

## ORIGINAL RESEARCH

# Estimation of Salivary and Serum Calcium Levels in Smokers and Nonsmokers with Chronic Periodontitis

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## ABSTRACT

Studies in the past investigated the role played by minerals in the etiology and/or progression of periodontal disease for more than four decades. Various host defense factors are present in the saliva. It also influences calculus formation and thus periodontal disease. Recent studies have shown a significant relationship between dietary calcium, serum calcium, and periodontal disease. Smoking marks a major risk factor for both periodontal disease and several systemic diseases. So, the aim of the present study is to estimate salivary and serum calcium levels in smokers and nonsmokers with chronic periodontitis.

**Materials and methods:** The study comprised 30 subjects, equally divided into three groups of clinically healthy periodontium, nonsmokers with chronic periodontitis, and smokers with chronic periodontitis. Clinical measurements, saliva, and blood samples were obtained. Biochemical analysis for the estimation of calcium was performed. Blood samples were collected through venipuncture method.

**Results:** Comparison of mean serum calcium between the three groups by analysis of variance showed highly statistically significant difference ( $F = 23.92, p = 0.001$ ).

**Conclusion:** Smokers with periodontitis exhibited reduced levels of calcium as compared with nonsmokers with periodontitis and the differences were statistically significant.

**Keywords:** Calcium, Chronic periodontitis, Smokers and nonsmokers.

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## INTRODUCTION

Calcium is the most abundant minerals in humans. About 99% of the total calcium in the human body exists in the bones and teeth, providing a structural function; the

remaining 1% is found in tissues and fluids and is crucial for the maintenance of cell metabolism, nerve transmission, and muscle contraction.

Researchers have been exploring the role played by minerals in the etiology and/or progression of periodontal disease for more than four decades. Recent studies have shown a significant relationship between dietary calcium, serum calcium, and periodontal disease.

Confounding and effective modification are of increasing importance as periodontal research addresses putative associations between periodontal disease and systemic disease. This is especially pertinent when dealing with smoking, as smoking is a major risk factor for both periodontal disease and several systemic diseases.<sup>1</sup> However, evidence from physiological and clinical studies regarding the mechanism by which calcium and magnesium are associated with periodontal disease, adjusted for smoking habits, is lacking. The purpose of the present study was to evaluate the independent association between serum and salivary calcium, taking smoking habits into consideration.

## MATERIALS AND METHODS

The study comprised 30 subjects, equally divided into three groups of clinically healthy periodontium, nonsmokers with chronic periodontitis, and smokers with chronic periodontitis. Clinical measurements, saliva, and blood samples were obtained. The study was approved by Institutional Ethical Committee of Al-Badar Dental College and Hospital, Gulbarga, Karnataka, India. Written consent was taken from patients prior to commencement of the study.

### Inclusion Criteria

Age above 18 years; systemically healthy patients; and patients with pockets more than 4 mm.

### Exclusion Criteria

Systemic diseases using medications affecting salivary secretions and calcium levels; pregnant and lactating women with severe immune deficiency.

### Determination of Salivary Calcium

Calcium determination was carried out by BioMérieux (France) calcium kit using spectrophotometer end-point

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method and reading results by a spectrophotometer (CECIL 1021, England) at 650 nm wavelength.<sup>2</sup> The principle is dependent on the reaction of Arsenazo III, which reacts with calcium in a slightly acidic medium to form blue-purple complex. The intensity of the color is proportional to calcium concentration. The measurement of optical density (OD) was carried out at 650 nm against the blank. The calculation was done according to the following equation:

$$\frac{\text{OD of sample}}{\text{OD of standard}} \times \text{Standard concentration} = \text{Concentration of calcium in mmol/L}$$

## Blood Collection

An elastic band around subject's upper arm was wrapped to stop the flow of blood. It is easier to put a needle into the vein because the veins beneath the band appear larger. The needle site was cleaned with alcohol and the needle was punctured into the vein. More than one needle stick may be needed and a tube was attached to the needle to fill it with blood. The band was removed from the subject's arm when enough blood is collected. The normal values listed here, called a reference range, were just a guide. These ranges may vary from lab to lab. Normal blood calcium is 2.2 to 4.5 meq/L.

## STATISTICAL ANALYSIS

All the three group's salivary and serum calcium levels were reported as mean and standard deviation (SD) of mg/dL.

The statistical significance of differences in salivary and serum calcium levels between healthy smoking and nonsmoking groups were estimated by one-way analysis of variance (ANOVA) followed by Scheffe's multiple comparison tests.

A p-value of < 0.05 was accepted as significance.

## RESULTS

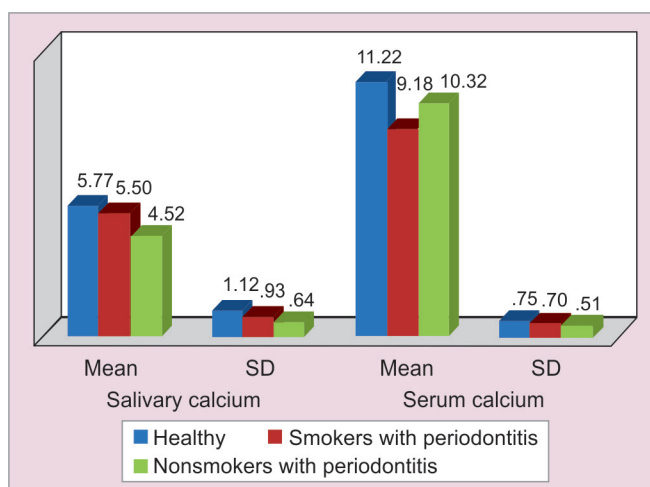
From Table 1, it is clear that the group 1 participants with clinically healthy periodontium had higher mean of  $5.76 \pm 1.11$  of salivary calcium followed by group 2 nonsmokers with chronic periodontitis ( $5.49 \pm 0.93$ ), and group 3 smokers with chronic periodontitis ( $4.52 \pm 0.64$ ). Further comparison of mean salivary calcium between the three groups by ANOVA showed statistically significant difference ( $F = 5.07, p = 0.014$ ).

**Table 1:** Comparison of salivary calcium among different study groups by ANOVA

Salivary calcium	n	Mean	SD	Std. error	F	Sig.
Group I	10	5.76	±1.11	0.354	5.05	0.014
Group II	10	5.49	0.93	0.294		
Group III	10	4.52	0.64	0.202		

**Table 2:** Comparison of serum calcium among different study groups by ANOVA

Serum calcium	n	Mean	SD	Std. error	F	Sig.
Group I	10	11.22	0.74	0.236	23.92	0.001
Group II	10	9.18	0.70	0.222		
Group III	10	10.32	0.51	0.161		



**Graph 1:** Mean and SD of serum and salivary calcium in different study groups

Similarly, group 1 study participants with clinically healthy periodontium had higher mean value ( $11.22 \pm 0.74$ ) of serum calcium followed by group 3 smokers with periodontitis ( $10.32 \pm 0.51$ ), and group 2 nonsmokers with chronic periodontitis ( $9.18 \pm 0.70$ ). Comparison of mean serum calcium between the three groups by ANOVA showed highly statistically significant difference ( $F = 23.92, p = 0.001$ ), as shown in Table 2. Mean and SD have been depicted in Graph 1.

## DISCUSSION

Salivary phosphorous and calcium have been associated with the deposition of tartar and formation of calculus over the teeth. Apparently, high level of salivary calcium is responsible for the resistance to dental decay. In comparison with other minerals, human body contains more calcium, as much as 1200 gm in a 70 kg adult. The most skeletal calcium is deposited in the form of hydroxyapatite  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ . Salivary calcium is related to plasma levels and under resting condition it is about 3 mmol/L.

Many previous reports indicated a significant association between smoking and periodontal disease. Nicotine derivatives are also known to be vasoconstrictive not only on peripheral vessels but also on coronary, placental, and gingival blood vessels.<sup>3</sup> Tobacco use may reduce the functional activities of polymorphonuclear leucocytes including chemotaxis and phagocytosis.<sup>4</sup>

Likewise, smoking also has significant systemic effects on plasma IgG levels.<sup>5</sup> Saliva is a major influencing

factor for plaque initiation, maturation, and metabolism. The composition of saliva and its flow influence the formation of calculus and periodontal disease.

Plaque is composed of inorganic components, predominantly phosphorous and calcium with trace amounts of other minerals, such as potassium and sodium. Primarily the source of inorganic components of supragingival plaque is from saliva. As the mineral components increase, the plaque mass gets calcified, which results in the formation of calculus.<sup>6</sup>

Sewón et al<sup>7-12</sup> with their series of studies have shown that oral mineralization potential of saliva plays an important role in periodontal health and disease. Mineralization-favoring factors are known to maintain the integrity of enamel surfaces, and intraoral mineralization capacity has been a matter of scientific interest for decades.<sup>13,14</sup>

In the present study, compared to nonsmokers lower content of Ca was observed in smokers in both serum and saliva. Except plaque index and gingival Index, there was no statistically significant difference in the mean levels of clinical parameters between the groups. These results are similar to the study conducted by Zuabi et al<sup>15</sup> and in contradiction to those obtained by Erdemir and Erdemir.<sup>16</sup> The differences could be attributed to the different techniques that were employed for biochemical analysis.<sup>17</sup>

## CONCLUSION

Smokers with periodontitis exhibited reduced levels of calcium as compared with nonsmokers with periodontitis and the differences were statistically significant.

More studies including prospective trials are necessary to understand the exact nature of the relationship between periodontal disease and serum calcium, taking smoking habits into consideration.

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