ORIGINAL RESEARCH

# Comparison between Serum and Salivary Albumin and Calcium Levels in Adolescent Age-group with Dental Caries

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## Abstract

**Aim:** The aim of our pilot study is to analyze the relationship between dental caries and albumin and calcium levels in serum and saliva in adolescent patients with varying caries experience as determined by their caries progression between the age-group of 9 and 19 years.

**Materials and methods:** A total of 20 subjects were selected based on their level of caries progression and divided into four groups of subjects, five in each as follows: group I—enamel caries, group II—dentinal caries, group III—pulpal caries, and group IV—control group. The saliva and serum were collected under standardized conditions from selected patients and analyzed for the presence of albumin and calcium, and then they were correlated to the same level in serum. The statistical analysis was done using the Chi-squared test.

**Results:** According to the present study, there is an increase in the levels of caries with a decrease in the levels of salivary albumin and calcium. Serum albumin and calcium levels were also found to be decreased in caries-prone individuals; hence, a significant correlation between serum and salivary albumin and calcium levels was found.

**Conclusion:** According to a review of the literature, we found an inverse relationship between the levels of albumin and calcium in serum and saliva with dental caries patients. So, it confirms the importance of albumin and calcium levels in inhibiting carrier progression. So it may be used as a biochemical indicator to evaluate the susceptibility of caries.

**Keywords:** Dental caries, Salivary albumin and calcium; Serum albumin and calcium.

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## Introduction

Saliva is the human body's easily accessible and noninvasive biofluid.[1] It is a heterogeneous fluid that continuously bathes the teeth' oral mucosa and maintains oral health. It contains proteins, glycoproteins, electrolytes, tiny organic molecules, and substances carried from the blood.[2] Saliva contains a wide range of biological components that aid in remineralization and shield the cementum, enamel, and dentin from the formation of caries. The quantity and makeup of secretions determine saliva's capacity to influence the development of dental caries.[3] However, until its quantity or quality is changed, it is rarely noticed. Various studies have been performed, and serum and salivary molecules (organic and inorganic) were contrasted and assessed to demonstrate their association, particularly with dental caries. Numerous investigations have established that enamel primarily shields against demineralization. by the inhibitory effects of protein and its substances like albumin, alkaline phosphatase, etc.[4,5] In addition, the inorganic components like calcium, phosphate, fluoride, etc., also play an important role in balancing the demineralization and remineralization process of the enamel, like during an acidic environment. Organic components also formed the pellicle formation on the teeth' surface to provide protection.[5,6]

According to recent data, saliva reflects several systemic changes in the body and is a good indicator of overall health. This synchronization with blood may aid in the evaluation of numerous parameters present in blood, and saliva may serve as a substitute sampling medium.

As a result, a lot of researchers have been motivated to look into the causes of dental caries as well as the protective mechanisms against it. One may anticipate that it will be a helpful indicator for oral diagnosis.

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Shahrabi et al. also reported that a number of organic and inorganic components found in serum and saliva, such as calcium, phosphate, fluoride ions, and bicarbonate buffer systems, may have protective effects.[6]

## Materials and Methods

This study was approved by the ethical committee RUHS Dental College and Hospital of the Dental Sciences, Jaipur, Rajasthan, India. A healthy individual was randomly selected from the Department

of Oral Medicine under the age-group between 9 and 19 years. Who fulfiled with the absence of any local or systemic illnesses that affected the salivary secretion in the mouth was a requirement for inclusion. The World Health Organization (WHO) criteria were used to determine the status of caries, and these criteria included and measured the decayed, missing, filled surfaces (DMFS)/decayed, filled, surfaces (dfs) score of caries patients.

Based on DMFS/dfs score, the caries group is divided into two groups:

- Patients with at least five decaying tooth surfaces who are currently in active care.
- Patients without caries whose teeth have a DMFS score of zero.

Patients who met the exclusion criteria for the trial included those with diabetes, radiation and gingivitis, systemic disease of the vital organs, history of long-term immunosuppressive medication use, and physical disability. Patients who met the inclusion criteria were chosen, and they were asked to provide a written agreement in which they were informed of the study's purpose and objectives as well as their role in it.

Based on their degree of caries progression, a total of 20 subjects were chosen (Department of Oral Medicine, RUHS College of Dental Sciences and Hospital, Jaipur) and divided into four groups of five subjects each—group I was for enamel caries, group II was for dentinal caries, group III was for pulpal caries, and group IV was a control group overseen by a single examiner.

Here, the patients were examined in natural light while one mouth mirror, dental explorer, and recommended radiograph were used. After using a soft bristle brush to clean and dry the teeth's smooth and occlusal surfaces, enamel caries were assessed using probing techniques. While dentinal and pulpal caries were evaluated radiographically. Patients with no cavities at all or with prior restorations comprised the caries-free group. Each patient had saliva and blood samples taken, and the contents of each were examined for the presence of calcium, alkaline phosphate, albumin, and total protein.

## Methods

Collecting of saliva samples in the morning, 1 hour after breakfast. Unstimulated saliva was collected in a sterile disposable plastic cup under standardized conditions by spitting method and stored at −4°C. Prior to 1 hour of sample collection, subjects were also advised to avoid brushing their teeth, drinking, and using mouthwash.

A blood sample was collected using a simple vial containing 5 mL of venous blood (without anticoagulant). Following the application of a tourniquet, take the appropriate aseptic measures. Blood samples were processed the same day, and serum analysis was done by a fully automatic biochemistry analyzer (RX Imola©).

After the centrifuge process was completed, the supernatant layer of saliva was diluted with normal saline up to 5 mL and analyzed by analyzer (Stat Fax 2000) (Fig. 1), using albumin and calcium kit (Merril diagnostic kit).

All the reagents are ready to use and are stable till the expiry date mentioned on the bottle label at 2–8°C.

## Procedure

A sample of the saliva and blood was centrifuged for about 15–20 minutes at 3000 rpm by a centrifuged machine, the REMI R-8C. A supernatant layer of saliva was collected and diluted with normal saline for up to 5 mL. Blood serum also separated from the plasm.


**Figs 1A to D:** (A) Stat Fax 2000; (B) Saliva samples; (C) Calcium test; (D) Albumin test

### Calcium Test Procedure

Kit consists of two reagents 1 and 2, and calcium standard concentration as stated on the label. The principal mechanism is that the substance o-Cresolphthalein complex combines with the calcium at alkaline pH to form a purple color complex, which is measured by the spectrophotometer at 578 nm. Here, three test tubes were labeled with blank, standard, and sample and, respectively, filled with 10 µL distilled water, 10 µL standard, and 10 µL sample diluted. Then, 0.5 mL of each reagent 1 and 2 were combined and incubated at 37°C for 1 minute. Based on the endpoint, compare the absorbance of the samples and the standard to the reagent blank.

### Albumin Procedure

Reagent 1 and albumin standard are included in the kit. The mechanism's basic idea is that the amount of albumin present is closely correlated with the amount of dye that bromocresol and albumin react to generate the bromocresol green-albumin complex at an acidic pH. Absorbance at 620 nm was determined by the spectrophotometer. The same method of calcium is followed to take three test tubes with blank, standard, and sample and, respectively, filled with 10 µL distilled water, 10 µL standard, and 10 µL sample diluted. Then, 1 mL of reagent was mixed and incubated for 1 minute at 37°C. Based on the endpoint, compare the absorbance of the samples and the standard to the reagent blank.

## Results

### Statistical Analysis

Primers and Statistical Package for the Social Sciences (SPSS) trial version 23 were the computer programs used for the statistical studies. The quantitative data were presented as mean and standard deviations, while the qualitative data were reported as proportions and percentages. The student *t*-test for parametric data was used to evaluate the difference in means between the groups, and the Chi-squared test was used to analyze the difference in percentage. A significance threshold of 95% ($p < 0.05$) was established for the tests.

Table 1 shows the serum and salivary albumin mean value, standard of deviation, and *p*-value as follows. Serum albumin values in this case are 4.35 ± 0.36 standard deviation (SD) for group I, 3.9 ± 0.48 SD for group II, 4.44 ± 0.16 SD for group III, and 4.24 ± 0.58 for group IV, with a *p*-value of 0.24 indicating statistical nonsignificance (*p*-value is significant and > 0.05).

Here, the salivary albumin value in group I mean value of 1.94 ± 0.19 SD, group II mean value of 1.38 ± 0.26 SD, group III mean value of 1.32 ± 0.35 SD, and group IV mean value of 2.11 ± 0.35 and *p*-value 0.006s which is significant statistically (significant *p*-value > 0.05). Figures 2 and 3 show the comparing value between the control and caries groups in serum and salivary albumin levels in various groups. Here, salivary albumin value in dental and pulpal caries shows a significant difference.

**Table 1:** Comparision of serum and salivary albumin levels in caries patients with the control group

| *Study group* *N = 20* | *Serum albumin (0.5–5 mg/dL)* | | | | *Salivary albumin (0.3–4 mg/dL)* | | | |
|---|---|---|---|---|---|---|---|---|
| | *Minimum* | *Maximum* | *Mean* | *±SD* | *Minimum* | *Maximum* | *Mean* | *±SD* |
| Group I enamel caries | 3.8 | 4.7 | 4.35 | 0.36 | 1.7 | 2.2 | 1.94 | 0.19 |
| Group II dentinal caries | 3.3 | 4.6 | 3.9 | 0.48 | 1 | 1.7 | 1.38 | 0.26 |
| Group III pulpal caries | 4.23 | 4.61 | 4.44 | 0.16 | 0.9 | 1.7 | 1.32 | 0.35 |
| Group IV control | 3.3 | 4.79 | 4.24 | 0.58 | 1.4 | 2.11 | 1.87 | 0.35 |
| *p*-value LS | 0.24 NS | | | | 0.006 S | | | |

S, significant [$p < 0.05$]; NS, non significant [$p > 0.05$]; LS, level of significant

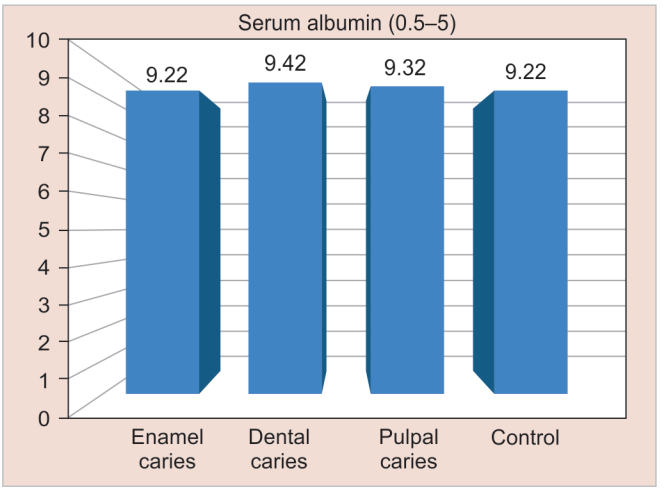


**Fig. 2:** Comparision of serum albumin level in between caries and control group

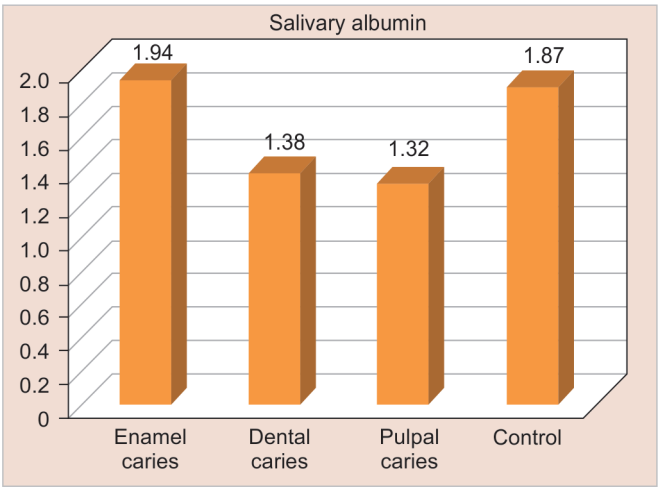


**Fig. 3:** Comparision of salivary albumin in between caries and control group

**Table 2:** Comparision of serum and salivary calcium levels in caries patients with the control group

| *Study group* *N = 20* | *Serum calcium (7–11 mg/dL)* | | | | *Salivary calcium (2–6 mg/dL)* | | | |
|---|---|---|---|---|---|---|---|---|
| | *Minimum* | *Maximum* | *Mean* | *±SD* | *Minimum* | *Maximum* | *Mean* | *±SD* |
| Group I enamel caries | 8.8 | 9.8 | 9.22 | 0.38 | 2.1 | 3.1 | 2.5 | 0.3 |
| Group II dentinal caries | 8.9 | 10.2 | 9.42 | 0.49 | 2 | 2.8 | 2.49 | 0.34 |
| Group III pulpal caries | 8.9 | 9.8 | 9.32 | 0.32 | 1.49 | 2.56 | 2.12 | 0.39 |
| Group IV control | 9.1 | 9.3 | 9.22 | 0.22 | 2.88 | 3.6 | 3.26 | 0.42 |
| *p*-value LS | | | 0.006 S | | | | 0.001 S | |

S, significant [$p < 0.05$]; NS, non significant [$p > 0.05$]; LS, level of significant

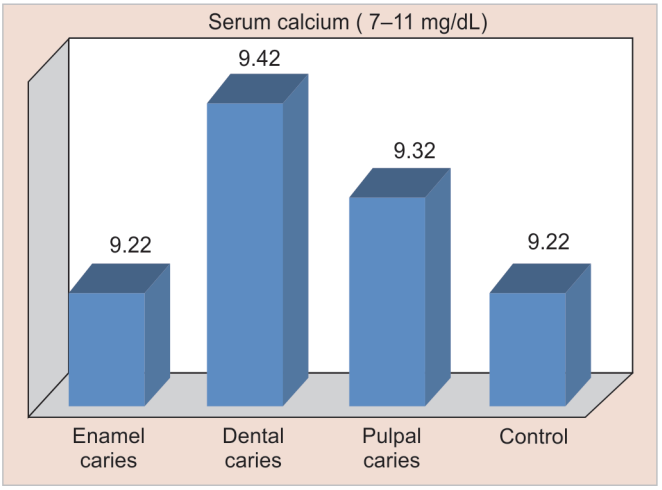


**Fig. 4:** Comparision of serum calcium level in caries and control group

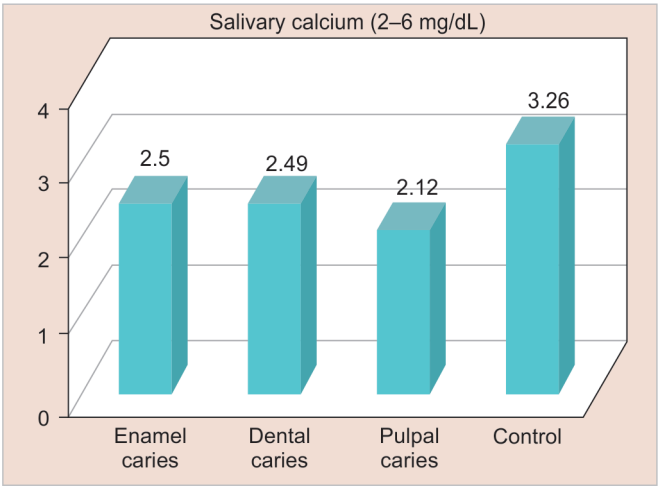


**Fig. 5:** Comparision of salivary calcium level in caries and control group

Table 2 shows the serum and salivary calcium mean value, standard of deviation, and *p*-value as follows. Here, the serum calcium value in group I mean value of 9.22 ± 0.38 SD, group II mean value of 9.42 ± 0.48 SD, group III mean value of 9.32 ± 0.32 SD, and group IV mean value of 9.22 ± 0.22 and *p*-value 0.0.006s which is significant statistically (significant *p*-value > 0.05).

Here, the salivary calcium value in group I mean value of 2.5 ± 0.3 SD, group II mean value of 2.49 ± 0.34 SD, group III mean value of 2.12 ± 0.39 SD, and group IV mean value of 3.26 ± 0.42 and *p*-value 0.001s which is also significant statistically (significant *p*-value > 0.05). Figures 4 and 5 show the comparison of the values between the control and caries groups in serum and salivary calcium levels in various groups. Here, salivary calcium value in enamel, dental, and pulpal caries shows significant differences.

## Discussion

Saliva has a crucial role in preserving the soft tissues and teeth.[1] It is a viscous, hypotonic fluid whose proteins and electrolytes regulate the microbiota's oral habitat and stop the tooth enamel from eroding. Albumin, the total protein in the oral cavity, is thought to be a serum ultrafiltrate to the mouth.[7,8]

Saliva contains a variety of proteins that have multiple functions, including albumin, histatins, cystatins, statherin, and acidic proline-rich proteins. Numerous studies have demonstrated their role in remineralization.[8] It has a buffering agent and a number of antibacterial components that work to preserve and protect the integrity of oral tissues.

A prooxidant-antioxidant balance that is out of balance is called oxidative stress. Proteins, albumin enzymes, and other antioxidants are found in lower amounts than oxidizable substrates, including glutathione peroxidase, uric acid, superoxide dismutase, and carotenoids. There is a dynamic equilibrium that is maintained in normal physiology. Oxidative stress occurs when this balance is shifted in favor of reactive oxygen species, either by a decrease in antioxidant defense or an increase in the synthesis or activity of reactive oxygen species. Thus, this is where dental cavities, plaque, calculus, and chronic periodontal illnesses begin to emerge.[2,9]

A sample of unstimulated saliva was obtained for our study. Due to the stimulated saliva, the composition of the saliva.

Stookey, in 2008, noted that saliva's composition can change when it's flow is stimulated.[10]

Anytime a white spot lesion with an intact surface layer and subsurface demineralization of the inorganic components is observed clinically. Thus, it displays the early caries lesion that is well-established and capable of being remineralized and re-established.[11,12]

Normal calcium levels vary from 6 to 7 mg/dL in unstimulated saliva to 7–11 mg/dL in serum. It is among the most effective buffers for controlling the pH of bodily fluids. It also has a significant impact on the mineralization and remineralization of tooth enamel. as opposed to phosphates, which are more resilient to the plaque's pH dropping to a crucial level.

The amount of calcium in saliva varied significantly in our investigation. In comparison to the caries-active group, it was discovered to be higher in the caries-free group. The comparison

of caries intergroups in enamel, dentin, and pulpal revealed a noteworthy distinction between the enamel and pulpal cries groups.

A 2006 study by Tulunoglu et al. likewise found that the caries-free group had higher calcium levels.[9]

Elevated protein levels in youngsters who are actively involved in caries can be the reason for their increased total antioxidant values. Thus, it follows that there needs to be a linear relationship between salivary antioxidant levels and total protein levels.[9]

Increased caries levels have been linked to both prenatal and postnatal nutritional deficits, according to some experimental research on the relationship between protein energy malnutrition and dental caries. It was proposed that enhanced enamel solubility was a mechanism underlying this increased susceptibility to caries. When considered collectively, the single Thai kid study conducted by Kanchanakamol et al. in 1996 revealed a favorable correlation between primary dentition caries, enamel hypoplasia, and malnutrition.[13–15]

A 1997 study by Robinson et al., in their opinion, unequivocally shows that intact albumin is present in enamel caries lesions *in vivo*. The lack of smaller species that cross-react suggests that either albumin is not greatly broken down inside the lesion or broken pieces either do not get into carious tissue or, if they do, do not stick to the mineral. When undamaged molecules are attached to a mineral, they might be largely shielded from deterioration.[16]

The results of a large-scale study that correlated the dental state with different biochemical tests in children indicate that the generation or prevention of dental caries is not largely influenced by blood calcium and phosphorus levels or acid-base relations. This also applies to the majority of the saliva's inorganic components. There seems to be a clear and significant relationship between the tooth's resistance to decay and how well calcium and phosphorus are retained. The research presented here provides no proof that phosphorus deficit in the diet is a significant contributor to dental caries or that various levels of acid-base imbalance brought on by a regular diet are to blame. They suggest that the organism's overall metabolic efficiency and the tooth's ability to fend against decay are closely correlated. They provide proof that the tooth's ability to fight decay is mostly determined by internal processes. Additionally, the data imply that optimal calcium and phosphorus retentions might be higher than those often regarded as sufficient.

Due to its endocrine, autocrine, and paracrine properties, vitamin D is linked to a wide range of biological processes.[17] Its purported roles involve controlling the metabolism of calcium and phosphate as well as their accumulation in mineralized tissues. Due to their crucial function in the development of teeth and bones, children and adolescents are especially susceptible to the clinical signs of vitamin D deficiency. In the basic intervention research, 206 children had their dental state examined, making 85 of them total. Around 28% of people had low vitamin D levels at baseline, compared to 11% following the intervention, and 34% of people said they were still taking vitamin D supplements. After adjusting for potential confounders, there was a slight reduction of the weak negative link between vitamin D status at age 6 and caries 2 years later (odds ratio 0.96; $p = 0.024$) according to logistic regression analysis. The correlation between low vitamin D levels and dental caries and between high vitamin D levels and dental caries-free status was validated by multivariate projection regression. Saliva LL37 levels were favorably correlated with vitamin D status at age 6, but it was unrelated to enamel abnormalities.

Antioxidant micronutrients are critical for reducing excessive production of cytokines as a consequence of extended immune response activation, in addition to minimizing oxidative damage and tissue damage.

When several antioxidants work together, they can fend off attacks from reactive oxygen or nitrogen species more effectively than when they work alone. Since the total antioxidant capacity takes into account the combined impact of all antioxidants found in bodily fluids, it may yield more biologically significant information than data acquired from measuring individual components. Children in both age-groups who were actively involved in dental caries had higher overall antioxidant capacities, according to our research. Increased antioxidant levels were also noted in the caries active group, according to a 2006 study by Tulunoglu et al.[9]

## Conclusion

A significant element that influences the development of dental caries has been identified by research, and it is known as saliva or the "bloodstream of the tooth." Additionally, saliva contains a number of enzymes that may affect dental cavities. Several salivary indicators have been used often, but not particularly successfully, to assess the risk of developing dental caries. An attempt was made in the current study to predict caries activity by relating serum and salivary calcium and albumin activity.

In the current investigation, we discovered that dental caries patients' serum and salivary calcium and albumin levels were inversely correlated. Thus, it confirms the significance of calcium and albumin levels in preventing the advancement of cardiomyopathy. Thus, it could be employed as a biochemical marker to assess the vulnerability to dental caries. However, more research in this area might be necessary.

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