

Correlation of Total Salivary Protein and Glucose in Children with Dental Caries Yielding *Candida* Species

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Abstract

Dental caries (DC) is a major oral health concern affecting a large population, primarily children. Children worldwide are affected by dental caries due to food habits or oral hygiene methods. It is seen that in healthy individuals, *Candida* species occur as normal commensal in the oral cavity. However, it becomes a potential opportunistic pathogen when associated with dental caries. Only a few studies have examined the variety of *Candida* species in children with dental caries. This study aims to evaluate the presence of *Candida* species in children with dental caries and correlate with total protein and salivary glucose present in those patients. 20 saliva samples were taken from children between 5 to 10 years of age. The sample was divided into two groups: Group 1 had Children with dental caries, and Group 2 had children without dental caries as control. The Biuret method was used to determine the salivary total protein concentration, and a modified calorimetric anthrone-sulfuric acid-glucose reaction was used to quantify the salivary total glucose content. Sabouraud dextrose agar medium was used to isolate and develop *Candida* colonies. Children with caries had higher salivary glucose content, total protein concentration, and even the prevalence of *Candida* species compared to children without DC. These salivary components may be employed as indicators for oral health based on the findings of these investigations. It is possible to evaluate additional etiological variables, salivary components, and enzymes for future research.

Keywords: *Candida* infections, Dental caries, Glucose, Oral health, Saliva, Total protein.

Introduction

Dental caries is a global healthcare burden affecting the overall quality of life. Globalization has been connected to rising sugar consumption and obesity in middle- and low-income nations, mainly due to changing dietary trends over the past few decades. Extensive research indicates that it is caused by a bacterial infection in the host body and poor nutrition (1). Saliva is the body's most powerful defence system, although it contains a variety of characteristics and proteins whose roles remain unknown. It is one of the essential biofluids that help in lubrication, swallowing, maintaining pH, taste, protection, cleansing, and digestion. The biochemical properties of saliva, specific gravity, and presence of anti-bacterial components like IgA, lysozyme, lactoferrin and the salivary peroxidase-hypo thiocyanate system are responsible for maintaining the oral microflora (2, 3). Saliva becomes an essential sample of choice,

particularly in paediatric dentistry, as its collection is non-invasive and painless, making it ideal for children. It also provides vital information on oral health and other systematic diseases like diabetes, microbial infections, and hormonal imbalances. Saliva is easy to process and preserve, making it an ideal choice of clinical sample for oral health diagnosis. Moreover, it provides information about children's dietary habits and nutritional deficiencies, which becomes crucial for treating oral diseases. It can also be used for genetic studies.

The WHO's efforts to reduce the prevalence of dental caries are hampered by the prevalence of the disease, which shows extreme differences across various factors and the need for more data on the topic. Research has been related to these characteristics of salivary composition to children's vulnerability to dental caries (4). Studies

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have shown significant differences in total protein and salivary glucose levels in children with Early Childhood Caries (ECC). Children with ECC had higher salivary glucose and total protein levels, demonstrating a relationship between salivary glucose and total protein in ECC. Thus, making it a risk indicator for dental caries and evaluating other etiological variables, salivary components, and enzymes for future research (5).

Major oral health issues include dental caries, most prevalent in socially disadvantaged communities. Primary school students around the world are affected. One of the most significant elements impacting a person's vulnerability to caries development is the quality and amount of their saliva (6). The healthy mouth of a person is frequently colonized by *Candida* species, which have several characteristics that affect the development of caries. *Candida* does not directly cause dental caries; however its presence and interactions in the oral cavity can influence oral health and may play a role in certain oral infections and conditions, particularly in immune compromised individuals or those with an imbalanced oral microbiota (7). These traits include the capacity for adhesion to tooth surfaces, the degradation of proteins and the extracellular matrix, colonisation of the oral mucosa, carbohydrate fermentation (contributing to oral acidity), and the production of extracellular enzymes, all of which increase the pathogenicity of the species. The sugar level of saliva is crucial for maintaining the balance of bacteria in the mouth cavity. Protein levels in saliva are crucial for maintaining homeostasis and good oral health. (7, 8).

Sugar exacerbates the symptoms and complicates treating oral candidiasis since it fuels and facilitates *Candida* development in the oral mucosa. Reducing sugar is crucial for controlling and avoiding these fungal diseases (7, 8). Those who consume large amounts of sugar, either through food or beverages like soda or juice, may find it more difficult to control and treat oral candidiasis successfully. Consuming sugar can

enhance fungal development and reduce the efficacy of antifungal therapies. Numerous studies have been done on pre-schoolers, but no studies have examined or correlated the amounts of salivary glucose and total protein with dental caries and those with healthy oral tissues. This study aims to evaluate the presence of *Candida* species in children with dental caries and correlate with total protein and salivary glucose present in those patients (7, 8).

Materials and Methods

A case-control prospective study was conducted on children, divided into two groups. The Group 1 study had children with dental caries; in Group 2, healthy children were controls between ages 5 to 15 years - presence of chronic illness oropharyngeal candidiasis oral bacterial infections as periodontitis Dental caries. Oral viral infections, refusal to participate, and children younger than five years were excluded from the study. The basic details of the participants from both groups were recorded in an Excel sheet, which included their age, gender, diet habits, frequency of milk intake, and sweet intake.

Sample Collection and Storage

The saliva sample was collected from the selected patients, and they were instructed to avoid any food intake at least 60 minutes before collection. The 2-3 ml of unstimulated saliva was collected in the Eppendorf tube and stored at 4°C until further investigation. It was made sure that the saliva samples were processed within 2 hours of the collection for microbiological and biochemical investigation.

Biochemical Evaluation

The total protein concentration in saliva was calculated using the Biuret method. Similarly, saliva's total amount of glucose was calculated using a modified colourimetric Anthrone-sulfuric acid-glucose reaction (Figure 1). A spectrophotometer and a colourimetric technique were used to calculate the total glucose and protein concentrations in both samples collected.



Figure 1: Principle of Glucose Estimation

Microbiological Evaluation

1 mL of saliva collected from each sample was homogenised with a vortex mixer. After that, phosphate buffer saline was used to dilute it. Both diluted and undiluted saliva samples were plated on Sabouraud Dextrose Agar (SDA) medium containing tetracycline to allow the formation of *Candida* colonies (Figure 2). After that, the plates

were incubated for 48–72 hours at 37°C. The expanding colonies were then sub-cultivated on Sabouraud Dextrose agar medium and kept for later identification after incubation. The isolated colonies were further subjected to a germ-tube test to identify *C. albicans* colonies.



Figure 2: Growth of *Candida* on Sabouraud Dextrose Agar

Table 1: Patient Details

Patient Details	Caries (Group A)	Non-Caries (Group B)
Age: Mean (Range)	7.5 (6-10)	6.5 (5-10)
Gender	Male= 5; Female= 5	Male= 7; Female= 3
Diet	Vegetarian= 0; Mixed Diet= 10	Vegetarian= 3; Mixed Diet= 7
Brushing Frequency	Once Daily= 8; Twice Daily= 2	Once Daily= 4; Twice Daily= 6
Frequency Of Drinking Milk	Once Daily= 8; Twice Daily= 2	Once Daily= 6; Twice Daily= 2
Does Not Drink=0		Does Not Drink= 2
Frequency Of Eating Sweets/Chocolates	More= 8; Less= 2	More= 2; Less= 8

Results

Table 1 summarizes the basic details of the participants from both groups. In Group A, which comprises the children with caries, 5 males and 5 females participated in the study, with a mean age of 7.5, and all the participants had mixed diets. Further, 8 participants brushed daily and took milk once daily, whereas 2 brushed twice and took milk twice daily. Similarly, 8 participants had high sugar intake in the form of sweets or chocolates, and 2 persons had controlled sugar intake. Likewise, the data from Group B was recorded. It comprised children with no caries; 7 males and three females participated in the study, with a mean age of 6.5.

Three participants had vegetarian diets, and 7 had mixed diets. Further, 4 participants brushed daily, and the other six brushed twice daily. Additionally, 6 participants took milk once daily, whereas 2 took it twice, and the remaining two did not have the habit of taking milk. Similarly, 8 participants had controlled sugar intake in sweets or chocolates, whereas two had high sugar intake (Table 1). *Candida* species were identified based on their colony characters based on their colony, which was creamy white colour on SDA. On germ-tube test, *C. albicans* is characterised by differentiating yeast cells into hyphal forms. In the Anthrone Test, glucose dehydrates after forming hydroxymethyl

furfural from a reaction with concentrated sulfuric acid. This furfural further interacts with anthrone to produce a complex that is bluish-green. The concentration of glucose in the sample increases with colour intensity. Similarly, in the biuret test,

the peptide interacts with dilute copper sulphate when the biuret reagent is applied to the sample, forming a dark purple material. An adverse outcome shows that there was no protein in the sample.

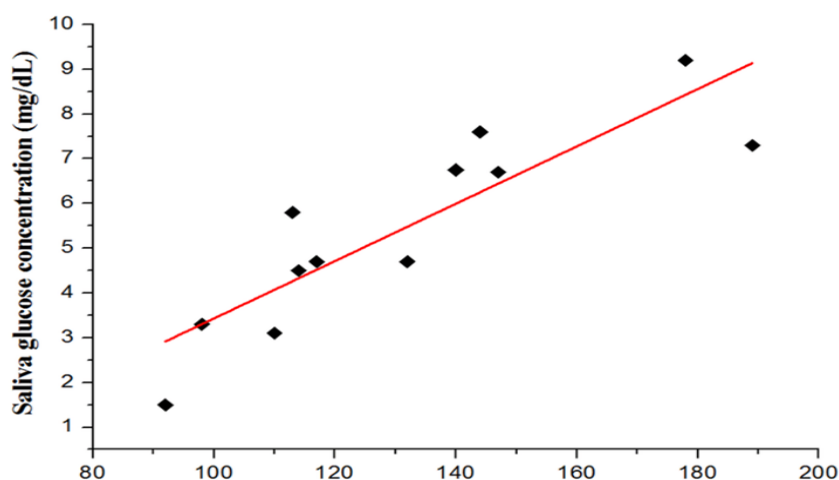


Figure 3: Salivary Glucose Estimation of Saliva Samples

In Figure 3 the black dots represent the non-*Candida* saliva samples, the green dots represent the *Candida* saliva samples, the saliva glucose concentration and the absorbance value obtained from the spectrophotometer. The glucose concentrations in the *Candida* samples were higher than those in the caries saliva samples, which did not result in the growth of *Candida* on SDA. Like Figure 3 the graph in Figure 4 shows the total protein content relative to the spectrophotometer's measured absorbance value, with orange dots denoting saliva samples free of *Candida* and red dots denoting samples that do. Therefore, the protein concentrations in the Caries

saliva samples with *Candida* growth were higher than those without caries.

Discussion

These findings demonstrate the potential utility of salivary glucose and protein concentration as a non-invasive oral health technique. Also, salivary proteins, such as proline-rich proteins and mucins, as well as oral epithelial transglutaminase, can either promote *Candida albicans* growth by encouraging adherence to oral tissues or inhibit growth through immune exclusion by binding and aggregating fungal cells to facilitate their clearance by swallowing (9).

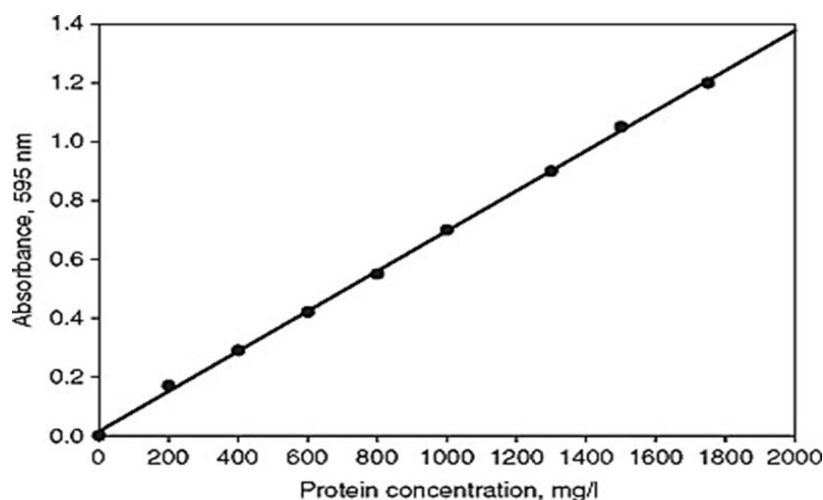


Figure 4: Total Protein Estimation of Saliva Samples

Candida species can modify the immune response, particularly in dysbiotic conditions. It is well established that higher sugar levels lead to dysbiosis in the oral cavity, impairing immune cells and leading to further infections. Similarly, high glucose levels lead to the glycation of salivary proteins, which alters their structure and functions and decreases their antimicrobial properties (7, 8). Lenander *et al.*, examined the potential connection between salivary glucose levels and tooth caries. The findings revealed increased salivary glucose levels and the dental caries index. Additionally, the rate of dental caries increased with advancing age. It has been related to maternal dental health and child nutrition (10). Dental caries is a complicated process influenced by internal and exterior factors, including diet, microbial flora, dental hygiene, and fluoride. Internal factors include saliva, the morphology of the tooth surface, general well-being, and hormonal and dietary states. Saliva serves various purposes, including guarding the oral mucosa and tooth surfaces. The concentration of secretory immunoglobulin A (SIgA) in saliva has been measured in some research on salivary composition. These studies' findings revealed that children with ECC had considerably greater salivary SIgA levels than healthy participants (11). According to a study by Soriano *et al.*, salivary glucose levels fluctuate throughout time depending on the person's level of diabetes rather than staying constant. According to a study, individuals with poor metabolic control were more likely to develop dental caries (12).

Research by Cury *et al.*, (13) found that plaque generated in sucrose was significantly more cariogenic than in the presence of both fructose and glucose. Further research is needed to determine how additional sugars in saliva affect the development of dental caries in children with ECC. Studies have found a highly significant link between the prevalence of caries in pre-schoolers and salivary and microbial proteins, such as PRP, IgA, IgG immunoglobulins, and histatin peptides, compared to those without caries. As a result, these salivary components could be employed as ECC biomarkers (13).

In research by Pitts N, other salivary protein components did not correlate with predicting caries risk (14). Because saliva has numerous structural and function redundancy levels, there is probably no link between salivary proteins and

tooth decay. It has been proven that dental caries and total salivary protein content are related in kids with ECC. More research is needed to determine the value of total salivary protein concentration as a predictor of young children's caries risk (14). Similar research was done on *Candida albicans*' prevalence and *Candida* non-*albicans*' carriage in preschool children's saliva based on their caries status (6). This study aimed to identify correlations between the prevalence of *Candida* infection, the variety of *Candida* species present, and the caries statuses among pre-schoolers (15). A single professional examiner evaluated 61 children between the ages of two and five. The International Caries Detection and Assessment System II (ICDAS) requirements divided them into the caries-free, moderate caries, and severe caries groups. To determine the *Candida* carriage rates, saliva samples were taken from each group's participants and plated on Sabouraud agar plates. The preliminary screening was conducted using Chrome agar *Candida* medium. To distinguish between the various *Candida* species in the samples, biochemical tests or CR/sequencing were used. The differences were considered significant if the p-value was less than 0.05 (15).

Candida carriage rates and the number of species of this fungus were higher in the group with the most severe levels of caries ($p < 0.05$). *Candida dubliniensis* and other unusual species of *Candida* non-*albicans* were only found in children with the most caries. However, the most common *Candida* species found in all children's saliva was *Candida albicans*. When some species of this fungus are present in their saliva, and there is a high rate of *Candida* carriage in pre-schoolers, their dental caries seem to be more severe (such as *C. albicans* and *C. dubliniensis*) (16).

Due to its prevalence, dental caries is one of the most widespread disorders. With the interaction of microbes, dietary variables, and salivary composition, it is frequently viewed as a complex disease. This study showed a link between oral *Streptococci* and *Candida* sp., particularly *C. albicans*, and a high rate of dental caries in young children, allowing it to cling to the enamel pellicle. Saliva contains crucial proline-rich proteins that serve as receptors for *C. albicans*, as O Sullivan *et al.*, established (17, 18). A carbohydrate-rich diet provides a rich supply of nutrition for *Candida*,

which undergoes fermentation and creates acidic by-products. As a result, these microbes maintain salivary pH below neutral, which is linked to dental cavities. All the evidence points to the colonization and persistence of *C. albicans* in the mouth. However, it is still unclear which factors, based on the standard metabolic functions carried out by the dominant bacteria linked to dental caries, are functional contributors to *Candida* and which factors directly contribute to the pathogenesis of dental caries (19-21). Hence, all studies prove the role of salivary protein and sugar as an additive effect of *Candida* infection in children with dental caries. However, some critical factors must be considered before establishing a diagnostic and treatment protocol for children suffering from dental caries. They are dietary habits, oral hygiene practices, fluoride exposure, genetic factors, systematic disease meditations, and socio-economic status. These variables are critical for accurately assessing the reason for dental caries in children (7, 8, 22).

Conclusion

A significant difference in total protein and glucose levels in saliva was found in children with dental caries, demonstrating the relationship between total protein and salivary glucose and offering a potential risk indicator for dental caries. Additionally, *Candida* has an ecological impact on the development of caries. Sugar consumption may be advised because a high-sugar diet encourages *Candida* growth. More *in situ* and *in vivo* caries models must be developed to understand how and when *Candida*'s contributing aspects are expressed during caries' onset and development.

Abbreviations

SIgA: Secretory immunoglobulin A

SDA: Sabouraud Dextrose Agar

Author Contributions

SP, SRP, and SR conceptualized the study. SP and SR conducted the study and collected the data. SRP and SR analysed the results and drafted the manuscript. SP and SRM critically evaluated and finalized the manuscript.

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Conflicts of Interest

None.

Ethics Approval

This study was conducted after the approval of the Institutional Ethical Committee, IDS, with reference no IEC-IDS/IDS/SOA/2023/I-02, dated 10th October 2023.

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