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Comparison Of Salivary Calcium Levels In Children With And Without ECC

Research Article

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Abstract

Background: Early Childhood Caries (ECC) is a chronic disease of childhood affecting the primary dentition. It is also termed as nursing caries or baby bottle tooth decay. It has a multifactorial etiology and saliva and its components is one of the important etiological factors of ECC. Salivary calcium protects the tooth from demineralization and maintains oral homeostasis. The present study was done to compare the salivary calcium levels in children with and without ECC.
Materials And Methods: It is a cross-sectional observational study conducted at Saveetha Dental College and Hospitals. Children between 37 to 72 months were examined and 15 caries free and 15 children with ECC were recruited for the study. The examination and sample collection were done by a single qualified Pediatric dentist. The samples were collected and analysed for calcium levels by colorimetric method.
Results: The results showed that there was a statistically significant difference in salivary calcium level in children with and without ECC. In children with Early Childhood Caries the mean calcium concentration in samples of unstimulated saliva was z4.96 ±1.64
The mean calcium concentration caries free children were found to be 9.14± 3.5
Conclusion: Salivary calcium concentration was high in Caries free group and found to be low in children with ECC. The present study emphasize that the salivary calcium definitely influences the development of carious lesion.

Keywords: Early Childhood Caries (Ecc); Saliva; Salivary Calcium.

Introduction

Oral cavity is a distinctive ecosystem, which harbours wide range of microorganism. Dental caries is the chronic disease and considered as a serious public health problemin many developing countries.[1,2] Dental caries affecting primary dentition is called as Early childhood caries (ECC) and defined 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 72 months of age or younger. [3] AAPD also states that, in children less than 3 years of age, any sign of smooth-surface caries is an indicative of severe ECC (ECC-S). From 3 to 5 years of age, 1 or more cavitated smooth surfaces, lost (because of caries), or clogged in the anterior maxillary temporary teeth index ≥ 4 (at 3 years of age), ≥ 5 (at 4 years of age), or ≥ 6 (at 5 years of age) corresponds to ECC-S. [4] The concepts of ECC and Early Severe Childhood Caries (S-ECC) have been used for over ten years to describe the state of caries present in children under 6 years. The most common reason for ECC is improper bottle feeding and hence ECC is also been termed as bottle caries," "nursing caries," "baby bottle tooth decay," or "night bottle mouth." ECC develops as a white-spot lesion in the upper primary incisors along the gingival margin and later progresses to complete destruction of the crown [5,6] The maxillary incisors are commonly affected and the mandibular incisors are least affected due to the protection of tongue and saliva from salivary glands. [7]

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The main causative agent of ECC is Mutans Streptococci namely Streptococcus mutans and streptococcus sobrinus. Streptococcus Mutans and Streptococcus Sobrinus initiate the carious lesion whereas, lactobacilli plays and important role in progression of carious lesion. [8] Apart from microbial factors, diet also plays an imperative role in ECC. Prolonged intake of sugary substances, fermentable carbohydrates is the main source of ECC. School going children are more vulnerable for the development of ECC due to the change in dietary habits. [9,10] Though, these factors play an important role in initiation of caries, but cannot be considered as a sole factor to develop a carious lesion in all cases. Hence there are several other contributing factors like salivary factors also play a major role in development of caries.

Saliva is the mixed glandular secretion which is constantly exposed to the teeth and the oral mucosa. The three major salivary glands are the parotid, submandibular and sublingual. The saliva also contains secretions from minor salivary glands. Saliva plays an immense role in the prevention of dental caries and protection of oral mucosa. Saliva can be classified as unstimulated or stimulated. The unstimulated saliva consists of a mixture of secretion from major and minor salivary glands. Its continuous presence in oral cavity throughout the day helps to protect the teeth and oral mucosa and hyposalivation leads to development of carious lesion. Hence, it reflects the importance of saliva in development of dental caries. [11,12]The salivary flow and its composition play an important role on the development of dental caries. It is important to consider the role of saliva in demineralization and remineralization process of tooth exposed to the oral cavity

Salivary calcium and phosphateplay an imperative role in oral homeostasis. Calcium is a bivalent ion excreted along with salivary proteins into the lumen of the acinus. Hence, the concentration of salivary calcium is dependent on the salivary flow rate. The ionized calcium fraction is about 50%, and it is strongly dependent on the salivary pH. Saliva consists of various inorganic compounds like calcium and phosphate, which helps in rehardening softened, demineralized enamel. Demineralization occurs at a low pH when there is a disproportionate in the mineral content between the oral environment and in the tooth. During these series of event, the enamel crystal is dissolved by the acids produced by the micro-organisms and leads to loss of calcium in the saliva leading to demineralization of tooth. Low level of salivary calcium and phosphate levels affects the equilibrium between demineralization and remineralization process and ultimately leads to caries development. Hence it explains the importance of salivary calcium in maintaining and preserving the tooth integrity from demineralization. These inorganic ions also influence the driving force for the precipitation of calcium hydroxyapatite (HAP), the principal inorganic component of dental hard tissues. [13] It also helps in post-eruptive maturation of enamel and increase resistance to demineralization. The concentration of salivary calcium in a healthy individual is 5.8mg/dl (2.2-11.3mg/dl) in resting saliva and 6mg/dl in stimulated saliva as reported by Jenkins. [11] Parotid saliva contains more phosphate and less Ca+2 ions compared to mixed saliva. On contrast, the level of Ca+2, Mg+2, and Zn+3 in mixed saliva are significantly higher than the parotid saliva.

Few studies carried out pertaining to the mechanism of how mineral concentration of saliva influence plaque during sugar challenge. The studies reported that, during sugar intake, inorganic phosphorus concentrations in saliva may fall with increases in flow rate, while the Ph of the saliva drops and it becomes desaturated with respect to calcium phosphates. Simultaneously, mineral concentrations in the fluid phase of plaque may rise and allow calcium and/or phosphate to diffuse out. High concentrations of these constituents in stimulated saliva may reduce outward diffusion, whilst low concentrations may encourage movement of ions out of plaque. [14,15]

Several conflicting reports have been published on the salivary calcium level and its role in ECC. Aruna S et al reported an increase in salivary calcium level in caries-free children compared to children with ECC.[16] On contrary, Turtola et al and Elizarova and Petrovich reported an increase in salivary calcium in children with increased caries activity.[17,18] Due to the existence of contradictory results, the present study was done to study thepossible relationship of salivary calcium level and ECC.

Aims And Objectives

1.To estimate the levels of calciumin the salivary samples of children between 3-6 years of age.
2.To compare the salivary calcium levels in children with and without ECC.

Hypothesis

There are significant differences in salivary calcium levels in children with and without ECC.

Main Aim

Establish whether there are significant differences in the salivary calcium level in children with and without ECC**.**

Materials And Methods

Type Of Study

The present study is a cross-sectional case control study. Children between 3-6 years of age, who reported to the Department of Pediatric and Preventive Dentistry, Saveetha Dental College, were included in the study.

Ethical Approval

This study "Comparison of Salivary calcium levels in children with and without ECC" has been approved by the Ethics Committee of the Saveetha Dental College and Hospitals.

The study was carried out following the principles of the Declaration of Helsinki. We invited children to be a part of the study after informing the parents/care givers about the clinical measurements and surveys to be applied, and their potential risks and benefits. The agreement was registered through an informed consent endorsed by the Ethics Committee of the Saveetha Dental College and Hospitals. 15 children with ECC were selected based on dmft index and 15 caries free children were recruited to participate in the study

Inclusion Criteria

Children of both genders, between 3-6 years of age.
Parents who agree to take part in the study, by signing an informed consent

Exclusion Criteria

Uncooperative children who do not allow the examination and/or collection of saliva.

Children with systemic diseases and/or pharmacological treatment.

Clinical Examination

Examination for dental caries was carried out by a single qualified Pediatric Dentist using mouth mirror and dental explorer. The decayed, missing, and filled teeth (dmft) scores for primary teeth were recorded using WHO criteria 18and the data's was entered by an assistant on data collection forms. Teeth extracted for trauma reasons were not included in dmft score. Restored teeth with recurrent caries and teeth filled with temporary materials were considered as decayed. The dmft value should be more than 1 for caries active children and 0 for caries free children.

Those children who fulfilled the inclusion criteria were screened for dental caries and categorized into 2 groups depending on their caries status

1. Group I: Caries free group
2. Group II: Children with ECC

Collection of Salivary Samples

The children were instructed not to eat or drink anything 1 hour before the sample collection to prevent contamination. [19-21] The children were asked to rinse their mouth to remove any food debris present. The salivary samples were collected between 10.00 am -11.00 am to avoid circadian variation. Unstimulated saliva was collected in sterile tube. Saliva was collected based on the technique explained by Wu et al. [22] The child was allowed to sit in well-ventilated and well-lit atmosphere. The children were asked to keep their head at 45°C, with one hand holding the sterile tube. The saliva was allowed to drip into the tube and allowed till sufficient for analysis without measuring the froth the quantity. The samples were transferred immediately to the laboratory. The samples were stored at -20°C until analysis.

Laboratory Procedures

Analysis of the samples was done on the same day. Samples were centrifuged at 5000 rpm for 5 minutes to remove bacteria and other debris. [23] Each sample was then estimated for calcium concentrations. The measurement of salivary calcium was done by colorimetric method. In colorimetric method, calcium and methyl-thymol blue makes a colour that is proportional with the calcium ion in solution. [24]These values were tabulated and subjected to statistical analysis.

Statistical Analysis

All data was analysed by using the SPSS 20.0 software. Descriptive statistics that included mean, standard deviation and minimum and maximum values were determined for 2 groups. A p value of 0.05 or less was considered for standard significance.

Results

The mean scores and standard deviations for salivary calcium are depicted in Table 1. In Group I (caries free) the mean concentrations of calcium was found to be 9.14 mg/dl and in Group II (Caries - active), the mean concentrations of calcium were4.96 mg/dl respectively.

Discussion

ECC is a chronic disease of childhood and remains as a major health problem in many developed countries. Several etiological factors were attributed to the development of ECC and the low salivary calcium levels remains as one of the etiological factors in development of ECC. Salivary calcium plays an important role from the beginning of eruption of primary teeth to permanent teeth through diffusion of calcium ions in the tooth enamel and increases the resistance of the tooth. It influences the precipitation of hydroxyapatite crystals of enamel and hence it acts as an important factor in post eruptive maturation. [11,13,26,27]Thus, the deficiency of salivary calcium could be an additional cause for development of ECC. Studies state that the critical Ph is lower for children and demineralizationoccurs at a low pH. [19] As a result of this, there will be undersaturation of mineral ions of saliva as compared to tooth's mineral content. During these series of events, the carbonated apatite crystals of enamel get dissolved by organic acids and results in loss of ca ions in tooth enamel. If any

Table 1: Comparison of Salivary calcium levels in Caries Free children and in children with ECC

Salivary Calcium	Caries Free Group I (N=15)		Group II (n=15)		p Value
	Mean	SD	Mean	SD	
	9.14	3.5	4.96	1.64	<0.001*

p>0.05 Not significant
p<0.001 highly significant
SD – Standard deviation

timely action taken by usage of remineralizing agent, the ca ions of the enamel get restored.

Saliva consists of supersaturated calcium and helps in remineralization, whereas undersaturated saliva leads to development of incipient lesion. Similarly, the quantity of salivary calcium varies between different salivary glands. For instance, parotid saliva has less calcium content than submandibular and sublingual salivary glands. Few studies carried out pertaining to the mechanism of how mineral concentration of saliva influence plaque during sugar challenge. The studies reported that, during sugar intake, inorganic phosphorus concentrations in saliva may fall with increases in flow rate, while the Ph of the saliva drops and it becomes desaturated with respect to calcium phosphates. Simultaneously, mineral concentrations in the fluid phase of plaque may rise and allow calcium and/or phosphate to diffuse out. High concentrations of these constituents in stimulated saliva may reduce outward diffusion, whilst low concentrations may encourage movement of ions out of plaque. [14,15,27,28]In the present study, the mean level of salivary calcium is increased in caries-free children when compared to caries-active children and is found to be statistically significant. (Table 1) This high concentration of salivary calcium levels could be a reason for remineralization of incipient lesion in caries free children. The saliva that is super saturated with calcium acts as a reservoir and enhances remineralization and overrides demineralization.

Several conflicting reports have been published on the salivary calcium and phosphate level and its role in ECC. A study done by Aruna S et al reported an increase in salivary calcium level in caries-freechildren compared to children with ECC. [16] On contrary, Turtola et al and Elizarova and Petrovich reported an increase in salivary calcium in children with increased caries activity. [17,18] On the other hand, few studies insisted that there was no difference in salivary calcium in caries-free and caries-active children. [29][32] A study done by Kargul et al found no significant differences in salivary calcium concentrations between caries- free and caries active children children. [33]

Shaw Let al evaluated calcium levels in saliva and plaque and found that the calcium level in saliva and plaque to be significantly higher in caries-free than a high caries group. [34] Similarly, Kittner D et al reported an increase in salivary calcium levels in persons with a low caries experience. [35]In regards to caries activity and calcium levels, Ashley found that the salivary calcium increased with decreasing caries activity, whereas Karshan observed that salivary calcium concentration decreased with increasing caries activity. [36,37]Preeti et al and Aryeh et al investigated salivary calcium level and found that the salivary concentration of calcium tends to be lower in children as compared to adults. This clearly explains that the salivary calcium balance is lower in children; hence it suggests that a deficient in calcium levels make them more susceptible to development of caries. [19,38]

An invitro study done by Amaechi B et al had found that, a chewing gum comprised of calcium and phosphorus would supersaturate the saliva with these ions and inhibit demineralization. 36Hence with the usage of this chewing gum, there will be a stimulation of saliva and thereby the levels of inorganic ions would increase and possibly inhibit demineralization[40].

The limitation of the study includes, smaller sample size. Hence in future, studies are needed with a greater number of children from the same socioeconomic stratum, to have a more representative and homogeneous sample.

Conclusion

Salivary calcium concentration was high in Caries free group and found to be low in children with ECC. The present study emphasize that the salivary calcium definitely influences the development of carious lesion. However, clinical interpretation of the results obtained in the present study should be made carefully as it involved only one of the host factor components of the multifactorial etiology of dental caries.

This article is of importance to paediatric dentist as:

•As ECC is considered as a major oral health problem among children, this paper evaluated the role of calcium and its protective function
•The salivary calcium can be considered as a useful parameter to measure caries experience in children along with other host factors.

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