Evaluation of Salivary Carbonic Anhydrase VI as a Biomarker for Early Childhood Caries

Dr. Sheetal Kumar Sahoo[1], Dr. Subhasmita Bhol[2], Dr. Sovamayee[3], Dr. Artabandhu Tripathy[4*]

[1]Associate Professor, Department of Pediatric & Preventive Dentistry, Hi-tech Dental College and Hospital, Bhubaneswar, Odisha.

[2]Professor, Department of Pediatric & Preventive Dentistry, Hi-tech Dental College and Hospital, Bhubaneswar, Odisha.

[3]Tutor, Department of Pediatric & Preventive Dentistry, Hi-tech Dental College and Hospital, Bhubaneswar, Odisha.

[4*]Tutor, Department of Pediatric & Preventive Dentistry, Hi-tech Dental College and Hospital, Bhubaneswar, Odisha.

***Corresponding Author: Dr. Artabandhu Tripathy, Tutor, Department of Pediatric & Preventive Dentistry, Hi-tech Dental College and Hospital, Bhubaneswar, Odisha. Email: artabandhut@gmail.com**

ABSTRACT

Background

Salivary carbonic anhydrase is an enzyme that contains zinc which helps in the regulation of the pH of the saliva using the production of bicarbonate. However, literature data is scarce for evaluation of salivary carbonic anhydrase VI as a biomarker for early childhood caries.

Aim

The present study was aimed to evaluate the activity and concentration of Salivary Carbonic Anhydrase VI level in preschool child subjects having white spot lesions and its correlation with Streptococcus mutans.

Methods

The study included 80 child subjects aged 3-6 years that were assessed using Nyvad's criteria and were randomly divided into 2 groups of children with 40 subjects with white spot lesions and 40 subjects without white spot lesions. Salivary samples were collected after stimulation. Determination of Carbonic Anhydrase VI level was done using ELISA (enzyme linked Immunosorbent Assay) kit. Culture and identification of S. mutans was done. Data gathered were statistically analyzed.

Results

The results of the present study depicted that mean Carbonic Anhydrase VI concentration was lower with a higher activity in child subjects aged 3-6 years and had white spot lesions. Also, the mean CFUs (colony forming units) for S. mutans were higher in white spot lesion group and the difference was statistically significant with $p<0.05$.

Conclusion

The present study concludes that salivary carbonic anhydrase enzyme is a potent marker for assessing the susceptibility of the dental caries in preschool children aged 3-6 years. Also, salivary carbonic anhydrase can be used as a reliable indicator of caries risk for prediction of the likelihood for development of the new lesions.

Keywords: Biofilm, carbonic anhydrase VI, caries susceptibility, saliva, Streptococcus mutans.

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Introduction

Dental caries is an acid demineralization of the dentin or enamel which is caused by the acid demineralization affected by the biofilm and is mediated by the saliva. ECC or early childhood caries is a dynamic and complex process where a variety of factors contribute to dental caries including socioeconomic factors, microbiota, poor oral hygiene, inadequate salivary flow, and/or dietary habits. Initial WSL or white spot lesions are generally the first lesions to appear after formation of the dental plaque which is manifested as thin and persistent layer that builds up on teeth at a noticeable extent within 24-48 hours and is a primary contributing factor for development of a carious lesion. The acquired enamel pellicle is a thin organic matter layer seen in between the bacterial plaque and enamel.[1]

A pH of 5.5 is considered as a critical pH where tooth mineral functions as a buffer and loose phosphate and calcium ions to the plaque. However, when pH goes below 5, subsurface demineralization is seen that leads to incipient caries in the intact enamel surface. A white opaque appearance in rough surface is seen in enamel lesions that are clinically manifested as active white surface lesion resulting from superficial decalcification. Clinically, visible white surface lesions need a significant demineralization which is particularly minimum of 300-500 µm. Despite cavitated lesions have a higher impact on quality of life in a child, diagnosis of

white surface lesions is vital for implementation of early strategies for intervention and to overcome aggressive nature of early carious lesions.[2]

Initial caries depicts principal microorganism as S. mutans. Acidic nature appears as most consistent factor associated with S. mutans and is linked with the carious nature. CA VI (Carbonic anhydrase VI) is the only secretory isoenzyme for mammalian Carbonic anhydrase family which is solely expressed in the serous acinar cells of submandibular and parotid glands. It is major salivary zinc metalloprotein responsible to regulate salivary pH hemostasis and buffer capacity by catalyzation of the hydration reaction. The enzymatic ability helps in maintaining the pH and allows acid neutralization in the oral environment.[3]

Despite current understanding concerning role of Carbonic anhydrase VI in saliva and caries occurrence, there is scarce literature data assessing concentration of Carbonic anhydrase VI and children activity with white surface lesions and its correlation to S. mutans.[4] Hence, the present study was aimed to evaluate the activity and concentration of Salivary Carbonic Anhydrase VI level in preschool child subjects having white spot lesions and its correlation with Streptococcus mutans.

Materials and methods

The present cross-sectional observational study was aimed to evaluate the activity and concentration of Salivary Carbonic Anhydrase VI level in preschool child subjects having white spot lesions and its correlation with Streptococcus mutans. The study was done at Department of Pediatric & Preventive Dentistry, Hi-tech Dental college and Hospital, Bhubaneswar, Odisha. Verbal and written informed consent were taken from all the subjects before study participation.

The study assessed 80 child subjects aged 3-6 years that were divided into two groups of 40 children each where Group I comprised of subjects with ECC and active white surface lesions in incisor teeth and other 40 subjects with no white surface lesions. Stimulated samples of saliva were collected to assess Carbonic anhydrase VI concentration, S. mutans count, and activity of Carbonic anhydrase VI.

The inclusion criteria for the study were healthy child subjects aged 3-6 years from both the genders, no caries lesions and 3–4 incisors affected with active white surface lesions, active caries with intact surface (Surface of enamel is whitish/ yellowish opaque with loss of luster that feels rough when the tip of the probe is gently moved across the surface based on Nyvad's criteria score 1, and the subjects whose parents consented for study participation. The exclusion criteria were subjects on antibiotic therapy, not willing to participate, and received fluoride topical application/remineralizing agents during the last 3 months.

For clinical assessment, using aseptic protocol, an explorer and mouth mirror was used on dental chair using adequate light. White surface lesions were assessed using Nyvad's criteria. The surfaces of teeth were cleaned using pumice and air dried for 5 seconds followed by clinical examination.

For collection of the salivary sample, stimulated saliva was collected from all participants at same time and minimum 1 hour after any meal preferably the breakfast. Subjects were made to sit in upright position and asked to chew on paraffin gum for 5 minutes. Saliva collected in first minute was discarded and remaining saliva was collected in Eppendorf tubes. All samples were labelled, transferred, and incubated at -20ºC till further assessment.

To assess activity and concentration of Carbonic anhydrase VI, ELISA (Enzyme-linked immunosorbent assay) kit was used where 50 µL of the antibody cocktail and 50 µL of each sample were added to corresponding wells. They were sealed and plates were placed on shaker at 400 rpm and allowed to sit at room temperature for an hour. After incubation wash buffer PT was used for washing each well. The optical density was recorded at 450 nm.

To determine the count of S. mutans, microbiological analysis was done to identify and culture the organism on blood agar using Todd–Hewitt broth incubation at 37°C in a 5% CO_2 environment for 24–48 h. Organisms were identified based on gram staining and colony morphology.

Collected data were subjected to statistical evaluation using the chi-square test, Fisher's exact test, Mann Whitney U test, and SPSS (Statistical Package for the Social Sciences) software version 24.0 (IBM Corp., Armonk. NY, USA) using ANOVA, chi-square test, and student's t-test. The significance level was considered at a p-value of <0.05.

Results

The present cross-sectional observational study was aimed to evaluate the activity and concentration of Salivary Carbonic Anhydrase VI level in preschool child subjects having white spot lesions and its correlation with Streptococcus mutans. The study included 80 child subjects aged 3-6 years that were assessed using Nyvad's criteria and were randomly divided into 2 groups of children with 40 subjects with white spot lesions and 40 subjects without white spot lesions. Salivary samples were collected after stimulation. The mean age of the study subjects was 4.23±1.14 years. There were 65% (n=26) subjects in 3-4 and 35% (n=14) in 5-6 years. There were 55% (n=22) females and 45% (n=18) male subjects in the study (Table 1).

It was seen that for comparison of mean concentration of carbonic anhydrase VI in two study groups, in subjects that had white spot lesions, mean

Carbonic anhydrase VI concentration was 440.30±64.97 which was significantly lower compared to subjects that had no white spot lesions where mean Carbonic anhydrase VI concentration was 1026.295±73.33pg/ml depicting a statistical difference with p<0.001 (Table 2).

The study results showed that for activity of Carbonic anhydrase VI, in subjects that had white spot lesions, it was 2.1993±0.25 which was significantly lower when compared to the subjects that had no white spot lesions where Carbonic anhydrase VI mean activity level of 1.6388±0.23. The difference was highly statistically significant with p<0.001 (Table 3).

Concerning the comparison of mean CFUs (colony forming units) of S. mutans in two study groups, in subjects that had white spot lesions, mean CFUs for S. mutans were 106.87±8.235 which was significantly higher when compared to the subjects that had no white spot lesions where mean CFUs of S. mutans were 33.400±3.389 with a p-value of <0.001 (Table 4).

Discussion

The present study included 80 child subjects aged 3-6 years that were assessed using Nyvad's criteria and were randomly divided into 2 groups of children with 40 subjects with white spot lesions and 40 subjects without white spot lesions. Salivary samples were collected after stimulation. The mean age of the study subjects was 4.23±1.14 years. There were 65% (n=26) subjects in 3-4 and 35% (n=14) in 5-6 years. There were 55% (n=22) females and 45% (n=18) male subjects in the study. These data were comparable to the previous studies of van Meijeren-van Lunteren AW et al[5] in 2021 and Marsh PD et al[6] in 2010 where authors assessed study subjects with demographics comparable to the present study in their studies.

The study results showed that for comparison of mean concentration of carbonic anhydrase VI in two study groups, in subjects that had white spot lesions, mean Carbonic anhydrase VI concentration was 440.30±64.97 which was significantly lower compared to subjects that had no white spot lesions where mean Carbonic anhydrase VI concentration was 1026.295±73.33pg/ml depicting a statistical difference with p<0.001. These results were consistent with the findings of de Souza TR et al[7] in 2024 and Borghi et al[8] in 2017 where results reported by the authors for comparison of mean concentration of carbonic anhydrase VI in subjects with and without white surface lesions were comparable to the results of the present study.

It was seen that for activity of Carbonic anhydrase VI, in subjects that had white spot lesions, it was 2.1993±0.25 which was significantly lower when compared to the subjects that had no white spot lesions where Carbonic anhydrase VI mean activity level of 1.6388±0.23. The difference was highly statistically significant with p<0.001. These findings were in agreement with the results of de-Sousa ET et al[9] in 2021 and Picco DD et al[10] in 2022 where activity of Carbonic anhydrase VI higher in subjects without white surface lesions comparable to the present study was also reported by the authors.

On comparison of mean CFUs (colony forming units) of S. mutans in two study groups, in subjects that had white spot lesions, mean CFUs for S. mutans were 106.87±8.235 which was significantly higher when compared to the subjects that had no white spot lesions where mean CFUs of S. mutans were 33.400±3.389 with a p-value of <0.001. These results were in line with the findings of Al-Mahdi R et al[11] in 2023 and Picco DC et al[12] in 2019 where authors also reported higher mean CFUs (colony forming units) of S. mutans in subjects with white surface lesions as in the present study.

Conclusion

Considering its limitations, the present study concludes that salivary carbonic anhydrase enzyme is a potent marker for assessing the susceptibility of the dental caries in preschool children aged 3-6 years. Also, salivary carbonic anhydrase can be used as a reliable indicator of caries risk for prediction of the likelihood for development of the new lesions.

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S. No	Characteristics	Number (n)	Percentage (%)
	Mean age (years)	4.23±1.14	
	Age range (years)		
	3-4	26	65
	5-6	14	35
	Gender		
	Females	22	55
	Males	18	45

Table 1: Gender and age characteristics in the study subjects

S. No	Variables	Mean	p-value
	Carbonic anhydrase VI concentration		**<0.001**
	White spot lesion group	440.30±64.97	
	No white spot lesion group	1026.295±73.33	

Table 2: Comparison of mean concentration of carbonic anhydrase VI in two study groups

S. No	Variables	Mean	p-value
1.	**Carbonic anhydrase VI activity**		**<0.001**
a)	White spot lesion group	2.1993±0.25	
b)	No white spot lesion group	1.6388±0.23	

Table 3: Comparison of mean activity of carbonic anhydrase VI in two study groups

S. No	Variables	Mean	p-value
	CFUs of S. mutans		**<0.001**
	White spot lesion group	106.87±8.235	
	No white spot lesion group	33.400±3.389	

Table 4: Comparison of mean CFUs of S. mutans in two study groups