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Estimation of Salivary Calcium and Phosphorus in Children with different caries status – A Cross-Sectional observational study

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Abstract

Background and Objectives

Early Childhood Caries (ECC) is a chronic disease of childhood affecting the primary dentition. ECC begin early in life and can affect the quality of life of child. It is also termed as nursing caries or baby bottle tooth decay. It has a multifactorial etiology and salivary parameters are considered as one of the important etiological factors of ECC. The present study was done to determine the level of salivary calcium and phosphorus in children with ECC, severe ECC and caries free. The main objective of the study is to determine the correlation between calcium and phosphorus level and to compare it with the three groups of children with different caries experience.

Materials and Methods:

It is a cross-sectional observational study conducted at Saveetha Dental college and Hospitals. Children between 3-6 years were examined and 20 caries free, 20 children with ECC and 20 children with Severe 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 salivary calcium and Phosphorus.

Results: The results showed that there was a statistically significant differences in salivary calcium and phosphorus levels among caries free, ECC and Severe ECC children.

Conclusion:

1. ***There was a significant difference in salivary calcium levels among caries free, ECC and Severe ECC children***
2. ***There is a significant difference in salivary phosphorus level among caries free, moderate ECC and Severe ECC children***

Key Words: Saliva, Salivary Calcium, Salivary Phosphorus, Early Childhood caries

Introduction

Despite continuous research for more than a century, Early childhood caries(ECC) still remains as a prevalent disease in children. ECC begin early in life and can affect the quality of life of child. ECC is defined as “the presence of one or more decayed (non-cavitated or cavitated lesions), missing teeth (due to caries), or filled tooth surfaces in any primary tooth in a child 71 of months age or younger. Any sign of smooth surface caries in a child less than

3 years of age is considered as ECC. From age 3 to 5, presence of one or more decay, missing teeth, or filled surfaces (dmfs) in primary anterior teeth is indicative of Severe ECC. Similarly, dmfs score of >≥4 (age 3), ≥5 (age 4), or ≥6 (age 5) surfaces indicates S-ECC. [1,2]Children require healthy teeth and chewing ability while transition from weaning diet, as dietary intake is important to maintain the nutritional status of children. [3,4] ECC is associated with pain and swelling and can affect the chewing ability of children, thereby indirectly affect the nutritional status of the children. Hence, it is of paramount importance to treat ECC at earlier stage to prevent the negative health impacts.

Saliva plays a vital role in maintaining the integrity of teeth and oral structures. Whole saliva is composed of 90% of secretion from major salivary glands (Parotid, submandibular and sublingual) and 10% of secretion from minor salivary glands and gingival crevicular fluid. [5] Saliva is composed of 99% water and less than 1% of inorganic and organic components. Though, these inorganic and organic contents are less than 1%, they play an imperative role in maintaining the integrity of tooth and preventing remineralization process. The functions of saliva include, aggregation and elimination of microorganisms, neutralize acids through buffering action, elimination of food debris, anti-microbial action, and participate in acquired enamel formation. [6,7]

Calcium and phosphate ions maintain the saliva in supersaturated state with respect to hydroxyapatite crystals of enamel and maintain the integrity of tooth. [8] If this environment is maintained, it will inhibit the demineralization process and enhance remineralization.

Inaddition, these ions influence the precipitation or dissolution of calcium hydroxyapatite (HAP), the principal inorganic component of dental hard tissues. [9] Salivary Calcium and phosphorus play a pivotal role in the post-eruptive maturation of the enamel and helps in rehardening softened, demineralized enamel. [10,11] Demineralization occurs when there exist a disproportionate in the mineral content between the tooth and oral environment. During these series of event, the enamel crystal is dissolved by the acids produced by the micro-organisms and leads to loss of these ions 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 and phosphorus ions in maintaining and preserving the tooth integrity from demineralization. Jenkins et al reported that, the level 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 [5] 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 is significantly higher than the parotid saliva.

There have been numerous studies conducted to explore the relation between Salivary calcium and phosphorus level and ECC. Turtola et al and Elizarova and Petrovich reported an increase in salivary calcium in children with increased caries activity, [12,13] whereas a pilot study done by Aruna et al reported an increase in salivary calcium levels in caries free children. [14] Studies had reported an increase in salivary phosphorus level in caries active children. [15,16] Since wide disparity exist in the results obtained from the above-mentioned studies, the aim of this research is to determine the level of salivary calcium and phosphorus

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in children with ECC, severe ECC and caries free. The main objective of the study is to determine the correlation between calcium and phosphorus level and to compare it with the three groups of children with different caries experience.

Materials and Methods

Type of Study

The present study is a cross-sectional observational 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 "Estimation of Salivary Calcium and Phosphorus in Children with different caries status – A Cross-Sectional observational study" has been approved by the Ethics Committee of the Saveetha Dental College and Hospitals. The study protocol was registered through an informed consent endorsed by the Ethics Committee of the Saveetha Dental College and Hospitals. 20 children with ECC and 20 children with severe ECC (S ECC) were selected based on dmft index and 20 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

Children were divided 3 groups; Group I – Caries free (dmfs=0), Group II- ECC(dmfs=1-3) and Group III- Severe ECC (dmfs>3) with 20 children in each group based on AAPD Guidelines. A total of 60 children, aged between 3-6 years were randomly selected, who reported Saveetha Dental College and Hospitals. The children were included in the study after obtaining informed written consent from the parent/guardian.

Clinical Examination
The caries status was recorded using dmfs (decayed, missing, filled surfaces) index based on WHO Criteria. [17] The examination was done under dental chair light using a sterile mouth mirror and probe. The dental examination was completed within 10 min and an assistant recorded the clinical findings.

Saliva Collection
To avoid circardian variation, the salivary samples were collected between 10.00 am -11.00 am. The children were refrained from eating or drinking anything 1 hour before the salivary sample collection to prevent contamination. [18-20] The children were asked to rinse their mouth to remove any food debris present.Saliva was collected based on the technique given by Wu et al.[21]The child was allowed to sit in well-ventilated and well-lit atmosphere. The

children were asked to keep their head at 45ºC, 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. About 5ml of unstimulated saliva was collected from all the children in a plastic rube.

The salivary samples were transferred to the laboratory immediately to estimate the amount of salivary calcium and phosphorus. The salivary calcium was estimated using Spinreact colorometric kit and salivary phosphorus was estimated using Spinreact phosphorus-UV kit. Salivary Calcium reacts swith Arsenazo III (1,8-Dihydroxy-3,6-disulpho-2,7- naphthalene-bis (azo)-dibenzenearsonic acid), at neutral pH, yields a blue colored complex. The intensity of the colour formed is proportional to the calcium concentration in the salivary sample. [22-24] The inorganic phosphorus reacts in acid medium with ammonium molybdate to form a phosphomolybdate complex with yellow colour. The intensity of the color formed is proportional to the inorganic phosphorus concentration in the sample.[25,26]

Statistical Analysis

All data was entered and analysed by using the SPSS 20.0 software. One way ANOVA was done to determine the difference of salivary calcium and phosphorus between the groups. Post-hoc Tukey test was done to determine the intergroup significance. A P value of < 0.05 was considered as statistically "significant" and a $P < 0.001$ was considered as statistically "highly significant."

Results

The mean and standard deviation value for salivary calcium is depicted in Table 1. In Group I (caries free) the mean concentrations of calcium was found to be 2.19+/-0.52 mg/dl , Group II (ECC), the mean concentrations of calcium were 2.69+/-1.11 mg/dl and Group III (S ECC), the mean concentrations of calcium were 3.46 +/-1.05 mg/dl respectively. There was a significant difference in the mean salivary calcium levels among the study groups ($P < 0.05$). Post hoc Tukey test revealed that, Group III had a significantly higher mean salivary calcium levels than that of Groups I and II. However, no significant difference was seen between the mean salivary calcium levels of Group I and Group II. (Table 2)

The mean and standard deviation value for salivary phosphorus is depicted in Table 3. In Group I (caries free) the mean concentrations of calcium was found to be 5.45+/-0.89 mg/dl , Group II (ECC), the mean concentrations of calcium were 7.83 +/-1.39 mg/dl and Group III (S ECC), the mean concentrations of calcium were 7.86 +/-2.72 mg/dl respectively. There was a significant difference in the mean salivary calcium levels among the study groups ($P < 0.05$). Post hoc Tukey test revealed that, Group III had a significantly higher mean salivary calcium levels than that of Groups I and II. There was a significant difference was seen between the mean salivary calcium levels of Group I and Group II.However, no significat difference was evident between Group II and Group III. (Table 4)

Discussion

ECC remains as a major public health problem due to the high prevalence in the world.[27] Salivary components namely inorganic and organic components plays an imperative role in protecting teeth and surrounding soft tissues. [28,29] Though several etiological factors

namely, microbial challenge, improper feeding patterns and improper oral hygiene measures plays a role in initiation of ECC, they alone are not sufficient to initiate ECC. So other salivary parameters also play a role in initiation and progression of ECC. Saliva has aninherent capacity to buffer strong acids and bases and provides calcium and phosphate ions needed to remineralize the teeth. The balance between demineralization and remineralization cycle and the amount of these ion supersaturated in saliva in of paramount important to prevent dental caries.

The Salivary Calcium Levels in our study were higher among children with Severe ECC than the children with ECC and caries free children. Group III had a significantly higher mean salivary calcium levels than that of Groups I and II. However, no significant difference was seen between the mean salivary calcium levels of Group I and Group II. The result was in accordance with the study done by Shahrabi M et al. [30] The author reported an increase in salivary calcium level in children with severe caries, but it was not statistically significant. Similary, Turtola et al and Elizarova and Petrovich et al. reported an increase in salivary calcium levels in children with increased caries activity. [12,13] On contrary, Aruna et al [14] and Jolly R et al [31] reported an increase in salivary calcium level in caries-free children. A study done by Vocel, Naujoks & Brudevo reported the possible reason for increase in calcium level in caries active children. [32] The possible reason could be, in a caries-active persons, an increased calcium content in the saliva might be due to an increase in the relative amount of submandibular saliva secreted. Another possible explanation could be a release of calcium from demineralized tooth, thereby increasing salivary calcium levels. [33,34]

The Salivary Phosphorus Levels in our study were higher among children with Severe ECC than the children with ECC and caries free children. Group III had a significantly higher mean salivary calcium level than that of Groups I and II. There was a significant difference was seen between the mean salivary calcium levels of Group I and Group II.However, no significant difference was evident between Group II and Group III. The results of the study were similar to the study done by Kaur A et al and Mahajana S et al, the authors reported an increase in salivary phosphate level in caries active children than the caries free children. [15,16] On the other hand, Shahrabi M et al reported an increase in salivary phosphorus level in caries free children. [30] In addition, Bagherian A et al and Abbas MJ et al reported no significant difference in salivary phosphorus level in children with and without ECC.[35,36]

Limitation

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

Conclusion

With the light of available evidence following conclusion can be drawn

1. There was a significant difference in salivary calcium levels among caries free, ECC and S ECC children.
2. There is a significant difference in salivary phosphorus level among caries free, ECC and S ECC children

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Table 1: Comparison of Salivary calciumlevels between the groups

Groups	N	Mean	SD	p value
Group I (Caries-free)	20	2.19	0.52	0.018*
Group B (ECC)	20	2.69	1.11	
Group C (Severe ECC)	20	3.46	1.05	

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

Table 2:Intergroup comparison of Salivary calcium levels

Groups	Group I (Caries-free)	Group B (ECC)	Group C (Severe ECC)
Group I (Caries-free)	-	0.46	0.05*
Group B (ECC)	0.46	-	0.17
Group C (Severe ECC)	<0.05*	0.17	-

p value < 0.05 – Significant – Post- hoc Tukey test

Table 1: Comparison of Salivary phosphoruslevels between the groups

Groups	N	Mean	SD	p value
Group I (Caries-free)	20	5.45	0.89	0.009*
Group B (ECC)	20	7.83	1.39	
Group C (Severe ECC)	20	7.86	2.72	

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

ISSN 2515-8260 Volume 06, Issue 01, 2019

Table 2:Intergroup comparison of Salivary calcium levels

Groups	Group I (Caries-free)	Group B (ECC)	Group C (Severe ECC)
Group I (Caries-free)	-	<0.05*	0.05*
Group B (ECC)	<0.05*	-	0.99
Group C (Severe ECC)	<0.05*	0.99	-

p value < 0.05 – Significant – Post- hoc Tukey test