari J M, Neiva R, Wang HL. Risk indicators for tooth loss due to periodontal disease. *J Periodontol* 2005;76:1910-8.
 15. Sewon L, Söderling E. Calcium concentration in dental plaque of patients with juvenile and adult periodontitis. *J Periodontol* 1998;58:611-8.
 16. Califano JV; Research, Science and Therapy Committee American Academy of Periodontology. Position paper: Periodontal diseases of children and adolescents. *J Periodontol* 2003;74:1696-704.
 17. Bergström J, Eliasson S, Dock J. Exposure to tobacco smoking and periodontal health. *J Clin Periodontol* 2000;27:61-8.
 18. han GJ, Mehmood R, Salah-ud-Din, Marwat FM, Ihtesham-ul-Haq, Jamil-ur-Rehman. Secretion of calcium in the saliva of long-term tobacco users. *J Ayub Med Coll Abbottabad* 2005;17:60-2.
 19. Kiss E, Sewon L, Gorzó I, Nagy K. Salivary calcium concentration in relation to periodontal health of female tobacco smokers: A pilot study. *Quintessence Int* 2010;41:779-85.
 20. Erdemir EO, Erdemir A. The detection of salivary minerals in smokers and non-smokers with chronic periodontitis by the inductively coupled plasma-atomic emission spectrophotometry technique. *J Periodontol* 2006;77:990-5.
 21. Mandel ID, Wotman S. The saliva secretions in health and disease. *Oral Sci Rev* 1976;25-47.