High Salivary Calcium Level Associated with Periodontal Disease in Indian Subjects – a Pilot Study

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**Objectives:** To evaluate the association of salivary calcium level with periodontal status in a case-control study model.

**Materials and Methods:** Fifty adult non-smoking subjects recruited from an Indian dental educational institution were categorised into case and control groups on the basis of a full-mouth periodontal examination. The case group was comprised of 25 subjects diagnosed with chronic generalised periodontitis and the control group included 25 periodontally healthy individuals. Basic demographic data was obtained and 3 ml of unstimulated saliva was collected. Salivary calcium levels were assayed by the ion selective electrode method.

**Results:** The mean age for cases (46.6 ± 6.9 years) was significantly higher than that for controls (42.4 ± 6.3 years). The mean number of teeth for the case group (28 ± 3) was significantly lower than that for the control group (31 ± 1). The mean salivary calcium level in the case group (2.11 ± 0.24 mmol/L) was significantly higher than in the control group (1.86 ± 0.25 mmol/L) when ANCOVA for age adjustment was applied.

**Conclusions:** Periodontal disease is associated with higher salivary calcium levels than that in periodontal health, indicating that the calcium level of saliva could possibly be a risk factor for development of periodontal diseases.

**Key words:** chronic periodontitis, periodontal disease, salivary calcium

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The primary etiological factor responsible for periodontal disease is ‘dental plaque’ – a polymicrobial biofilm established on tooth surfaces, and retained if not removed by frequent plaque removal methods (Bowen, 1976). As dental plaque is pivotal to periodontal disease, differences with respect to quality of dental plaque are a factor for variability in an individual’s risk for periodontal disease. Saliva is the primary source for mineralisation 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.

The rate of mineralisation of dental plaque and calculus formation is known to vary among individuals. The premise that high salivary calcium content would result in a more rapid rate of plaque mineralisation has been investigated (Sewón et al, 1998), since rapidly hardening plaque is difficult to clean and such individuals would be at increased risk for periodontal disease. Some earlier studies have shown that a positive correlation exists between high salivary calcium content and periodontitis (Sewón et al, 1995,1990; Kuraner et al, 1991) but others have contradicted this (Sewón et al, 1998, 1990).

As geographical differences contribute to differences in periodontal disease expression, as do dietary and lifestyle factors, there is a need to determine an association between salivary calcium levels and periodontal status in order to identify a putative risk.
factor in the multi-factorial aetiopathogenesis of periodontal diseases in different populations.

**OBJECTIVES**

The present pilot study was designed to compare salivary calcium levels in Indian subjects with chronic generalised periodontitis with those of periodontally healthy controls.

**MATERIALS AND METHODS**

The study was conducted at Dr DY Patil Medical and Dental College and Hospital, Pimpri, India. Fifty subjects participated in the study. Adult non-smoking subjects with at least 20 teeth present were included. Subjects suffering from hyposalivation of any origin, xerostomia, systemic disorders such as HIV/AIDS or other infections, diabetes mellitus, those undergoing radiation therapy, either pregnant or lactating and those under any medication likely to alter parameters under study, were excluded from participation. The study protocol was approved by the institutional ethical committee. Subjects were interviewed about their general health, oral hygiene and smoking habits, and informed consent was obtained from all subjects. Demographic data recorded included: age, gender, smoking status and body mass index (BMI), which was calculated by dividing weight in kilograms by height in metres squared.

**Clinical examination**

A single examiner carried out a full-mouth periodontal examination. Clinical periodontal parameters recorded at six sites on all teeth (except third molars) were probing pocket depth (PPD, in mm), clinical loss of attachment (LOA, in mm) and bleeding on probing (BOP), which was noted as ‘positive’ if presence of bleeding within 30 seconds of probing was recorded.

**Subject stratification**

Based on the periodontal condition, subjects were assigned to case and control groups. Each group was comprised of 25 subjects. LOA was the primary measure considered to differentiate between case and control groups. The case group included subjects having more than 30% of sites with LOA and more than 10% of sites with positive BOP, thus diagnosed with chronic generalised periodontitis (Armitage, 1999). The control group included periodontally healthy subjects. Those individuals having no sites with clinically evident LOA were regarded as periodontally healthy if they also had less than 10% of sites BOP positive. The addition of a 10% cut-off for the percentage of BOP positive sites for diagnosis of ‘periodontal health’ was set in order to exclude those with overt gingival inflammation, but no LOA.

**Saliva collection and analysis**

Three millilitres of unstimulated saliva was collected from subjects by having them expectorate for 5 minutes into a sterile container at the same time in the morning, after 1 hour of fasting, which was then stored on ice. Subjects had avoided the intake of any food or water 1 hour before the collection of the sample and all samples were collected at the same time, to avoid alterations in the salivary flow rate and electrolytes.

Calcium levels were measured in millimoles/litre by detection of calcium ion in saliva using a calcium ion selective electrode method with an AVL9180 ion selective electrolyte analyser. Prior to reading, the analyser automatically initiated a calibration cycle and a calibration curve was determined from two measured points of a standard solution of precisely known calcium ion concentration, as per the manufacturer’s instructions. It was also assured that samples and standards were at the same temperature. The frequent calibration was done to ensure good reproducibility of readings. All readings were taken once by a single technician.

**Statistical analysis**

A two-tailed unpaired t-test was used for comparing means of demographic variables and salivary calcium levels between the two groups and analysis of covariance (ANCOVA) with age as a covariate was performed to adjust for the difference in age between the two groups.
RESULTS

All data are reported as mean (± standard deviation [SD]). The mean age for cases was 46.64 (± 6.9) years and was significantly higher than that for controls (42.4 ± 6.3 years), while the mean number of teeth noted in the case group was significantly lower at 28 (± 3) teeth as compared to the control group (31 ± 1). The mean BMI for case and control groups were not significantly different (Table 1). Descriptive analysis of clinical periodontal parameters in the case group showed a mean of 56.29% BOP positive sites, versus 8.31% in the control group (Table 2). Pocket depth distribution was categorised within the case group as mean percentage of sites with moderate PPD (4–6 mm; 24.07%) and severe PPD (more than 6 mm; 8.3%), while the mean LOA in the case group was 5.28 (± 0.92) mm (Table 2). The mean salivary calcium level in the case group was 2.11 (± 0.24) mmol/L, which was significantly higher than that in the control group 1.86 (± 0.25) mmol/L, after ANCOVA using age as a covariate was performed ($P = 0.002$) (Table 3).

DISCUSSION

Analysis of saliva may offer a cost-effective approach to assess periodontal disease in large populations (Kaufman and Lamster, 2000) and salivary calcium may be important with regard to both dental and gingival health by way of effect on minerali-

The present pilot study was designed to verify this association in a group of ethnically similar Indian subjects. Ethnic-genetic factors are known to significantly affect skeletal bone mass (Pollitzer and Anderson, 1989), which has shown correlation with salivary calcium levels (Sewón et al, 2004). It also is known that salivary ionised calcium levels correlate well with those in serum (Rehak et al, 2000). Moreover, salivary flow rates, which in turn may affect electrolyte levels, have been shown to vary among different ethnic groups (Björnstad and Crossner, 2007). Ethnicity may significantly impact plaque composition and subgingival calculus, and hence periodontal disease expression (Booth and Ashley, 1989.) To the authors’ knowledge this is the first reported study of its kind in a group of subjects from western India.

Smokers and tobacco chewers were excluded as smoking is reported to independently increase salivary calcium levels by decreasing skeletal bone density (Sewón et al, 2004; Avşar et al, 2009; Erdemir and Erdimir, 2006; Taguchi, 2005; Laine et al, 2002). Moreover, the constant exposure of taste receptors to tobacco presumably affects the salivary reflex, and in turn salivary calcium levels (Khan et al, 2005). Patients with systemic diseases such as diabetes were excluded as these may influence salivary electrolyte levels and flow rate (Moreira et al, 2009) as well as increase the rate of periodontal destruction. As age-related differences have been noted in salivary electrolyte levels (Sevon and Laine, 2008) including calcium levels (Yalçın et al, 2005), age was considered as a covariate. However, as the groups were not age matched, the effect of age could not be completely eliminated as a possible confounder. The higher mean age of the case group could also be linked with increasing prevalence of periodontal disease and risk of tooth loss with increasing age (Grossi et al, 1994; Al-Shammary et al, 2005). The rationale behind recording BMI was that calcium metabolism shows variance with obesity, due to a change in the binding of calcium to plasma proteins and an increase in parathyroid hormone levels (Lind et al, 1993; Nahid et al, 2004) and the fact that obesity is also associated with higher incidence of periodontal disease expression (Khader et al, 2009; Pischon et al, 2007). The periodontal parameters noted in the case group indicate individuals with severe and extensive periodontal disease, within the category of severe generalised chronic periodontitis (Armitage, 1999). Thus the study compares two ‘extreme ends’ of the periodontal disease spectrum, similar to that in a previous study (Sewón et al, 1990).

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 (Mandel, 1987), in contrast to other studies (Sewón et al, 1995, 1998; Laine et al, 2002). A possible drawback could be that the collection of true unstimulated saliva may be difficult to standardise due to a variance in local stimuli and daily variation (Mandel and Wotman, 1996; Polland et al, 2003), which could be a reason why many studies (Sewón and Makela, 1990) have used stimulated saliva for analysis. 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 (Larsen et al, 1999).

The significantly higher salivary calcium level in the case group as compared to the control group, has been demonstrated in similar reports by previous studies (Sewón et al, 1990, 1995; Kuraner et

<table>
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<td>Unpaired t test (P value, significance)</td>
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<td>Cases</td>
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<td>Controls</td>
<td>1.86 ± 0.25</td>
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SD: standard deviation

Table 3 Comparison of salivary calcium levels between case and control groups
al, 1991) but differs from reports that observed no relation of salivary calcium with periodontal bone loss in young adults (Sewón et al, 1990, 1998). This is likely to be due to the differences in the age group of the subjects evaluated. The relatively high mean incidence of BOP positive sites in the case group validates earlier findings that higher calcium levels are associated with poor gingival health (Sewón et al, 1990; 1998). Plaque and salivary calcium levels are highly correlated (Matsuo and Lagerof, 1991) and inefficacy of plaque control measures in removal of a more rapidly calcifying plaque mass would confer a higher risk for periodontal disease, along with a decrease in caries risk due the increase in remineralisation potential of saliva and plaque (Sewón, 1998). Although this cross-sectional study doesn’t indicate causality, the present data indicates a relationship between periodontal disease outcome and salivary calcium levels. Prospective studies designed to evaluate healthy individuals with high salivary calcium with respect to periodontal disease development could help in identifying the true contribution of salivary calcium to periodontal disease risk.

The pH, buffering capacity and flow rate were not examined in this study and could possibly have added to the conclusions. Most studies evaluating salivary pH and buffer capacity have used stimulated saliva as these parameters vary widely in resting saliva (Moritsuka et al, 2006), which was evaluated in this study. Increasing salivary flow rate and its resultant increase in pH above neutral (Ferguson and Fort, 1974) is associated with an increase in both salivary calcium content (Mandel, 1980) and periodontitis associated microorganisms (Bradshaw and Marsh, 1998). However, dietary and systemic factors also affect salivary calcium, and certain studies (Armitage, 1999) have inferred that variations in salivary pH and flow rate may not necessarily correlate with salivary calcium content (Rokenbach et al, 2006; Almstahl andWikström, 2003), which may be modulated by systemic factors like renal function (Davidovich et al, 2009) and variances in calcium transport in salivary glands (Homann et al, 2006). Additionally, the present study measured ionised calcium levels in saliva, which could vary from the total calcium levels. Further research along these lines is required to elucidate the relationship of salivary variables, including electrolytes, with periodontal disease risk, so that more optimal preventive treatment regimes can be designed for such groups.

CONCLUSION

Within the limits of the present preliminary study, it is possible to conclude that among a sample of Indian subjects, the calcium level of saliva is higher in chronic generalised periodontitis compared to that in periodontally healthy individuals. This finding supports the view that higher salivary calcium could act as a risk factor for the development of periodontal diseases, possibly by raising the mineralisation potential of dental plaque. Large-scale prospective studies including other salivary parameters are essential to further assess this relationship.

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REFERENCES


