Estimation and Comparison of Serum and Salivary Calcium Levels in Periodontitis Patients and Healthy Subjects: A Clinicobiochemical Study

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ABSTRACT

Background: Calcium (Ca) mineral is the fifth most abundant and essential element present in the bones and teeth, offering a structural function. It is vital for proper development and maintenance of calcified oral tissues. The deficiency of serum calcium is a risk factor periodontal disease and 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. Therefore, the present study was conducted to estimate and compare the serum and salivary calcium levels in healthy subjects and periodontitis patients.

Aim: The aim of this study is to estimate and compare serum and salivary calcium levels in healthy subjects and patients with periodontitis.

Material and methods: The study group comprises individuals attending to the outpatient Department of Periodontics, Sri Sai College of Dental Surgery. The study population consists of 30 subjects, divided into group A (aggressive periodontitis), group B (chronic periodontitis) and group C (healthy subjects). Serum and salivary calcium levels are detected for each patient by absorption spectroscopy.

Results: Results obtained showed a statistically significant increase in the levels of serum and salivary calcium in periodontitis patients in comparison to healthy group.

Conclusion: Based on these results, it can be assumed that there exists a clear and a significant association between high serum and salivary calcium levels and periodontitis.
also significantly with regard to dental health. It is one of the most intensely studied potential markers for periodontal disease in saliva.7

Therefore, the present study conducted to estimate and compare the serum and salivary calcium levels in healthy subjects and periodontitis patients.

MATERIALS AND METHODS

The study group consists of individuals attending to the outpatient Department of Periodontics, Sri Sai College of Dental Surgery. The duration of the study spanned over a period of 2 months i.e., January 2015 to February 2015. Ethical clearance was obtained from Institutional Ethics Committee Board (EC Board number: 496/ss-cds/IRB-E/OS/2015). The study included 30 subjects both males and females in the age group of 20–45 years. They were divided into three groups consisting of 10 patients each.

Group A: Comprises aggressive periodontitis patients (Armitage, 1999)

Group B: Comprises chronic periodontitis patients (Armitage, 1999)

Group C: Comprises healthy subjects with periodontal pocket depth ≤3 mm, clinical attachment loss equal to zero and absence of gingival inflammation and bleeding on probing.

Serum and salivary calcium levels detected for each patient by absorption spectrophotometry.

The patients were informed about the procedure of the study, and an informed consent was signed before their participation in the study. The selection of patients was done on the same day before the collection of sample.

Exclusion Criteria

1. Periodontal surgery within the last year,
2. Scaling and root planing as part of initial periodontal therapy within the past six months,
3. History of any systemic diseases, conditions, or use of medications that might affect bone and mineral metabolism and periodontal health.
4. Treatment with oestrogen and bisphosphonates and
5. Subjects under treatment with antibiotics.

At the initial examination, each subject received a complete periodontal examination, which included: modified gingival index8 (MGI), probing depth (PD), clinical attachment loss (CAL) and calculus severity index9 (CSI).

Armamentarium Used

2 ml syringe, 10 ml test tubes, spirit, cotton, ERBA liquixx calcium (arsenazo III method, end point).

METHODOLOGY

Two millilitres of blood was collected from the antecubital vein under aseptic conditions and then transferred into sterile test tube with anticoagulant in it to prevent coagulation of blood. Unstimulated whole saliva samples were collected following a brief rinsing of the mouth with water. The saliva samples were collected from the lower vestibular sulcus by a plastic syringe. Two millilitres saliva sample was collected. The samples were then transferred to the laboratory immediately for determination of calcium levels using Arsenazo III method, end point.

Calcium Estimation

Biochemical assays of saliva samples were carried out to quantify the calcium levels. The kit consisted of arsenazo III reagent and calcium standard.

Principle: Arsenazo III is chemically stable and has a very high affinity for calcium in a neutral pH range. In this assay system, Arsenazo III forms a blue Arsenazo III – calcium complex with an absorbance maximum at 650 nm. The concentration of calcium is proportional to the absorbance of the blue coloured Arsenazo III – calcium complex. Arsenazo III has a high infinity (ko =1 ×107) for calcium ions and shows no interference from other cations normally present in serum, plasma or urine.

Test parameters:

Wavelength: 650 nm
Temperature: 37°C
Sensitivity: the lower limit of detection is 0.04 mg/dl.

Reagents used:

Phosphate buffer, pH7.5
8- hydroxyquinoline-5-sulfonic acid
Arsenazo III
Detergents

RESULTS

In aggressive and chronic periodontitis group the mean age range was found to be 28.5±7.43 years and 41±6.50 years, respectively. The mean age range in the healthy group was found to be 34.2±5.71 years (Table 1). Comparison of modified gingival index among three groups was done. The mean MGI score in group A was 1.92, group B was 1.92 and group C was 0.50 (Table 1). The comparison of periodontal pocket depth (PPD), clinical attachment loss (CAL) and calculus severity index (CSI) are presented in Table 1. It indicates that high salivary calcium levels may be associated with calculus formation in periodontitis groups.

The normal serum calcium level in humans is 9–11 mg/dl. Serum calcium levels are increased in group A and B with a mean value of 10.20 and 10.10. There is
Comparison of saliva calcium levels between three groups showed the following results. (Table 3).

Table 3: Comparison of three groups (A, B, C) with mean saliva calcium scores by one way ANOVA.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>9.51±0.62</td>
</tr>
<tr>
<td>Group B</td>
<td>9.99±1.04</td>
</tr>
<tr>
<td>Group C</td>
<td>7.23±0.28</td>
</tr>
</tbody>
</table>

F-value 1.7718  
P-value 0.1892  
Pairwise comparisons by Tukey’s multiple post hoc procedures

<table>
<thead>
<tr>
<th>Comparing Groups</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A vs. Group B</td>
<td>0.3107</td>
</tr>
<tr>
<td>Group A vs. Group C</td>
<td>0.0036*</td>
</tr>
<tr>
<td>Group B vs. Group C</td>
<td>0.0041*</td>
</tr>
</tbody>
</table>

*p<0.05

Graph 2: Comparison of mean saliva calcium scores.

DISCUSSION

The present study was an attempt to estimate and compare serum and saliva calcium levels in healthy subjects and patients with periodontitis. In the current study, it has been elucidated that an increased level of serum and saliva calcium levels were observed in patients with periodontitis. Periodontitis is an inflammatory condition due to bacterial challenge, which leads to alveolar bone loss. Due to bone loss, there can also be loosening of teeth.

The serum calcium level is generally maintained within a narrow range of 9 to 11 mg/dl (2.25–2.75 mol/l), and the mean total serum calcium levels observed in our study were within the normal range. However, the total serum calcium was statistically significantly higher in periodontitis group when compared to healthy group. It has been evident that, low dietary intake of calcium results in bone loss to meet the needs of the body for normal functional activities. Low serum calcium promotes secretion of parathyroid hormone (PTH), resulting in bone resorption which might be a cause for increased serum calcium level. In periodontitis affected individuals, due to alveolar bone destruction there can be

Table 1: Comparison of age, modified gingival index, PPD, CAL, and CSI.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Mean±SD</th>
<th>Mean±SD</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>28.5±7.43</td>
<td>41±6.50</td>
<td>34.2±5.71</td>
</tr>
<tr>
<td>MGI</td>
<td>1.92±0.17</td>
<td>1.92±0.14</td>
<td>0.50±0.14</td>
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<tr>
<td>PPD</td>
<td>5.91±0.69</td>
<td>5.08±0.76</td>
<td>-</td>
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<tr>
<td>CAL</td>
<td>6.17±0.61</td>
<td>5.46±0.66</td>
<td>-</td>
</tr>
<tr>
<td>CSI</td>
<td>9.20±1.75</td>
<td>14.20±1.75</td>
<td>4.80±1.48</td>
</tr>
</tbody>
</table>

Table 2: Comparison of three groups (A, B, C) with mean serum calcium scores by one way ANOVA.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>10.20±0.72</td>
</tr>
<tr>
<td>Group B</td>
<td>10.10±0.53</td>
</tr>
<tr>
<td>Group C</td>
<td>9.34±0.21</td>
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</table>

F-value 5.6633  
P-value 0.0088*  
Pairwise comparisons by Tukey’s multiple post hoc procedures

<table>
<thead>
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<th>Comparing Groups</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Group A vs. Group B</td>
<td>0.7287</td>
</tr>
<tr>
<td>Group A vs. Group C</td>
<td>0.0025*</td>
</tr>
<tr>
<td>Group B vs. Group C</td>
<td>0.0091*</td>
</tr>
</tbody>
</table>

*p<0.05

Graph 1: Comparison of mean serum calcium scores.
increased serum calcium level. The obtained results were in accordance to the studies conducted previously by Nishida et al.\textsuperscript{1} and Pushparani et al.\textsuperscript{10}

The study was also undertaken to examine the relationship of salivary calcium, with regard to periodontal disease. Unstimulated whole saliva was collected from the lower lingual sulcus. A positive correlation between the level of calcium and the severity of disease was observed. The results showed that the subjects in the periodontitis group had the highest levels of salivary calcium which was statistically significant when compared to healthy group subjects. These results are consistent with the findings of Sewon et al.\textsuperscript{11} who found that there was a higher calcium concentration in the saliva in the periodontitis subjects as compared to the periodontitis free subjects. They opined that periodontitis affected subjects had a higher re mineralisation potential than individuals with no signs of periodontal disease. It seems that salivary calcium, due to its affinity for being readily taken up by plaque, is an important factor, with regard to onset of periodontitis.

Patients with aggressive periodontitis showed higher mean salivary Ca values (9.51) with a significant $P < 0.05$ when compared with healthy controls, while patients having aggressive periodontitis showed lower mean salivary Ca level, when compared with patients having chronic periodontitis. Sewon et al.\textsuperscript{12} 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.

In the present study, salivary calcium levels were correlated with calculus formation by using calculus severity index. The mean value of CSI of chronic periodontitis group is higher when compared to other two groups and it is also consistent with salivary calcium level. The mean CSI score of aggressive periodontitis group is 9.20 which is lower than chronic periodontitis group which can be correlated to low level of local deposits in that group and highlights the importance of quality of plaque.

**CONCLUSION**

Within their limits of the study showed a clear and a significant association between high serum and salivary calcium levels and periodontitis. It is therefore, suggested that monitoring for change in serum and salivary composition might be a useful tool to establish periodontal health status. Further studies may be required to determine calcium homeostasis with its regulatory mechanism in periodontitis patients.

**REFERENCES**