Quantitative Assessment of Calcium Profile in Whole Saliva From Smokers and Non-Smokers with Chronic Generalized Periodontitis

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ABSTRACT

Background: Measures of in vivo calcium status are important in understanding the mineralization capacity as it is an essential mineral component of both teeth and bone; and also play a vital role in the lipid profile and hormonal balance.

Aim: To evaluate the existence of any disturbances in calcium metabolism and absorption induced by smoking, by quantitatively assessing the variations in the salivary calcium level between smokers and non-smokers with periodontitis and relating to their periodontal status.

Materials and Methods: A total of 50 male patients were selected and categorized as Group I (smokers with chronic generalized periodontitis) and Group II (non-smoker/ non-

tobacco users with chronic generalized periodontitis). Clinical parameters such as Calculus Index and Community Periodontal Index were assessed. Subsequently two ml of unstimulated whole saliva was collected and subjected to biochemical analysis for the estimation of salivary calcium which was carried out in the next 20 min.

Results: Salivary calcium levels were significantly higher in Group I (2.2700) compared to Group II (1.7260). Higher calculus index and CPI index score were also seen in Group I when compared to Group II.

Conclusion: Elevated salivary calcium level among the Group I emphasize the decreased calcium absorption efficiency among the smokers. High salivary calcium content hardens plaque more rapidly, indirectly influencing the level of oral hygiene.

INTRODUCTION

"A cigarette is the only consumer product which when used as directed kills its consumer" states Dr. Gro Harlem Brundtland. High levels of tobacco consumption among disadvantaged population groups may lead to a doubling of the disease burden in these social groups from chronic illnesses related to tobacco consumption as well as from communicable and nutrition related diseases, which still account for a large share of total disease burden in the disadvantaged social groups in India.

The primary etiological factor responsible for periodontal disease is 'dental plaque'– a polymicrobial biofilms established on tooth surfaces, and retained if not removed by frequent plaque removal methods. As dental plaque is pivotal to periodontal disease, differences with respect to quality of dental plaque is a factor for variability in an individual's risk for periodontal disease. Saliva is the primary source for mineralization of supragingival plaque (Poff et al., 1997) and salivary minerals; calcium and phosphate, in particular, are taken up by plaque covering the cervical area of teeth. The rate of this process depends on salivary mineral content concentration [1].

Whole saliva is mainly composed of fluid synthesized by major and minor salivary glands. Major salivary glands consisting parotid, submandibular, and sublingual glands, are known to secrete fluid transported from serum as well as surrounding glandular tissues. This selective transportation within salivary glandular tissue is regulated by both acinar and tubular epithelial cells. Beside the secretions from salivary glands, oral mucosa, periodontium, as well as oral microflora also contribute to the final content of whole saliva. Hence, whole saliva represents a complex balance among local and systemic sources. This favours the application of saliva in the diagnosis not only for salivary gland disorders but also, for oral diseases and systemic conditions [2].

The non invasive and simple nature of procuring saliva allows for repetition and multiple sample collection that can potentially aid in early diagnosis, monitoring disease progression, or treatment responses with minimally trained personnel. This advantage of using saliva fascinates investigators who seek for an alternative form of body fluids to simplify a diagnostic procedure [2].

Calcium is an essential ion within the human body. The maintenance of a constant free ionised calcium concentration is biologically important for the function of excitable tissues. Variations in serum calcium values may have profound effects on neurological, gastrointestinal and renal function. Normal calcium concentration is maintained as a result of tightly regulated ion transport by the kidneys, intestinal tract and bone. This is regulated by calcaemic hormones, in particular parathyroid hormone and the active form of Vitamin D [3].

Positive correlations have been shown between high salivary calcium content and periodontitis [4] and between high salivary calcium content and chronic cigarette smoking [5].

Measures of in vivo calcium status are important in understanding the mineralization capacity as it is an essential mineral component of both teeth and bone, and also plays a vital role in the lipid profile and hormonal balance. Hence, this study aims at evaluating the existence of any disturbances in calcium metabolism and absorption induced by smoking, by quantitatively assessing the variations in the salivary calcium level between smokers and non smokers with periodontitis and relating to their periodontal status.

MATERIALS AND METHODS

A total number of 50 male patients were selected from the out–patients reporting to Department of Periodontics, Yenepoya Dental College & Hospital Mangalore, who gave consent to participate in the study. Sample size was determined based on the pilot study and

estimated to be 50 with 90% power and 95% confidence interval. Subjects who fulfilled selection criteria for the study were equally divided into two groups; Group I smokers with chronic generalized periodontitis (25) and Group II non smoker/ non-tobacco users with chronic generalized periodontitis (25). Ethical clearance was duly obtained from the institutional ethics committee.

Selection criteria

Patient population: 50 males

Inclusion Criteria

- Study consists of two groups with 25 subjects each.
- Group I (smokers with chronic generalized periodontitis) : smokes 10 cigarettes per day for a minimum of three years.
- Group II (non-smokers/ non-tobacco users with chronic generalized periodontitis) : who have not used any form of tobacco till date.
- Group I (smoker): smokes 10 cigarettes per day for a minimum of three years.
- Group II (non-smoker): who have not used any form of tobacco till date.
- Age Group: 20-50 years.
- Subjects of study: males.
- Subjects with chronic generalized periodontitis: more than 30% of the sites presented with loss of attachment as assessed by CPI index
- Minimum of 15 permanent teeth, including the index tooth or its substitutes.

Exclusion Criteria

- History of any periodontal therapy undertaken for the past 6 months.
- History of any systemic diseases.
- Disorders involving any salivary gland duct.
- History of any hormonal replacement therapy.
- History of any routine medications, supplemental vitamin and mineral intake for the past 6 months.
- Habit such as alcohol consumption.

All subjects followed a regular oral hygiene regimen, brushing the tooth once daily in the morning with tooth brush and tooth paste using horizontal/ scrub technique of brushing. None of the subjects reported using any form of interdental aids/ mouth washes.

Armamentarium used includes mouth mask, disposable latex gloves, mouth mirror, tweezers, No.5 explorer (Shepard's hook), CPITN-probe, kidney tray, cotton rolls, 2ml disposable plastic vials for transfer of specimen to the laboratory, saliva measuring unit, digital stopwatch, salivary calcium estimation kit (Bhat Bio-Tech India (Pvt) Ltd.), sterilized test-tubes, spectrophotometric unit.

The clinical parameters assessed included Calculus component of Oral Hygiene Index Simplified (OHI-S) and Community Periodontal Index (CPI) [6,7].

Subsequently a 2 ml of unstimulated whole saliva was collected; during 8 am-12 pm; at least 30 min after the last meal and also restraining from smoking for the past one hour. Initially the subjects were asked to swallow the residual saliva and allow the saliva to be accumulated in the floor of the mouth in the next two minutes. The sample collected in a vial was subjected to biochemical analysis by spectrometric method for the estimation of salivary calcium which was carried out in the next 20 min.

The same operator recorded all the clinical data which was entered in Microsoft excel sheet. The data in this study was analyzed by using unpaired t-test and Mann-Whitney test by SPSS 17. A p-value of <0.05 was considered to be statistically significant.

RESULTS

The examinations on the 50 male subjects revealed that the mean calcium content of the saliva was significantly higher (p< 0.0005) for the Group I (2.2700) than for the Group II (1.7260) as demonstrated in [Table/Fig-1]. [Table/Fig-2] highlights the elevated extent of calculus in Group I (p<0.0005). On comparing the CPI scores and the loss of attachment, a statistically significant relationship were found between Group I and Group II [Table/Fig-3,4].

Groups	N	Mean	Std. Deviation	Mean Difference	p-value	95% Confidence Interval of the Difference
Group I	25	2.2700	24838	0.544	<0.0005	0.43449 to 0.65351
Group II	25	1.7260	0.10275			

[Table/Fig-1]: Comparison of level of salivary calcium (mmol/l) between group I and group II
unpaired t-test

Groups	N	Mean	Std. Deviation	Mean Difference	p-value	95% Confidence Interval of the Difference
Group I	25	2.472	0.3973	0.9708	<0.0005	0.7340 to 1.2076
Group II	25	1.501	0.4347			

[Table/Fig-2]: Comparison of calculus index between group I and group II
unpaired t-test

Groups	N	Minimum	Maximum	Percentiles		
				25th(q1)	50th (Median)q2	75th(Q3)
Group I	25	4	4	4.00	4.00	4.00
Group II	25	2	4	3.00	3.00	3.00

[Table/Fig-3]: Comparison of CPI index between group I and group II
Mann-Whitney U *p<0.0005

Groups	N	Minimum	Maximum	Percentiles		
				25th(Q1)	50th (Median)Q2	75th(Q3)
Group I	25	3	4	3.50	4.00	4.00
Group II	25	2	3	2.00	2.00	2.00

[Table/Fig-4]: Comparison loss of attachment between group I and group II
Mann-Whitney U *p<0.0005

DISCUSSION

Although salivary calcium is increased in periodontitis [8], its significantly higher rates seen in smokers compared to non-smoker, highlights the role of smoking in the progression of periodontitis; as it seems that salivary calcium, due to its affinity for being readily taken up by plaque, is an important factor with regard to calculus formation and also the level of attachment loss as revealed by the present study.

Cigarette tobacco varies in its nicotine content, but common blends contain 15-25 mg per cigarette. Cotinine, an alkaloid, is one of the metabolite of nicotine. Robson et al., stated that collection of unstimulated saliva produced highest salivary nicotine concentration, whereas stimulated technique results in the highest salivary nicotine concentration [9]. Because of the ease of collection and storage, effect of calcium on stimulated saliva of smokers over non-smokers has well been documented. Far less attention has been paid with respect to unstimulated saliva, hence this study was carried out to quantify the calcium profile in whole saliva, from smokers and non-smokers with chronic generalized periodontitis; followed by comparison of the salivary calcium and the periodontal status between both the groups.

Analysis of saliva may offer a cost-effective approach to assess periodontal disease in large populations (Kaufman and Lamster,) and salivary calcium may be important with regard to both dental and gingival health by way of effect on mineralization of plaque (White) [10].

Plaque with high calcium content rapidly hardens and is therefore an indirect cause of poor oral hygiene. Calcium concentration of plaque significantly increases due to smoking. It has been shown previously that smokers have difficulty in performing efficient tooth brushing.

Bergström et al., observed supragingival calculus was strongly associated with smoking [11]. A dose-dependent relation exists between the influences of smoking on the amount of supragingival calculus. Supra-and sub gingival calculus is known to be specifically favourable for microbial growth and retention [12].

Sevón states, subgingivally retained, rapidly mineralizing plaque may be a significant reason for periodontitis susceptibility [13]. Hence, it appears that one of the main oral side effects of smoking is rapid mineralization of plaque and associated disease progression when compared to non-smokers [12].

Salivary calcium, due to its affinity to be readily taken up by plaque, is a principle factor not only with regard to the initiation of periodontitis but also significantly with regard to dental health [14]. The calcium levels of saliva may also reflect the fluctuations in dietary calcium and general calcium turnover. Thus monitoring for change in salivary calcium might be a useful tool to establish favourable response to periodontal therapy (Zuabi et al.,) [15]. The assay of salivary calcium level could perhaps be also used in future risk assessment of periodontitis (Sewon et al.,) [16]. Assessment of salivary calcium from both Group I and Group II revealed increased salivary calcium in both the groups compared to the health. Although calcium levels were significantly higher (p<0.0005) in Group I compared to Group II. This difference was statistically significant, which suggests an existence of an altered calcium metabolism and absorption among the periodontitis subjects and is seen pronounced among the smokers. This is in accordance with the findings of Sevón et al., and Macgregor et al., though the assessment was done using stimulated saliva [5,17]. A positive correlation was also exhibited by female smoker patients with periodontitis and higher salivary calcium (Endre Kiss), which may also be an influence of the hormonal shifts exhibited by the females [12]. Since the subject population was of middle aged females (30-62 years), decline in bone mass as a consequence of osteoporosis is accelerated with the onset of menopause and hormonal variations in these age group individuals. Salivary sample collected is of stimulated in origin. Hence the saliva obtained depends on which gland is been stimulated. Calcium concentration is about 45% higher in the submandibular glands than in the parotid glands. A study by Mandel et al., has shown high concentration of plaque and submandibular saliva has been found in heavy calculus formers than in light calculus formers, signifying the need for unstimulated salivary sample [18]. Whereas Zuabi et al., stated decreased calcium concentration of non-stimulated saliva of tobacco smokers. According to Sewón diminished skeletal bone density, a known adverse effect of smoking, may reflect elevated levels of salivary calcium highlighting the existence of disturbances in calcium metabolism and absorption [12,15,16].

In health, secretion of saliva occurs in two stages-first, secretion occurs into the glandular acini which is approximately similar to extra cellular fluid (ECF) and then this primary secretion flows into the acinar duct where reconditioning occurs, some substances are actively reabsorbed while some are actively secreted but during maximal salivation, there is not much time for the reconditioning process to occur and hence, the total concentration of solute in saliva reduces [19]. Smoking in general increases the activity of salivary glands and a level of tolerance develops to the salivatory effects of smokers resulting in habitual smokers to salivate minimal compared to novice smokers [20]. Hence elevated salivary calcium may also be linked to decreased salivary flow among the smokers and may also predict calcium dissolution from teeth matrix. Bone resorption is also directly regulated locally by ionized calcium generated as a result of osteoclastic resorption [21].

On comparing the calculus scores (Simplified Oral Hygiene Index), the mean value for the Group I was 2.472 and for Group II, it was 1.501 which is statistically significant (p<0.0005); emphasizing that plaque sample with increased calcium content mineralizes rapidly. The community periodontal index score and loss of attachment were statistically significant among both Group I and Group II, in which smokers showed increased periodontal pockets and these results are in accordance with the study by Baharin B, et al. It was found that smokers demonstrated higher proportion of sites probing ≥ 5mm and more bone loss in all regions. In a longitudinal study, Machtei et al., states that smokers had three times more attachment loss and two times more alveolar bone loss than non smokers [22,23].

Within the limitations, the findings of the study support the view that increased salivary calcium reflecting altered calcium turnover, fluctuations in dietary calcium, enhanced mineral dissolving action of osteoclast induced bone resorption among the smokers could act as a risk factor for the development and progression of periodontal diseases, possibly by raising the mineralization potential of dental plaque and hampering the normal structure and function of alveolar bone. The clinical significance of these findings needs, however, to be determined in further, large scale controlled studies.

CONCLUSION

Cigarette smoking is a major risk factor for periodontitis. It is often associated with high susceptibility, comparatively early onset disease, and more severe and wide spread periodontal destruction and treatment failure. Elevated salivary calcium in smokers, emphasizes the role of smoking in the progression of periodontitis; as it seems that salivary calcium plays a significant role with regard to calculus formation and also the level of attachment loss revealed by our present study. Whilst the limitations of this study include severity of periodontitis was not standardized and serum calcium level was not assessed.

Hence, the usage of salivary biomarkers appears promising for future application to diagnose periodontal diseases and to prognosticate periodontal treatment outcomes. The present study clearly demonstrates that high salivary calcium concentration seems to be a characteristic feature of periodontitis, which is worsened by the synergistic action of smoking, revealing the significance of habit cessation. This highlights the supportive role of dentist in an attempt to improve the oral and general health status of an individual.

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