Comparison of Salivary Protein Profile and Dietary Habits in Children With and Without Early Childhood Caries

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Authors’ contributions

This work was carried out in collaboration among all authors. Author DR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PR and RG managed the literature searches and analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background and objectives: The purpose of this study was to evaluate the relationship between the total salivary protein concentration and dietary habits in Caries-free, Early childhood Caries and Severe Early Childhood Caries children.

Materials and methods: The present study included Thirty children who were divided into three groups. They were further divided into Caries-free, Early childhood Caries and Severe Early Childhood Caries with 10 children in each group. Information was collected regarding oral hygiene practices, feeding habits and dietary pattern through a face-to-face interview with the parent/caretaker of the child. Unstimulated saliva was collected and total protein concentration was estimated. Multivariate logistic regression analysis was performed to assess the relative contributions of dietary and feeding patterns between children with and without ECC. ANOVA was done to identify the difference in mean protein concentration between the groups.

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1. INTRODUCTION

The American Academy of Pediatric Dentistry (AAPD) defines Early Childhood caries (ECC) as the presence of one or more decayed (non-cavitated or cavitated), missing (due to caries), or filled tooth surfaces in any primary tooth in a child 71 months of age or younger [1]. It is a multi-factorial disease with clinically manifested initially as decalcification of dental hard tissues. Improper feeding patterns and bacteria are considered as a major cause for the carious lesion. Though dietary management remains as a barrier to control the development of ECC, there is a need to identify the other possible intrinsic etiological factors, namely salivary factors to develop an individualized preventive measure in near future.

Saliva is a unique biologic fluid containing numerous immunologic and non-immunologic components, which function together to prevent the development of dental caries. Saliva is secreted at a rate from 0.3 ml to 7 ml/min [2,3]. The volume of the saliva and its components depend on activity of the salivary gland, stimulation of the salivary gland, physiologic stimuli, circadian rhythm, age and gender of the patient [4-13]. The important functions of saliva include lubrication of oral cavity, preserving the soft tissue from abrasion during mastication, promotes digestion of carbohydrates, mechanical flushing to remove food debris, maintains remineralization and demineralization cycle by chemically maintaining pH, calcium and phosphate levels and salivary proteins and immunoglobulins exhibit antibacterial action by neutralizing bacterial toxins and enzymes [14]. Though saliva contains numerous salivary components, each has more than one protective function and all don’t contribute to same functional role. For instance, statherin and Proline rich proteins inhibits calcium phosphate precipitation, whereas histatins and mucins plays a lesser role in in inhibiting precipitation [15].

Salivary gland secretion varies according to individual’s diet patten and studies stated that species with different dietary habits showed a variation in their salivary proteomes [16,17]. Studies found that children with protein calorie malnutrition had reduced level of Salivary Immunoglobulin A levels (IgA) and increased rate of caries [18,19]. Lamy E et al and de Costa et al found that the amount of polyphenols and tannins in food have been linked to the changes in salivary glands and in the amounts of particular salivary proteins (e.g., proline-rich proteins and alpha amylase) [20,21]. In addition, Shaw and Griffiths conducted an animal study and inferred that rats fed with less protein diet during pregnancy and lactation developed tooth that were more prone to caries than the rat that was fed with high protein diet [22]. Furthermore, studies found that intake of milk products increase the saturation of calcium and phosphate in saliva and helps in remineralization of teeth [23]. Hence diet is indirectly related to the level of salivary proteins and rate of remineralization of tooth.

Literature provides little evidence for the existence of naturally acquired immunity to dental caries [24]. A longitudinal study done by Li Y et al had stated that 94% of children between 3-5 years of age, who had caries in their primary dentition developed caries in their permanent teeth 8 years later [25]. Levine M et al had stated that, differences between a cariogenic and noncariogenic microbiota could be due to variations in intrinsic immunity proteins expressed from an individual’s genome. Similarly, many children who consume cariogenic diet doesn’t develop dental caries, explaining the importance of other salivary factors namely organic and inorganic content of saliva plays an

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**Results:** Risk factors specific to the ECC group were Breast feeding <24 months (OR 2.1, 95% CI, 0.35-13.0), bottle feeding (21.0, 95% CI, 2.8-15.7), snacking more than 3 times per day (OR 1.22, 95% CI 0.28-5.73), consumption of less protein diet (OR 4.1, 95% CI, 1.99-8.81), consumption of less milk products (2.33, 95% CI, 1.67-6.77). Results also revealed that there was no statistically significant difference in total protein concentration between Caries-free, Early childhood Caries and Severe Early Childhood Caries group.

**Conclusion:** Within the limitation of this study, we can conclude that, Breast feeding less than 24 months, bottle feeding, snacking more than thrice per day, consuming low protein diet and low milk products had a high risk of developing ECC. There was no significant difference in the mean protein concentration between caries free, ECC and S ECC group.

**Keywords:** Saliva; total protein; early childhood caries.
imperative role in development of ECC in children. Salivary proteins and peptides are important in maintaining oral health and homeostasis, as many of the oral diseases are associated with qualitative and quantitative variation in salivary protein profile [26,27]. These salivary proteins and peptides namely, Proline rich proteins, histatins, mucin, lactoferrin exert antibacterial action and thereby affect the oral microflora [28,29]. Hence, Salivary proteins play an imperative role in the etiology of dental caries [30]. Saliva being a unique biological fluid, with relatively inexpensive to collect and store, and carries very low risk to both patients and staff. Though saliva carries several advantages, there exist certain difficulties in collecting and storing the samples. It is mandatory to used standard protocols while processing the salivary samples, as saliva contains components may come from salivary glands, epithelial cells or metabolism of microflora. Hence, centrifugation of salivary samples is important to remove bacteria and cell fragments. Saliva samples should be stored in an ideal temperature to prevent Proteolysis [31].

Few studies had been carried out on evaluating mean salivary proteins and provided contradictory results. de Farias DG et al and Bhalla S et al had stated that there was no significant difference in the mean protein concentration between the caries free and caries active children [32,33]. On contrary, Prabhakar AR et al had stated that mean salivary protein concentration was found to be higher in caries active children [34]. Since the literature provides varied results and there are only few studies related salivary protein profile and dietary patterns, the present investigation was undertaken to evaluate the role of dietary patterns and estimation of mean salivary protein in children with ECC and to compare it with non-carious children. In the present study, children with ECC were divided into Group II-ECC and Group III Severe ECC (S ECC).

2. MATERIALS AND METHODS

The present study is a descriptive cross-sectional study, conducted between September 2020 and November 2020 in the Department of Pediatric and Preventive Dentistry, Saveetha Dental College and Hospitals after obtaining Ethical approval from the Scientific Review Board. The procedures were explained to the parents of the children involved in the study, and an informed written consent was obtained prior to the investigation. Only those provided the informed consent were included in the study. Only one author performed the entire clinical examination and the collection of saliva samples. Ten children with ECC and 10 children with severe ECC (S ECC) were selected based on dmft index and 10 caries free children were recruited to participate in the study. Children of both genders, between 3-6 years of age group were included in the study. The children were divided into 3 groups, 10 children each, based on the dmfs score. Group I: Caries-free, Group II: ECC, Group III: S- ECC.

Inclusion Criteria:

- Children with growth and development
- Absence of congenital or systemic disease
- Children with no history of any medication intake for past 1 month

2.1 Methodology

Phase 1: Oral Health Questionnaires

In the first phase of the study, information was collected from the parents regarding the children’s oral hygiene practices, feeding habits and dietary pattern. The questionnaires were adopted from the previous study and used to obtain information regarding the children’s feeding practices (breast feeding, bottle feeding), dietary habits (snacking history, Protein diet, milk product consumption) oral hygiene practices (brushing frequency) [35,36].

Phase 2: Intraoral examination and Saliva Collection

The intraoral examination was performed in a dental chair, using a dental mirror and an explorer. The dmfs index was assessed. The children were refrained from eating or drinking for 1 hr before the sample collection. To prevent saliva contamination with toothpaste, water, or other substances, tooth surfaces were cleaned only with sterilized gauze prior to sampling. In group I, children were considered to be caries free, Group II: ECC, Group III: S ECCC based on AAPD Guidelines. Saliva collected was done between About 5ml of unstimulated saliva was collected Saliva was collected between 8:00 and 11:00 a.m., to prevent cicardian variation and a total of 5ml of saliva was collected. As the behaviour of the child influences the composition of saliva, [27] children who were exhibiting negative behaviour were excluded from the study. Hence, finally there were 10 children in each group.
The samples were transferred to the laboratory immediately and stored at -4°C to perform biochemical assays. The samples were centrifuged at 3000 rpm for 10 min for total protein estimation. The total protein concentration was estimated using Lowry method [37]. The technique as follows: Under alkaline condition, the nitrogen reacts with the copper ions and followed by reduction of Folin Ciocalteay phosphomolybdic phosphotungstic acid to heteropolymolybdenum blue by the copper-catalyzed oxidation of aromatic acids. The protein concentration is directly proportional to the rate of chelate formation.

2.2 Statistical Analysis

Data was collected and tabulated. Statistical analysis was done using SPSS software version 20.0. Multivariate logistic regression analysis was performed to assess the relative contributions of dietary and feeding patterns between children with and without ECC. One way ANOVA was done to determine the difference in mean protein concentration between the groups. The level of significance was regarded as p<0.05.

3. RESULTS

Table 1 shows the oral hygiene practices, feeding and dietary patterns of children with and without ECC. The caries free group data suggest 32% of children brushed only at morning and 40% of children brushed at morning and night. The ECC group data suggest 68% of children brushed at morning only and 60% of children brushed at morning and night. According to feeding practices, caries free group data showed, 22.2% of children were breast fed less than 24 months and 38.1% of children were breast fed for more than 24 months. In addition, 14.3% of children were not bottle fed and 77.8% of children were never bottle fed. The data of ECC group showed, 77.8% of children were breast fed for less than 24 months and 61.9% of children were breast fed for more than 24 months. 85.7% of children were bottle fed and 22.2% of children were not bottle fed. Snacking history data showed, 30.8% of children in caries free group had snacking history of more than thrice per day and 64.7% of children in ECC group had snacking history of more than thrice per day. Regarding diet patterns, ECC group data showed 87.5% of children in ECC group had less protein diet and 85% of children consumed less milk products. Multivariable models were fitted to estimate the independent association of each of the identified factors with ECC group status (Table 2). Risk factors specific to the ECC group were Breast feeding <24 months (OR 2.1, 95% CI, 0.35-13.0), bottle feeding (21.0, 95% CI, 2.8-15.7), snacking more than 3 times per day (OR 1.22, 95% CI 0.283-5.734), consumption of less protein diet (OR 4.1, 95% CI, 1.99-8.81), consumption of less milk products (2.33, 95% CI, 1.67-6.77).

Table 2 shows the comparison of mean protein concentration of different groups. When comparing the mean protein concentration between the groups, it was evident that the mean protein concentration in the caries-free group was 0.97 ± 0.59, ECC group was 0.86 ± 0.45 and S ECC group was 1.12 ± 0.51. The difference was not found to be statistically significant (p> 0.05).

4. DISCUSSION

ECC is a multifactorial disease and hence, immunologic and non-immunologic factors play an equal role in initiation and progression of ECC. The proteins that originate in whole saliva are derived mainly from secretions of the parotid, submandibular, sublingual, and minor salivary glands. In addition to that, small amount of protein is also derived from oral microorganisms, crevicular fluid, epithelial cells, polymorphonuclear leucocytes, and dietary compounds [38]. Salivary proteins are considered as "double edged swords", they may increase the colonization of oral microflora or they may provide protective role against dental caries [39]. Since studies found a correlation between dietary patterns and ECC and salivary protein concentration varies according to diet intake, this study aimed to find a possible association between dietary pattern and salivary protein concentration and the presence/absence of caries experience in early childhood.

Saliva was collected based on the technique suggested by Colin Dawes. This technique was simple and child's co-operation was obtained easily [40]. The child was asked to pool the saliva in the floor of the oral cavity and asked to spit intermittently. All the samples were collected between 8.00 AM and 11.00 am to prevent circadian variation. The salivary total protein levels were determined using Lowry method, based on the reactivity of the peptide nitrogen with the copper ions under alkaline conditions and the subsequent reduction of the Folin...
Table 1. Comparison between the children with and without ECC based on the analysed risk factors and risk ratio using the multiple logistic regression model

| Questionnaire | Caries-free (10) | ECC (20) | Risk Ratio |
|---------------|------------------|----------|------------|
| 1. Oral hygiene practices | | | |
| When do you brush? | | | |
| Morning only | 8(32%) | 17(68%) | 0.70 |
| Morning and bedtime | 2 (40%) | 3(60%) | |
| 2. Feeding practices | | | |
| a. Breast feeding | | | |
| Breast feeding < 24 months | 3 (22.2) | 8(38.1%) | 2.15 |
| Breast feeding > 24 months | 7(77.8%) | 12(61.9%) | |
| b) Bottle feeding | | | |
| Bottle fed | 3(14.3%) | 18(85.7%) | |
| Never Bottle fed | 7(77.8%) | 2(22.2%) | 21.0 |
| 3. Snacking | | | |
| Snacking < 3 times per day | 6(35.3%) | 11(54.7%) | |
| Snacking > 3 times per day | 4(30.8%) | 9(69.2%) | 1.22 |
| 4. Dietary pattern | | | |
| a. Protein diet | | | |
| High consumption of protein rich foods | 8(57.1%) | 6(42.9%) | |
| Low consumption of protein rich foods | 2(12.5%) | 14(87.5%) | 4.1 |
| b. Milk products | | | |
| High consumption of milk products | 7(70%) | 3(30%) | |
| Low consumption of milk products | 3(15%) | 17(85%) | 2.33 |

Table 2. Comparison of Mean Protein concentration (µg/ml) values between the groups

| Groups | N  | Mean  | SD   | F Value | p value |
|--------|----|-------|------|---------|---------|
| Group I (Caries-free) | 10 | 0.97  | 0.59 |        |         |
| Group B (ECC) | 10 | 0.86  | 0.45 | 0.61   | 0.547   |
| Group C (Severe ECC) | 10 | 1.12  | 0.51 |        |         |

p value was evaluated using ANOVA. p value < 0.05 – Significant * 

Ciocaltey phosphomolybdic phosphotungstic acid to heteropolymolybdenum blue by the copper-catalyzed oxidation of aromatic acids. The resulting increase in absorbency is monitored by a detector at 660 nm. The observed rate of chelate formation is directly proportional to the total protein concentration in the sample.

In the present study, snacking habits, feeding and dietary pattern showed an increased risk of caries development. Children who had a history of snacking more than 3 times a day had 1.2 times more risk of developing ECC than caries free children. The results were similar to study done by Mahesh R et al, where the author inferred that the snacking more than thrice per day increased the risk of ECC [35]. Children who were breast fed for less than 24 months had 2.15 times more risk of developing ECC. The result is contradictory to the study done by Tida H et al, where the author found no association between breast feeding and ECC [41]. Children who were bottle fed had 21 times more risk of developing ECC and the results are similar to research done by Mahesh et al and Davies et al. [35,42]. The authors reported bottle feeding as a risk factor of ECC. In addition, children who consumed less protein diet exhibited 4.1 times more risk of developing ECC and children who consumed less milk products presented with 2.33 times more risk of developing ECC. The result is in accordance with the study done by Zaki NA et al and Priyadharshini P et al, where the authors reported that, the dairy products and proteins such as whole grains have a protective role against ECC [43,44].

In the present study, there was no significant difference in the mean protein concentration between the caries-free, ECC and S ECC group (p > 0.05). Table 1 shows the mean protein concentration of different groups. The results were similar to the results obtained by de Farias.
DG et al and Bhalla S et al, where the authors found no significant difference in mean protein concentration in children with and without ECC. Whereas, Bagherian A et al (2008 & 2012) reported an increase in Salivary IgA levels in children with ECC [32,33]. Study done by Prabhakar AR et al, Preethi BP et al and Pandey P et al reported that there was a statistically significant difference in mean protein concentration between caries-free and caries active children [34,45,46]. Pyati SA et al compared the total salivary protein concentration in 6-12 year old caries-free and caries active children and stated that the salivary protein was increased in caries active children [47]. Vibhakar et al conducted a study to correlate the dmft values and the total protein concentration in a group of adult population and the author had concluded that the total salivary protein levels showed a positive correlation with the Decayed, Missing, Filled Total (DMFT) teeth index. The author suggested further studies with larger sample size and further analysis into specific salivary proteins and their role in protection against dental caries [38]. One of the possible reasons for this varied result could be due to the age selection criteria. In the present study children between 3-6 years were selected, whereas most of the above-mentioned studies included children and adults above 7 years of age. The other possible explanation could be the biological maturation of immune system, as the current study included children between 3-6 years, their biological immune system would not have matured as compared to children and adults included in the comparison studies [34,37,41-43].

Though, there was no significant difference in mean protein concentration between the groups, the less protein diet, low consumption of milk products and their feeding and snaking patterns could be a reason for developing ECC. Since protein intake plays a significant role in salivary IgA concentration and prevention of caries, further studies are needed to estimate and compare the protein intake and salivary IgA levels to explore the relationship between protein intake and ECC.

The limitation of the study includes, 1. The technique used for protein estimation. The Folin reagent is reactive for only a short period of time after addition and the technique is complicated, requires more steps and once the protein sample has reacted with the dye, the protein cannot be used for other assays. 2. Several environmental factors plays a role in initiation of ECC, namely, feeding habits, fluoride exposure, and oral hygiene measures. Hence it is difficult to select matching children from the above-mentioned criteria. Salivary proteins are only one of the factors that influence caries development as we could not fully control the rest of these variables. 3. Smaller sample size. Further studies are required with a larger sample size to evaluate individual salivary proteins and comparison.

5. CONCLUSION
To conclude, the present study identified

1. Breast feeding less than 24 months, bottle feeding, snacking more than thrice per day, consuming low protein diet and low milk products had a high risk of developing ECC.
2. There was no significant difference in the mean protein concentration between caries free, ECC and S ECC group.

Further studies are needed to understand the effects of protein diet, individual protein concentration, and its role in ECC with a larger sample size.

CONSENT
As per international standard, parental written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL
As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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