

Comparison of Salivary Calcium Level and pH in patients with Aggressive Periodontitis and Healthy Individuals: A Clinico -Biochemical Study

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Abstract

Background: The rich variety of molecules present in the salivary secretions renders saliva an attractive possible source of disease biomarkers. Salivary flow and composition influences calculus formation and periodontal disease. Therefore the present study was conducted to compare the salivary calcium level and pH in patients with aggressive periodontitis and healthy individuals.

Materials and Method: The study was conducted among 108 patients, divided into group I, Group II, and Group III. Clinical diagnosis of aggressive periodontitis was made with evident bone loss on radiograph. Probing depth and Clinical attachment loss were recorded using Williams's calibrated probe. Other signs of inflammation were recorded using; Gingival index (GI) and Plaque index (PI). After periodontal recordings, saliva samples were collected from all patients. Samples were then assessed by AVL9180 electrolyte analyzer (Roche, Germany) for calcium ion and pH by 'pH litmus test paper.

Results: Mean plaque index and gingival index values was found higher among Group III (1.92 ± 0.23) and Group II (1.77 ± 0.37). Salivary calcium levels and pH levels were found higher in Group III (2.62 ± 0.01) and (7.43 ± 0.62). When group I for salivary calcium was compared with other two groups (II and III), it showed statistically significant values ($P < 0.01$). However, for salivary pH values, the findings were statistically insignificant.

Conclusion: On comparison between the 3 groups, it was found that the group with smokers having aggressive periodontitis showed higher salivary calcium levels and salivary pH.

Key Words: Saliva, Periodontitis, Smokers, Calcium, Attachment loss

Introduction

Human saliva is a fluid with many biological functions essential for the maintenance of oral health [1]. The saliva circulating in the mouth at any given time is termed as whole saliva and it comprises of a mixture of secretions from the major and minor salivary glands and traces from the gingival crevicular fluid [2]. The rich variety of molecules present in the salivary secretions renders saliva an attractive possible source of disease biomarkers. Over the last few years salivary research workers have been developing salivary diagnostic tools to monitor both oral and systemic disease. Saliva has many apparent advantages over serum as a medium for clinical diagnosis [3,4].

Salivary flow and composition influences calculus formation and periodontal disease. Salivary calcium, due to its affinity to be readily taken up by plaque, is an important factor not only with regard to the onset of periodontitis but also significantly with regard to dental health. It is one of the most intensely studied potential markers for periodontal disease in saliva. The normal reference value of salivary calcium is in the range of 0.5-2.7 mmol/L. The intra-oral mineralization potential of periodontally healthy subjects with no marginal alveolar bone resorption is different from that of subjects who have already been treated for periodontitis [5-7].

Cross-sectional and longitudinal studies have provided strong evidence that smoking is a significant risk factor for

periodontal disease [8]. Epidemiological studies of dental disease have consistently found poorer oral hygiene in tobacco smokers than in non-smokers [9]. All of the surveys have reported increased quantities of calculus in smokers. It has long been known that smoking causes a marked increase in salivary flow rate as a simple reflex effect and this could explain the tendency of smokers to accumulate increased amounts of calculus. There is some evidence that smoking also increases the mineralizing potential of saliva [9].

Data on salivary calcium of different study populations such as patients with rheumatoid arthritis, heavy smokers and women in menopausal ages have shown that there is higher means of salivary calcium level when compared to age-matched counterparts because of decreasing bone mineral density. An elevated level of salivary calcium in smokers is related to a greater degree of bone loss and lower mineral density of bones than in non-smokers [10,11]. Saliva has a pH normal range of 6.2-7.6 with 6.7 being the average pH. Resting pH of mouth does not fall below 6.3. In the oral cavity, the pH is maintained near neutrality (6.7-7.3) by saliva [12]. Smokers have comparatively higher oral pH than non-smokers. Therefore, there is a great possibility for this pH to extract calcium from the scales deposited on the teeth (or even from their teeth) of these individuals which might result in the elevated levels of salivary calcium [13].

So far no studies have been done to estimate and analyze

the salivary calcium level in patients with aggressive periodontitis. Therefore the present study was conducted to compare the salivary calcium level and pH in patients with aggressive periodontitis and healthy individuals.

Materials and Methods

Ethical clearance and study population

A total of 108 subjects of both sexes (age range 25-55 years) were selected from the Outpatient department (OPD) of Department of Periodontology of a dental college in India on the basis of convenience sampling after obtaining ethical clearance from the Institutional Ethics Committee. Out of 108 selected patients 36 subjects having healthy gingiva were selected and included in **Group I** as a control group, 36 patients who were having aggressive periodontitis who were non- smokers were included in **Group II** and 36 patients who were smokers having aggressive periodontitis were selected and included in **Group III**. A detailed systemic and family history was recorded. Detailed systemic and family history was recorded. Only those voluntary subjects, who agree to give a written informed consent, were included in the study.

Inclusion criteria

1. Patient's age between 25-55 years.
2. Clinical diagnosis of Aggressive periodontitis with evident bone loss on radiograph and PD ≥ 3 mm or more at 30% of proximal sites, Clinical attachment loss(CAL) ≥ 1 mm.
3. No history of periodontal therapy in last 6 months.
4. Patients who are current smokers in group III.
5. Subjects with at least 20 permanent teeth present.

Exclusion Criteria

1. Presence of systemic disease that could influence the course of periodontal disease.
2. Intake of antibiotics or anti-inflammatory drugs 1 month before the study.
3. Pregnant or lactating women.
4. 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.
5. Any history of periodontal therapy in last 6 months.
 - Patients with current or past habit of tobacco smoking or chewing.
 - History of any type of oral cancer or surgery.

Clinical assessment

Clinical diagnosis of aggressive periodontitis was made with evident bone loss on radiograph, probing depth (PD) of ≥ 4 mm, clinical attachment loss (CAL) of ≥ 1 mm. Probing depth and clinical attachment loss were recorded using Williams's calibrated probe. Other signs of inflammation were recorded using Gingival index and Plaque index [14]. Healthy subjects who were having no signs of inflammation, no evident bone loss on radiograph with PI= 0, GI=0, probing depth (PD) ≤ 3 mm, no clinical attachment loss (CAL) were included in the study as control group.

Collection of saliva sample and analysis

After periodontal recordings, saliva samples were collected from all patients (from 10.30 am to 2.30 pm). Following a brief rinsing of the mouth with water, two milliliters of unstimulated saliva was collected from subjects by having

them instructed to expectorate for 1-2 minutes into a sterile container after 1 hour of fasting, which was then stored on ice. Subjects were avoided for the intake of any food 1 hour before the collection of the sample. Samples were then assessed by AVL9180 electrolyte analyzer (Roche, Germany) for calcium ion and pH by 'pH litmus test paper.

Estimation of ionized salivary calcium level by ion selective electrode method:

AVL9180 Electrolyte Analyzer: The AVL9180 electrolyte analyzer is a microprocessor- based instrument using ion selective electrodes for measurement of sodium, potassium, chloride, ionised calcium and lithium. The user is able to select any one of the measurement modes: whole blood, serum, urine, aqueous standard solution, QC material, acetate or bicarbonate dialysate depending on the sample type to be analyzed. The analyzer automatically processes the sample through the necessary steps, then prints and displays the result.

Principles of procedure: AVL9180 analyzer methodology is based on ion selective electrode (ISE) measurements principle to precisely determine the measurement values. There are six different electrodes used in AVL9180 electrolyte analyzer: sodium, potassium, ionised calcium, lithium and a reference electrode. Each electrode had an ion selective membrane that undergoes a specific reaction with corresponding ion contained in the sample being analyzed. The membrane is an ion exchanger, reacting to the electrical charge of the ion causing a change in the membrane potential or measuring voltage, which is built up in the film between the sample and the membrane.

A galvanic measuring chain within the electrodes determines the difference between the two potential values on either side of membrane. The galvanic chain is closed through the sample on one side by the reference electrode, reference electrolyte and the open terminal. The membrane, inner electrolyte and inner electrode close the other side. A difference in ion concentrations between the electrolyte and the sample causes an electrochemical potential to form across the membrane of the active electrode. The potential is conducted by a highly conductive, inner electrode close to an amplifier. The reference electrode is connected to ground as well as to amplifier. The ion concentration in the sample is then determined by using a calibration curve determined by, measured points of standard solution with precisely known ion concentration.

Specimen collection: To assay specimen, 2 ml of saliva collected was then centrifuged at 3500 rpm and clear solution is taken for analyzing ionized calcium. The AVL9180 Electrolyte analyzer accepts the samples directly from samples cup and, with the use of an adaptor, from capillary tubes or the AVL micro sampler. Then measuring mode is selected, the analyzer automatically processes the sample through necessary steps, then prints and displays the result.

Analyzing salivary pH: pH litmus test paper measures pH level between 5.5 to 8.0 pH. pH test strip contains an easy colour- coded chart to determine saliva pH levels. A drop of saliva is placed on pH paper strip by micro pipette just enough to moisten it. Saliva reacts with strip, causing it to change in colour. The colour of the strip is matched to the corresponding colour chart. The different shades of colour that the colouring

agent adopts allow the degree of a substance's acidity or alkalinity to be measured. Each shade of colour corresponds to a precise pH value. The value is not indicated on the pH strip itself, however, but on a colour chart that comes with the strips. This chart includes every shade of colour the strips can adopt, with its corresponding pH value next to it.

Statistical analysis

Completed questionnaires were coded and spreadsheets were created for data entry. The data was analyzed using SPSS 17 (SPSS Inc. Chicago, IL, USA) Windows software program. Descriptive statistics were used to summarize the demographic information and the survey data was analyzed using one way ANOVA test. Confidence level and level of significance were fixed at 95% and 5% respectively.

Results

Table 1 depicts the demographic profile of study subjects included in all the three groups. Majority of the subjects (76%) were in the age group of 25-35 years. The mean age of the study population was found to be 30.37 ± 4.39 years. There were 12 males and 24 females in Group I and gender distribution in Group III was completely opposite to Group I. Majority of the subjects (54.5%) were educated till high school, 42.5% were graduates and only 3% were post graduates.

Mean plaque index values in Group I was 0, in Group II mean PI was 1.35 ± 0.49 and in Group III mean PI was 1.92 ± 0.23 (Table 2). Mean gingival scores were highest in Group II patients (1.77 ± 0.37) and Group III patients (1.12 ± 0.38) respectively.

Findings depicted in Table 3 revealed that highest salivary calcium was noted in Group III with mean value of 2.62 ± 0.01 followed by Group II (2.11 ± 0.01) and least value was noted in Group I (2.01 ± 0.08). A similar trend was noted in salivary pH values among all the three groups.

When salivary calcium value of Group I was compared with other two groups (II and III), a statistically significant difference was noted (p<0.01). However, statistically insignificant result was found when salivary pH value of Group I was compared with other two groups (P>0.05) (Table 4).

Groups	Age	Gender	
	Mean ± SD	Male	Female
Group I	30.25 ± 4.03	12	24
Group II	30.31 ± 3.29	21	15
Group III	30.57 ± 5.87	24	12

Table 1. Demographic profile of study subjects in all the three groups.

Groups	Mean ± SD	
	Plaque Index (PI)	Gingival Index (GI)
Group I	0	0
Group II	1.35 ± 0.49	1.77 ± 0.37
Group III	1.92 ± 0.23	1.12 ± 0.38

Table 2. Mean values of Plaque Index and Gingival Index.

Groups	Mean ± SD	
	Salivary Calcium	Salivary pH
Group I	2.01 ± 0.08	6.83 ± 0.24
Group II	2.11 ± 0.01	7.00 ± 0.67
Group III	2.62 ± 0.01	7.43 ± 0.62

Table 3. Mean and SD values for Salivary Ca level and pH.

Groups	Salivary Calcium levels		
	Mean Difference	q	P value
Group I vs. Group II	-0.09	7.58	*** P<0.001
Group I vs. Group III	-0.60	42.42	*** P<0.001
Group II vs. Group III	-0.51	35.64	*** P<0.001
Salivary pH levels			
Group I vs. Group II	-0.16	0.93	ns P>0.05
Group I vs. Group III	-0.59	3.00	ns P>0.05
Group II vs. Group III	-0.43	2.16	ns P>0.05

Test applied: One-way Analysis of Variance (ANOVA); ***Statistically extremely significant at P<0.001, ns not significant at P>0.05.

Table 4. Comparison of Salivary Calcium levels and Salivary pH levels between groups.

Discussion

This study is the first of its kind that studied salivary composition and pH has in smokers and non-smokers having aggressive periodontitis.

Group II patients in our study includes Aggressive periodontitis patients and when we compared mean salivary calcium values of Group II patients with Group I, Group II patients showed higher mean salivary calcium values and the difference was statistically significant. Sewon et al has shown that calcium concentration of supragingival plaque was higher in adult periodontitis patients as compared to patients with juvenile periodontitis [15]. It also seems that elevated concentrations of Calcium in both resting saliva (most of it comprising submandibular saliva) and stimulated saliva could be associated with elevated concentrations of Calcium in plaque [16].

In aggressive periodontitis generally there is little supragingival plaque or calculus. In contrast, some investigators have found similar levels of plaque and calculus in patients suffering from aggressive periodontitis and some other periodontal disease. Although the quantity of calculus and plaque is limited, it seems that quality of plaques, that is the bacteria present, is of etiologic importance in aggressive periodontitis [17].

When mean salivary calcium level of patients in Group I was compared with Group II and Group III, Group III patients showed higher mean salivary calcium value as compared to Group II and Group I patients and the findings were statistically significant (p value<0.001). This increase in salivary calcium level of smokers having aggressive periodontitis can be because of the effect of smoking on the composition of saliva.

pH of the saliva also influences viscosity of saliva and precipitation of calcium-phosphate salts to form calculus [4]. The metabolism of nitrogenous substrates results in the formation of base at high pH levels which can lead to deposition and accumulation of calcium phosphate as calculus within the plaque. It has been shown that higher pH levels are found not only in plaques located in region of the same mouth exposed to a greater flow of saliva but also in plaques of individuals with higher flow rates of resting saliva [18]. Kleinberg1964 [19] emphasized that substrate availability is the underlying regulator of pH and the overall acid base metabolism. Thus dietary habits, frequency and composition of diet would be the main regulators of the pH and thus also a main factor regulating its mineralization or demineralization.

In the present study, it was decided that unstimulated whole saliva should be collected as it predominantly bathes the oral cavity most of the time, as opposed to stimulated

saliva [20] which is used in some other studies [21,22]. It has been suggested that in advanced periodontitis, unstimulated saliva is representative of pooled sub gingival plaque samples [3]. A possible drawback could be that the collection of true unstimulated saliva may be difficult to standardize due to a variance in local stimuli and daily variation [21,23] which could be a reason why many studies have used stimulated saliva for analysis [24]. Therefore, it may be of value to conduct such experiments by collecting individuals' saliva samples at different time periods, which may help reduce time-related intra-individual differences in salivary composition [25].

In our study, we collected all the saliva samples between 10.30 am to 2.30 pm. Our results clearly showed that subjects with high calcium levels had significantly higher mean plaque scores than their counterparts. Smokers had poorer oral hygiene than non-smokers. Moreover, smokers had significantly more plaque than non-smokers, and there was a trend towards increased plaque deposits with increasing cigarette consumption [26].

In our study mean gingival scores were lowest in Group III (mean GI value of 1.12 ± 0.38) as compared to Group II. Though smokers had more plaque values than others, they showed less gingival inflammation, this may be due to the effect of smoking on the gingival tissues [26]. Smoking does not normally lead to striking gingival changes. A reduction in clinical signs of gingivitis has been reported in smokers and this effect has been shown to be independent of plaque levels.

Saliva and crevicular fluid play a decisive role in the prevention of periodontal disease and indeed paradoxically in the induction of periodontal pathology. Thus many studies have been conducted keeping in view the importance of these

two factors. Salivary minerals, calcium and phosphate in particular, are taken up by plaque covering the gingival 1/3 of teeth; the rate of this process probably depends on salivary mineral content and on plaque pH, among other factors [27]. Rapidly-hardening plaque, characteristic of subjects with high salivary calcium is more difficult to clean, especially from the sulcus area, than the plaque formed in an environment low in calcium [16]. Calcifying plaque increases plaque retention limiting oral hygiene and hence causing gingivitis. The continuous, apically growing, calcifying plaque may be sufficient enough to cause periodontitis, despite further efforts to improve oral hygiene.

Conclusion

The present study concluded that a group with smokers having aggressive periodontitis showed higher salivary calcium levels and salivary pH; this suggests that trend towards increased mineralizing potential in the saliva of smokers. Smoking independently increases salivary calcium levels by decreasing skeletal bone density. This supports the view that higher salivary calcium could act as a risk factor for the development of periodontal diseases, possibly by raising the mineralization potential of dental plaque. In subjects with high salivary calcium, rapidly hardening plaque is more difficult to clean, especially from sulcus area and usually prolonged microbial irritation leads to periodontal disease. This is the first study where salivary calcium and pH is evaluated in aggressive non-smoker and smoker aggressive patients. Though, large-scale prospective studies including other salivary parameters are essential to further assess this relationship.

References

1. Mandel ID. The diagnostic uses of saliva. *Oral Medicine*. 1990; **19**:119-25.
2. M. Castagnola, PM Picciotti, Messasa C Fanali. Potential application of human saliva as diagnostic fluid. *Acta Odontológica Latinoamericana*. 2011; **31**: 347-57.
3. Sonalee S, Manpreet K. A study of analytical indicators of saliva. *Annals and Essences of Dentistry*. 2012; **4**: 9-18.
4. Sausan Al Kawas, Zuabaidah HA Rahim, David B Ferguson. Potential uses of human salivary protein and peptide analysis in the diagnosis of disease. *Archives of Oral Biology*. 2012; **57**: 1-9.
5. William V. Giannobile, Thomas Beikler, Janet S. Kinney, Christoph A. Ramseier, Thiago Morelli & David T. Wong. Saliva as a diagnostic tool for periodontal disease: current state and future directions. *Periodontology 2000*. 2009; **50**: 2-64.
6. 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 & Preventive Dentistry*. 2011; **9**: 195-200.
7. Sudhir S. Quantitative evaluation of salivary calcium, phosphorous, protein and Ph in health and diseased periodontium. *Annals and Essences of Dentistry*. 2010; **2**: 21-4.
8. Sewón L, Söderling E, Karjalainen S. Comparative study on mineralization related intraoral parameters in periodontitis affected and periodontitis-free adults. *Scandinavian Journal of Dental Research*. 1990; **98**: 305-12.
9. Calsina G, Ramo'n J-M, Echeverri'a J-J. Effects of smoking

on periodontal tissues. *Journal of Clinical Periodontology*. 2002; **29**: 771-6.

10. I. D. M. Macgregor and W. M, Edgar. Calcium and phosphate concentrations and precipitate formation in whole saliva from smokers and non-smokers. *Journal of Periodontal Research*. 1986; **21**: 429-33.

11. Sevón LA, Laine MA. Effect of age on flow rate, protein and electrolyte composition of stimulated whole saliva in healthy, non-smoking women. *Open Dentistry Journal*. 2008; **2**: 89-92.

12. Baliga S, Muglikar S, Kale R. Salivary pH: A diagnostic biomarker. *Journal of Indian Society of Periodontology*. 2013; **17**: 461-5.

13. Ivana S, Kristina P, Anica B, Krunoslav C, Kresimir B, Kata RG. Salivary calcium concentration and periodontal health of young adults in relation to tobacco smoking. *Oral Health & Preventive Dentistry*. 2012; **10**: 397-403.

14. Løe H. The Gingival Index, the Plaque Index and the Retention Index Systems. *Journal of Periodontology*. 1967; **38** Suppl: 610-6.

15. Sewon L, Soderling E. Calcium concentration in dental plaque of patients with juvenile periodontitis. *Journal of Periodontology*. 1998; **58**: 611-8.

16. Sewón L, Soderling E, Karjalainen S. Comparative study on mineralization related intraoral parameters in periodontitis affected and periodontitis-free adults. *Scandinavian Journal of Dental Research*. 1990; **98**: 305-12.

17. Position paper. Periodontal diseases of children adolescents. Research science and therapy committee of the American Academy

of Periodontology. *Journal of Periodontology*. 2003; **74**: 1696-1704.

18. F Agha-Hosseini, I Mirzaei-Dizgah, PP Moghaddam, ZT Akrad. Stimulated whole salivary flow rate and composition in menopausal women with oral dryness feeling. *Oral Diseases*. 2007; **13**: 320-3.

19. Kleinberg I, Jenking G. The pH of dental plaques in different areas of mouth before and after meals and their relationship to the pH and rate of flow of resting saliva. *Archives of Oral Biology*. 1964; **9**: 493-516.

20. Mandel ID, Wotman S. The saliva secretions in health and disease. *Oral Sciences Reviews*. 1976; **8**: 25-47.

21. Sewón LA, Karjalainen SM, Söderling E, Lapinleimu H, Simell O. Association between salivary calcium level and oral health. *Journal of Clinical Periodontology*. 1998; **25**: 915-9.

22. Sewon LA, Karjalainen SM, Sainio M, Seppa O. Calcium and other salivary factors in periodontitis-affected subjects prior to treatment. *Journal of Clinical Periodontology*. 1995; **22**: 267-70.

23. Polland KE, Higgins F, Orchardson R. Salivary flow rate and pH during prolonged gum chewing in humans. *Journal of Oral Rehabilitation*. 2003; **30**: 861-5.

24. Sewón L, Makela M. A study of possible correlation between high salivary calcium level and dental conditions in young adults. *Archives of Oral Biology*. 1990; **35**: 211S-212S.

25. Larsen MJ, Jensen AF, Madsen DM, Pearce EI. Individual variations of pH, buffer capacity and concentrations of calcium and phosphate in unstimulated whole saliva. *Archives of Oral Biology*. 1999; **44**: 111-7.

26. Markkanen H, Paunio I, Tuominen R, Rajala M. Smoking and periodontal disease in the Finnish population aged 30 years and over. *Journal of Dental Research*. 1985; **64**: 932-5.

27. FP Ashley, PY Coward, RA Jalil, RF Wilson. Relationship between inorganic calcium and phosphorus concentrations of both resting and stimulated saliva and dental plaque in children and adults. *Archives of Oral Biology*. 1991; **36**: 431-4.