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Original Research Article

An Analytical Assessment of the Salivary Calcium Level and Ph in Patients with Chronic Periodontitis and Healthy Individuals

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Abstract

Aim: The present study was conducted to compare the salivary calcium level and pH in patients with chronic periodontitis and healthy individuals.

Methods: A total of 120 subjects of both sexes (age range 25-55 years) were selected from the Outpatient department (OPD) of Department of Dentistry of Netaji Subhas Medical College and Hospital, Bihta, Patna, Bihar, India. Out of 120 selected patients 40 subjects having healthy gingiva were selected and included in Group I as a control group, 40 patients who were having chronic periodontitis who were non- smokers were included in Group II and 40 patients who were smokers having chronic periodontitis were selected and included in Group III.

Results: The demographic profile of study subjects included in all the three groups. Majority of the subjects (70%) were in the age group of 25-35 years. The mean age of the study population was found to be 30.40 ± 4.10 years. Mean plaque index values in Group I was 0, in Group II mean PI was 1.36 ± 0.50 and in Group III mean PI was 1.90 ± 0.25 (Table 2). Mean gingival scores were highest in Group II patients (1.78 ± 0.32) and Group III patients (1.12 ± 0.40) respectively.

Conclusion: The present study concluded that a group with smokers having chronic periodontitis showed higher salivary calcium levels and salivary pH; this suggests that trend towards increased mineralizing potential in the saliva of smokers.

Keywords: Saliva, Periodontitis, Calcium, Attachment loss

Introduction

Periodontitis is defined as "an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms, or group of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with increased probing depth, recession or both." 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. [1,2]

Saliva have a significant role in the establishment and progression of periodontal disease because of its importance in oral biofilm formation and

host defense. [3] 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. [4] 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. [5] Calcium is the widely studied inorganic constituent as a possible biomarker for periodontal disease.

The primary etiological factor responsible for periodontal disease is 'dental plaque'. The inorganic components of plaque are calcium, phosphorous and other minerals. Salivary calcium and phosphorous are readily absorbed by dental plaque forming calculus which can lead to periodontitis. The concentration of Alkaline Phosphatase (ALP), an intracellular enzyme released from secondary granules of neutrophils increase significantly with increasing inflammation and plaque accumulation. The increased activity might also be a consequence of destructive processes in alveolar bone and metabolic changes in inflamed gingiva. ALP enzyme is an indicator of higher level of cellular damage. [6,7]

The enzymes released from host cells can be easily obtained within the oral cavity either from Gingival Crevicular Fluid (GCF) or from whole saliva. Raised levels of various biochemical markers in GCF have predicted clinical attachment loss and alveolar bone loss. However, there are inherent problems in collecting GCF in a routine dental office setting. [7] The whole saliva is easy-to-use noninvasive diagnostic method and can be collected in large quantity, with less discomfort to patient and clinician when compared to GCF. [8]

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

Methods

A total of 120 subjects of both sexes (age range 25-55 years) were selected from the Outpatient department (OPD) of Department of Dentistry of Netaji Subhas Medical College and Hospital, Bihta, Patna, Bihar, India.

Inclusion criteria

1. Patient's age between 25-55 years.
2. Clinical diagnosis of chronic 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 1month 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.
6. History of any type of oral cancer or surgery.

Methodology

Out of 120 selected patients 40 subjects having healthy gingiva were selected and included in Group I as a control group, 40 patients who were having chronic periodontitis who were non- smokers were included in Group II and 40 patients who were smokers having chronic 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.

Clinical assessment

Clinical diagnosis of chronic 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.[8] 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 microsampler. 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: Demographic profile of study subjects in all the three groups

Groups	Age	Gender	
	Mean± SD	Male	Female
Group I	30.20 ± 4.06	15	25
Group II	30.49 ± 3.32	26	14
Group III	30.50 ± 5.09	28	12

Table 1 depicts the demographic profile of study subjects included in all the three groups. Majority of the subjects (70%) were in the age group of 25-35 years. The mean age of the study population was found to be 30.40 ± 4.10 years. There were 15 males and 25 females in Group I and gender distribution in Group III were 28 males and 12 females.

Table 2: Mean values of Plaque Index and Gingival Index

Groups	Mean± SD	
	Plaque Index	Gingival Index
Group I	0	0
Group II	1.36 ± 0.50	1.78 ± 0.32
Group III	1.90 ± 0.25	1.12 ± 0.40

Mean plaque index values in Group I was 0, in Group II mean PI was 1.36 ± 0.50 and in Group III mean PI was 1.90 ± 0.25 (Table 2). Mean gingival scores were highest in Group II patients (1.78 ± 0.32) and Group III patients (1.12 ± 0.40) respectively.

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

Groups	Mean± SD	
	Salivary Calcium	Salivary pH
Group I	2.04 ± 0.06	6.84 ± 0.26
Group II	2.10 ± 0.02	7.00 ± 0.70
Group III	2.60 ± 0.01	7.45 ± 0.64

Findings depicted in Table 3 revealed that highest salivary calcium was noted in Group III with mean value of 2.60 ± 0.01 followed by Group II (2.10 ± 0.02) and least value was noted in Group I (2.04 ± 0.06). A similar trend was noted in salivary pH values among all the three groups.

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

Groups	Salivary Calcium Levels		
	Mean Difference	q	P value
Group I vs. Group II	-0.08	7.60	P<0.001
Group I vs. Group III	-0.58	42.48	P<0.001
Group II vs. Group III	-0.50	35.65	P<0.001
	Salivary pH levels		
Group I vs. Group II	-0.15	0.95	P>0.05
Group I vs. Group III	-0.55	3.00	P>0.05
Group II vs. Group III	-0.46	2.18	P>0.05

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).

Discussion

Saliva plays an important role in maintenance of oral health. Changes in composition and output of saliva may have detrimental effects on oral health. Patients suffering from dry mouth experience dental caries and difficulty in maintaining oral hygiene. Saliva and GCF not only play a decisive role in preventing periodontal disease but also ironically in the induction of periodontal pathology. [9,10] Recent advances in diagnosis of oral and periodontal disease are moving towards use of various biomarkers, which can be used to identify and quantify the periodontal risk. The fluctuations in dietary calcium intake and general calcium turnover may reflect in the levels of salivary calcium.

Group II patients in our study includes Chronic 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. [11] 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. [12]

pH of the saliva also influences viscosity of saliva and precipitation of calcium-phosphate salts to form calculus. [13] 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. [14] Kleinberg1964 [15] 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 which is used in some other studies. [16-18]

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. In our study mean gingival scores were lowest in Group III (mean GI value of 1.12 ± 0.40) 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. [19] 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. [20]

Saliva and crevicular fluid play a decisive role in the prevention of periodontal disease and indeed paradoxically in the induction of periodontal pathology. 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 chronic periodontitis showed higher salivary calcium levels and salivary pH; this suggests that trend towards increased mineralizing potential in the saliva of smokers. 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.

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