ORIGINAL RESEARCH

Estimation and Association of Total Protein Concentration with Early Childhood Caries in 3–6-year-old Children: A Randomized Clinical Trial

Umapathy Thimmegowda[1], Soumya Pai[2], Nagarathna Chikkanarasaiah[3], Aishwarya Nanjappa[4]

Abstract

Background and objective: Caries is a common problem witnessed in children, early childhood caries (ECC) is the most predominant chronic disease which not only leads to distress and pain but also poor quality of life, thus affecting the overall well-being of children. Salivary protein plays a vital part in monitoring health status or disease. It was stated that the salivary proteins could regulate the equilibrium of oral health, preserve a stable ecosystem, and constrain the growth of cariogenic bacteria.

Aim: The aim of this study is to estimate the total protein concentration in saliva and its correlation to ECC.

Materials and methods: A total of 20 patients with ECC in the age-group of 3–6 years were selected as the experimental group and 20 patients without caries for the control group. Unstimulated saliva samples were collected and subjected to spectrophotometry. The data obtained was subjected to statistical analysis. Independent student's *t*-test was used for the comparison of mean salivary pH between the caries group and the control group. Mann–Whitney test was used for a comparison of salivary total protein concentrations between the two groups.

Results: The mean pH of the carious group showed a statistically significant slightly lower value than that of the noncarious group. On the contrary, the mean total protein concentration of the carious group presented a statistically significant higher value than that of the noncarious group. Age-wise comparison of mean salivary proteins in the carious group and the noncarious group showed an increase in the protein concentration in the children aged 4 years or younger.

Conclusion: Based on the results of this study, it can be concluded that there is a strong association between the total protein concentration in saliva and ECC. There exists a significant increase in the total protein concentration in children with ECC. As age increases, total protein concentration decreases with age.

Clinical significance: Total protein concentration and particular protein estimation and quantification help us in assessing the risk of caries in children at the earliest and prevention of caries through preventive measures. Estimation of total salivary protein concentration in children can be a marker for ECC in children.

Keywords: Early childhood caries, Saliva, Total protein concentration.

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Introduction

Caries is a common problem witnessed in children, early childhood caries (ECC), is the most predominant chronic disease which not only leads to distress and pain but also poor quality of life, thus affecting the overall well-being of children.

One of the most complex and varied microbial habitats is the mouth cavity.[1] A crucial role for salivary protein is in the surveillance of disease or health conditions. It was claimed that salivary proteins may maintain a stable environment, control the development of cariogenic bacteria, and balance dental health.[2] Many proteins found in saliva, including lysozyme, lactoferrin, and salivary peroxidase, are known to have a role in the maintenance of soft tissues and the defense against oral infections. These proteins influence plaque and bacteria in different ways, either directly or indirectly, modifying the tooth's vulnerability to dental caries.[3]

Saliva, despite being the strongest defense system, still has a wide array of properties and proteins whose role is yet not clearly known. Current caries research seeks to identify risk factors as well as natural oral defenses that may protect against or prevent caries development.

The first line of defense in the oral cavity involves not only the well-known major salivary glycoproteins, such as mucins, proline-rich glycoprotein, and immunoglobulins, but also a variety of smaller salivary (glyco) proteins, such as lysozyme, lactoferrin, agglutinin, and cystatins.[4] There appears to be a significant overlap in functionality between all of these proteins and peptides because they show a wide range of antibacterial action. This could explain the finding that the concentration of

[1–3]Department of Pediatric and Preventive Dentistry, RajaRajeswari Dental College and Hospital, Bengaluru, Karnataka, India

[4]Department of Pediatric and Preventive Dentistry, RajaRajeswari Dental College and Hospital, Bengaluru, Karnataka, India

Corresponding Author: Umapathy Thimmegowda, Department of Pediatric and Preventive Dentistry, RajaRajeswari Dental College and Hospital, Bengaluru, Karnataka, India, Phone: +91 9986478744, e-mail: umapathygowda@gmail.com

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a single component does not appear to be associated with the susceptibility to oral illnesses. Although the precise cause of this "redundancy" is unknown, a number of factors could be at play.[4] So the quantification of total proteins in saliva and its correlation to ECC is necessary. Further identification of each protein responsible for ECC is advised.

A subfield of electromagnetic spectroscopy called spectrophotometry measures a material's transmission or reflection characteristics quantitatively as a function of wavelength. Photometers, sometimes referred to as spectrophotometers, are used in spectrophotometry to measure the intensity of a light beam at various wavelengths. A technique called spectrophotometry relies on the quantitative examination of molecules based on the amount of light that colored compounds absorb.[5] The most used colorimetric technique for figuring out a solution's protein level is Lowry's assay. It was the most popular technique for estimating the protein content of biological materials. Copper ions in an alkali solution are used as a pretreatment for the proteins, followed by the acid found in the Folin reagent. Lowry's assay is 100-fold more sensitive in determining the absorbance when compared to the other methods such as the Biuret method.[6]

Numerous researches have examined the connection between total salivary protein content and dental caries; however, there are few studies conducted on children who are at high-risk for dental caries.[7] According to a recent analysis, there is still much to learn about the relationship between a variety of salivary variables and dental caries. A study reported higher levels of total salivary proteins in a caries-free group compared with a decayed, missing, filled teeth (dmft) group suggesting an effective protective function of salivary proteins.[7]

Additional research showed that children's caries activity was correlated with an increase in protein concentration.[8] The total salivary protein content did not differ statistically significantly between children with and without ECC, according to several additional studies.[9] There is unclear evidence linking the total salivary protein concentration with dental caries in kids with ECC. This hypothesis of increase or decrease of total salivary proteins in caries-free and carious children is still questionable. Therefore, the purpose of this research is to determine the total protein concentration in saliva and its correlation to ECC in children.

Materials and Methods

Study Design

The current study included 40 participants in total, ranging in age from 4 to 6 years, who were enrolled from September 2020 to 2021. The study sample was divided into the experimental group (caries group) which consisted of 20 patients and the control group (caries-free) also consisted of 20 patients each. Randomization was achieved by using the computer-generated allocation. The inclusion criteria included children with the presence of at least four or more carious lesions, children of 3–6 years of age who consented to the study, and children with no caries. Exclusion criteria included children with any medical conditions, children taking any medication, or children with special healthcare needs. The institutional ethical approval to conduct this study was obtained from reference no RRDCH/IEC21/61. Informed consent was taken from each caregiver before considering the children in the study. The study was double-blinded and the participants and data analysts were unaware of the interventions.

Dental Examination

The same examiner performed a comprehensive dental examination on each participant to assess their oral health using sterile, disposable diagnostic instruments. The experience with dental caries was evaluated in accordance with the 2013 World Health Organization (WHO) criteria.[10] The decayed, extracted, filled teeth/decayed, missing, filled teeth (deft/DMFT) indices were computed independently for each child, where (d/D) denoted a carious tooth, (e/M) a tooth extraction or loss owing to caries, and (f/F) a filling in primary or permanent teeth due to caries, respectively.

Saliva Collection

To lessen the impact of circadian fluctuation, unstimulated saliva was collected 2 hours after breakfast, between 10 and 11 am, following the dental check-up. The kids were instructed to fill a graded Eppendorf tube with their saliva while sitting straight and with their heads slightly inclined. In order to prevent protein degradation, the samples were kept at –20°C until they were needed, which was not >1 week. Every sample that was gathered was taken to the dextrose lab for additional analysis.

Saliva pH Measurement

After collection, all samples were transported to the laboratory for pH estimation. The Labman digital pH meter, which was placed atop a stable base stand, was used to test pH directly (to eliminate discrepancies in the values owing to handling movements). With its long, narrow stem and double junction gel-filled electrode, the pH meter allows for the measurement of small volumes of substances in tiny vials. The electrode was submerged in the sample within a sealed container, and after a few seconds, for the digital reading to stabilize, the salivary pH value was determined based on the last stable reading. The data collected were subjected to statistical analysis.

Protein Estimation

The stock solution and dilutions for the standard curve were prepared. The Lowry solution was brought to room temperature. The solution was vortexed well to mix and 0.5 mL of it was transferred to a 10 mL glass tube. A titration of 1,000 µL was performed. After adding 0.7 Lowry solution, the tube was left to stand at room temperature in the dark for 20 minutes. Folins phenol reagent was made with bovine serum during the final 5 minutes, and 0.1 mL was added to each tube. It was incubated in the dark at room temperature for at least 30 minutes or longer. The Beckman DU-68 spectrophotometer was turned on to warm up and stabilize. The samples were vortexed after 30 minutes and 1.3 mL of the samples were transferred to semimicro disposable curettes. At 660 nm, using the quantitative mode, the absorbance values of the samples were recorded. Readings were recorded for each sample.

Statistical Analysis

The 2013 release of Version 22.0 Armonk, New York: IBM Corp of the Statistical Packages for Social Sciences (SPSS) for Windows was used to statistically evaluate the data that had been gathered. The study employed the Independent student's *t*-test to compare the mean salivary pH levels of the caries and control groups. The concentrations of salivary total protein in the two groups were compared using the Mann–Whitney test.

Results

The mean age and standard deviation (SD) were 4.40 ± 0.99 and 4.30 ± 0.92 for caries and control groups, respectively ($p = 0.74$) (Fig. 1). The mean pH of the carious group presented a statistically significant slightly lower value than that of the noncarious group, 6.7 and 7.2, respectively with $p < 0.01$ (Table 1). On the contrary, the mean total protein concentration of the carious group presented a statistically significantly higher value than that of the noncarious group, 748 and 254, respectively with $p < 0.01$ (Table 2). Age-wise comparison of mean salivary proteins between noncarious (Fig. 2) and carious groups (Fig. 3) showed that there was an increase in the proteins in the children aged 4 years or younger.

Discussion

Saliva serves as the mouth cavity's natural defense mechanism and is crucial for shielding the tooth surfaces that are exposed. Through basic mechanical rinsing, antibacterial activity, buffering ability, calcium phosphate binding proteins, immunological surveillance, and the release of antimicrobial peptides, saliva can restore the demineralization of the exposed tooth surface.[11] It has been explained that salivary proteins have a dual function, acting as a protective mechanism based on the site, location, and action of the microorganism that colonizes them. This results in the production of mucin, which acts as a barrier against the development of caries and keeps the tooth from drying out.[12]

Saliva fractions have been employed in the majority of research describing the protein components of saliva. Samples are taken straight from the salivary glands, such as the parotid glands or minor salivary glands, and then parotid, entire saliva, and crevicular fluid are compared. Whole saliva is significant to study because it covers teeth naturally and because it is a uniform mixture of secretions from both the major and minor salivary glands. This allows for the observation of a wide range of biological and physiological properties of the saliva as well as population-specific variability.[13] In recent years, research using entire saliva has been recommended for the reasons outlined above.

A mixture of mucous and serous fluids, unstimulated saliva is mostly produced by the submandibular and minor salivary glands.[14] Saliva that has been stimulated differs greatly from saliva that has not, in terms of flow rate, percentage input from different glands, and protein composition. The saliva flow rate rises significantly in

Table 1: Showing comparison of salivary pH levels between carious and noncarious groups

Comparison of mean salivary pH between two groups using independent student t-test					
Group	*N*	*Mean*	*SD*	*Mean difference*	*p-value*
Noncarious	20	7.21	0.37	0.42	<0.001*
Carious	20	6.79	0.46		

* is statistically significant at $p < 0.005$

Table 2: Showing comparison of mean salivary total protein concentration between carious and noncarious groups

Comparison of mean salivary total protein concentration (in µg/mL) between two groups using the Mann–Whitney test					
Group	*N*	*Mean*	*SD*	*Mean difference*	*p-value*
Noncarious	20	254.24	83.54	–494.21	<0.001*
Carious	20	748.46	76.50		

* is statistically significant at $p < 0.005$

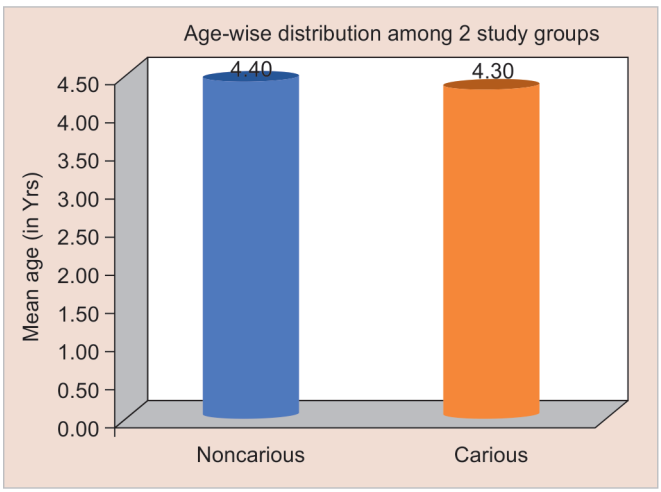

Fig. 1: Showing age-wise distribution among study groups

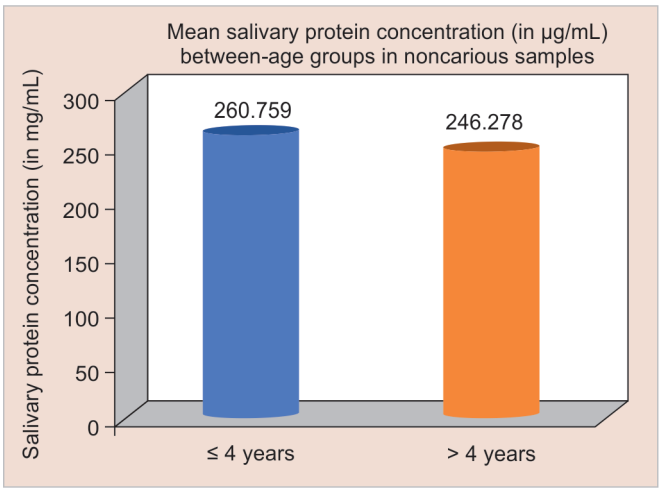

Fig. 2: Showing mean salivary protein concentration between age-groups in noncarious samples

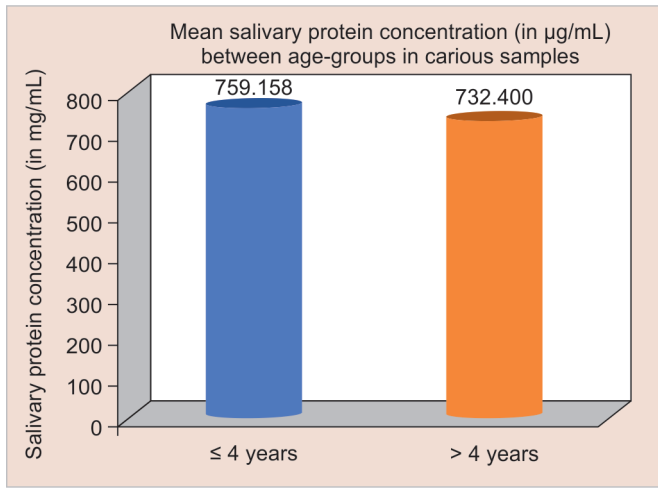

Fig. 3: Showing mean salivary protein concentration between age-groups in various samples

response to stimulation. Proteins, sodium, chloride, bicarbonate, and calcium concentrations rise with stimulation, while those of magnesium phosphate, urea, ammonia, and uric acid fall. Moreover, stimulation raises pH.[15]

Thus unstimulated saliva was taken in this study to quantitate the protein concentration to avoid varying results that increase in stimulated saliva.

The hydrogen bicarbonate balance in saliva determines both the pH and buffering capacity of saliva. Saliva has a pH of roughly neutral, which is maintained by buffering agents such as inorganic phosphate in resting saliva and the carbonic acid-bicarbonate system in stimulated saliva. Dental caries is caused by organic acids produced by bacteria fermenting carbohydrates, which dissolve minerals from the tooth surface. As a result, a pH drop to <7 is observed during caries. This interferes with the ion exchanges leading to the demineralization of enamel.[16] In our study the mean pH of the carious group showed a statistically significant slightly lower value than that of the noncarious group which is in comparison with the study done by a study that showed similar results where there was an increase in the pH in noncarious groups.[17] Children without dental caries produced saliva with a slightly higher pH level than children with severe dental caries, according to another study.[18]

The estimation of total protein concentration in the saliva of children with ECC can be a predictor of risk in children. So the study on the estimation of total protein concentration was done and found that the mean total protein concentration of the carious group presented a statistically significant higher value than that of the noncarious group, in accordance with our study another study, concluded that total protein concentration rises with proportion to the number of carious lesions.[19] Biological variability can explain the observed varying results of increased protein concentration with caries. Salivary protein concentration can vary depending on the source of saliva; higher salivary flow results in lower concentrations, higher concentrations, the state of stimulation, salivary flow, or circadian variation.[20]

Age-wise comparison of mean salivary total protein between carious and noncarious groups showed an increase in the protein concentration in the children aged 4 years or younger. Supporting our study, a study stated that as age increases, total protein concentration decreases.

In contrast to our research, another study found that although the mean values of amylase activity, total protein concentrations, and total immunoglobulin M (IgM) were comparable between the groups, children with ECC had considerably greater levels of total salivary IgA and IgG.[20]

Although a study found that women typically had greater total protein concentrations than males, our study did not compare results based on gender. Studies comparing youngsters based on gender have not yet been conducted. Since the identification of proteins necessitates the employment of particular antibodies and other necessary reagents, the scope of the study did not allow for this. Finding them is what needs to be done next to finish this study. Such research will make it possible to characterize the salivary components and their interactions in greater detail, which will be extremely helpful in determining the impact of salivary components on a dynamic, complex process like dental caries and aid in the hunt for therapeutic interventions to help manage the condition.

Conclusion

The study's findings indicate that there is a substantial correlation between ECC and saliva's total protein content. Children with ECC have a significantly higher concentration of total protein. The concentration of total protein decreases with aging. Since ECC is a complex disease, it is not possible to attribute its cause to a single risk factor. Nevertheless, more knowledge about the molecular epidemiology of salivary proteins may encourage the application of this technology as a diagnostic tool for early childhood oral health issues, including ECC.

Clinical Significance

Knowing the total protein concentration and particular proteins in situ helps us in assessing the child's caries risk and preventing caries through preventive measures. Estimation of total salivary protein concentration in children can be a marker for ECC in children.

Orcid

Umapathy Thimmegowda https://orcid.org/0000-0003-2426-5057
Aishwarya Nanjappa https://orcid.org/0000-0002-9879-8705

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