



## Original Research Article

## Urease activity in caries free and caries active children

Vinod Kumar<sup>1,\*</sup><sup>1</sup>Dept. of Pedodontics, Navodaya Dental College, Raichur, Karnataka, India

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## ABSTRACT

**Background:** Few studies have proven that urease has caries inhibiting effect. Urease enzymes hydrolyze urea into ammonia and carbon dioxide. This ammonia raises the pH value of plaque and saliva which will reduce caries activity. The aim of this investigation was to measure and compare the urease activity level before and after carbohydrate rinse in caries free and caries active children of 5-12 years.

**Materials and Methods:** 30 caries free and 30 caries active children aged 5-12 years were selected for the study. A child was not eligible if he/she has systemic illness and received antibiotics within the last three months. DMFT/dmft was recorded to define the caries status, unstimulated saliva was collected in the eppendorf tubes and supra gingival plaque was collected from all available smooth surfaces. Urease activity was measured following biochemical protocol. Mean, standard deviation, median, and range (min.-max.) of urease activity was calculated. The Independent sample t test<sup>2</sup> was done to compare urease activity in both groups. Paired sample t test<sup>3</sup> was applied to know the change in the urease activity pre and post oral sucrose rinse in saliva and plaque

**Results:** Increased saliva and plaque urease activity was found in caries free group compared to caries active group and in both groups urease activity increased after oral carbohydrate rinse for both saliva and plaque samples

**Conclusion:** The strategy can be developed for carbohydrate rinse to generate higher urease activity for caries control and urease activity measurement could be considered as parameter for caries risk assessment in children.

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## 1. Introduction

The dental caries affects 50% of the children between the ages of 5 to 12 years as per Dental council of India report in 2003. Dental council of India (DCI) highlighted the overwhelming increase of caries especially in children aged 5-12 years group. In the national oral health survey done by DCI in 2002-2003, it has observed that 50% of children in the age group of 5-12 years had dental caries. DCI has specified that early diagnosis and preventive care can bring down the prevalence rate of dental caries in children.<sup>1</sup> Few studies have proven that oral urease enzyme have caries inhibiting effect. Urease enzymes released by oral bacteria hydrolyze urea into ammonia and carbon dioxide. This

ammonia raises the pH value of plaque and saliva which will reduce caries activity.<sup>2-5</sup> Also many studies have proven that oral bacteria such as *S. Salivarius* and *A. Naeslundii* produce insignificant amounts of urease but during the presence of excess carbohydrates urease production will increase in oral cavity which in turn raises pH level of saliva and plaque thus causing reduction of caries activity.<sup>6,7</sup>

This research is primarily focused on oral urease level activity in children after carbohydrate rinse which can be a preventive mechanism of dental caries in children. Oral cavity provides a unique intraoral bacterial environment that may have particular effect on caries susceptibility. The working hypothesis in this research is that during the cariogenic challenge urease activity in the saliva and plaque will be increased thus inducing the caries protection

\* Corresponding author.

E-mail address: [dr.vinod990@gmail.com](mailto:dr.vinod990@gmail.com) (V. Kumar).

mechanism in oral cavity.

The specific aim of this investigation is to measure the urease activity level in plaque and in saliva of children following exposure to sugar solution. For the above said purpose, urease level in the plaque and in the saliva of 60 children aged 5 to 12 year was measured after fasting and after oral rinse of 10% sucrose solution.

## 2. Materials and Methods

### 2.1. Subject recruitment

The study subjects were recruited from the outpatient pool of the Pediatric dentistry. Because of lack of specific pilot studies the sample size was determined arbitrarily. 30 caries free and 30 caries active children in the age group of 5-12 years was selected for the study. DMFT/dmft was recorded to define the caries status of the study sample. The age group selected was between 5-12 years because DCI in its investigation has found that highest caries level was found in 5-12 years age group, which requires preventive measures.<sup>1</sup> So this age group has been selected to study the level of urease activity. The purpose of the study design was explained to the parents of the selected patients, and informed written consent of the parents was obtained. The study protocol was approved by the Institutional Ethics Committee (Ethical certificate No: NDC/IEC/2018/112). The study excluded subjects with low salivary flow (less than 0.5 ml per gland in 5 minutes), who had used antibiotics or chlorhexidine in the previous month, with systemic diseases that precluded dental treatment, who had received dental bleaching treatment in the previous month or with periodontal disease. Subjects were equally divided into two groups based on the DMFT/dmft score.

Group 1: 30 caries free children. (Baseline DMFT/dmft score=0)

Group 2: 30 caries active children. (DMFT/dmft score >1)

### 2.2. Sample collection

The study subjects were refrained from eating and drinking anything but water for a minimum of 12 hours prior to sample collection. They had also refrained from oral hygiene procedures for at least 6 hours prior to sample collection to achieve sufficient plaque accumulation for collection. All samples were collected between 8 am and noon.

Initially, supragingival plaque was collected from all available smooth dental surfaces (buccal surfaces) on the upper and lower right quadrants using Gracey curette. Plaque was placed in pre-weighed micro-centrifuge tubes (eppendorf tube). Saliva samples were collected using a mucous trap attached to the dental suction/plastic conical tube/ pre-weighed micro-centrifuge tubes (eppendorf tube). A minimum of 2 ml of unstimulated saliva was collected.

Following the first sample collection (saliva and plaque) the subjects were asked to take 5 ml of a sterile 10% sucrose solution and swirl it around for 1 minute then spit it out. After 1 minute, a temporary drop in plaque pH will occur due to bacterial metabolism of the carbohydrate. The pH returns to baseline levels normally within 30 minutes following the exhaustion of the carbohydrate source.<sup>2</sup> Thus, a second plaque and saliva sample was collected by the same method 30 minutes following the oral rinse from the quadrants on the opposite side of the mouth. Plaque and saliva samples were placed immediately on ice and had been transferred to the laboratory, where they were weighed again. The difference in the weight of the tubes between pre- and post-sampling corresponds to the net weight of the respective plaque sample.

### 2.3. Biochemical measurement of urease

The enzyme activity is easily determined by measuring the ammonia formed colorimetrically.

Principle: The reaction is stoichiometric and the enzyme urease splits urea liberating ammonia and carbondioxide.

Preparation of standard graph: Ammonium sulphate solution was prepared by mixing 20 mg ammonium sulphate powder with 100 ml of distilled water. Different aliquots of it were taken and the volume was made up to 3ml using distilled water. 1ml of Nessler's reagent was added to it. The solution was mixed properly and the color intensity was measured at 500nm. NH<sub>3</sub> forms a brown complex in presence of Nessler's reagent (K<sub>2</sub> Hg I). A standard graph was plotted keeping concentration of standard (micro gm/ml) in the X axis and optical density (at 500 nm) in the Y axis.

Enzyme assay: Pipette out 1ml of 3% Urea solution buffered with 1ml of 0.2M phosphate buffer (pH 7). 0.5ml of saliva samples and plaque samples were added in different test tubes. This was incubated at 55<sup>0</sup> Celsius for 15 minutes. The tubes were quickly placed in the ice at the end of incubation time. 1ml of 0.66N H<sub>2</sub>SO<sub>4</sub> was added to stop the reaction and to precipitate the protein, 1ml of 1M sodium tungstate solution was added. To remove the precipitate and to obtain supernatant they were centrifuged for 15 minutes. Two ml of supernatant was pipetted out and 250 microlitre of Nessler's reagent was added to it. Then aliquots of supernatant were assayed for NH<sub>3</sub>. The color change of the solution was observed. The solution uptake was then measured by using uv-via Spectrophotometry at λ 500 nm and the obtained values were plotted against the graph to obtain the urease enzyme activity.<sup>8</sup> The urease activity was measured by the quantification of ammonia. Urease enzyme is measured in units as nano moles of ammonia produced per minute per milligram of protein.

#### 2.4. Statistical analysis

The collected data has been entered into MS Excel followed by the analysis using SPSS trial version 22. Demographic characteristics such as age, gender etc has been represented using Mean, SD and Percentages. The comparison of salivary and plaque urease activity between caries active and caries free children has been done using “Independent sample t test”. The change in the urease activity before and after the oral sucrose rinse in saliva and plaque has been tested using “paired sample t test”. P value <0.05 has been considered to be statistically significant.

### 3. Results

Majority of the participants from both Caries free and caries active groups were females (53% and 57% respectively). The average age of the participants from both caries free and caries active group were 9.30 years and 9.00 years, respectively (Table 1).

Comparison of Pre- Post values of urease in saliva and plaque before and after oral sucrose rinse: in caries free group salivary urease activity significantly ( $P < 0.001$ ) increased after oral sucrose rinse and plaque urease activity increased non-significantly ( $P = 0.372$ ) post oral sucrose rinse; in caries active group salivary and plaque urease activity significantly increased after oral sucrose rinse (Table 2).

Comparison of the pre rinse urease values of saliva and Plaque: salivary urease activity in caries free group was significantly ( $P < 0.001$ ) high in comparison to caries active group; Plaque urease activity in caries free group is non-significantly ( $P = 0.008$ ) high in comparison to caries active group (Table 3).

Comparison of the post sucrose rinse urease values of saliva and Plaque: Salivary and plaque urease activity in caries free group is significantly high ( $P < 0.001$ ) in comparison to caries active children (Table 4).

### 4. Discussion

Our current investigation is established on biochemical reaction of urea into ammonia and carbon dioxide by urease enzyme which results into alkalization of pH of saliva and plaque leading to inhibition of cariogenic potential in oral cavity.

The findings of our investigation proved that a urease activity in both saliva and plaque was found to be higher in caries free children which was in agreement with the studies of Nascimento, et al. (2009)<sup>9</sup> and Gordan V, et al. (2010).<sup>10</sup>

Shu M (2007)<sup>11</sup> and Frostel in 1960 et al.<sup>12</sup> found higher urease levels in the plaque of caries-free subjects compared to a caries-active group.

Some previous research has proved that chewing the urea added chewing gum after sucrose rinse results into pH recovery in dental plaque (Imfield et al., 1995).<sup>13</sup>

In the nature urease enzyme is synthesized by many bacteria like *Bacillus pasteurii*, *Morganellamorganii*, and some isolates of *E. coli*. Urease expression in most of bacteria is regulated by urea and nitrogen availability (Mobley et al., 1995).<sup>14</sup>

Many invitro studies have proved that expression of urease enzyme in oral bacteria is controlled by the pH, glucose availability, and growth rate (Chen Y et al., 1995).<sup>6</sup>

The urease activity in *S. Salivarius* is decreased at neutral pH and low availability of sugars but it increases to 650 fold during the high sugar availability and at acidic pH. The expression of urease in response to pH is controlled at the transcriptional level. The oral bacteria *A. naeslundii*, is a pioneer organism in the oral cavity and a highly abundant species in dental plaque, the urease expression in this organism drops low when nitrogen sources increases. However, this organism can produce significant levels of urease activity when its grown the organism is grown under limited nitrogen sources, which correspond to conditions of carbohydrate-excess. Thus, *A. naeslundii* significantly contribute to the pH-moderating activity of oral biofilms helps in prevention of caries (Morou-Bermudez et al., 2000).<sup>7</sup>

Liu et al found that many environmental factors influence the urease activity of *A. naeslundii* growing under pH dilution rate, carbohydrate availability and nitrogen availability. They proved that urease expression in biofilms increases with higher glucose availability.<sup>1</sup>

Our results indicated that urease activity in both saliva and plaque of caries free and caries active group increased after carbohydrate rinse and the above observations from the previous studies indicate that oral bacteria have a greater capacity to produce excess urease when there is excess oral presence of carbohydrates and low acidic plaque pH. This in vivo study indicated that urease activity in saliva and plaque is an important endogenous caries preventing mechanism.

Finally, the most important evidence of this study is that the natural endogenous alkaligenic potential of the oral environment is associated with caries free children due to high urease activity and there was increase in urease activity after oral carbohydrate rinse in both caries free and caries active groups. This investigation is a pilot study which will be forerunner for larger studies with larger sample size. The findings of these investigations will provide an insight into the level of urease activity in caries free and caries active children and also urease activity after carbohydrate rinse, which will provides us information about the various levels of the protective mechanisms of dental caries in caries risk children.

The clinical implication of the study is to develop a strategy for carbohydrate rinse and to chew urea added chewing gums to generate higher urease activity which will recover alkaligenic potential of the saliva and plaque thus preventing caries through endogenous mechanism.

**Table 1:** Gender and age distribution

Groups	Males N (%)	Females N (%)	Minimum Age	Maximum Age	Mean Age	SD
Caries free	14(47%)	16(53%)	6	12	9.30	1.41
Caries active	13(43%)	17(57%)	7	12	9.00	1.08

**Table 2:** Comparison of Pre- Post values of urease in saliva and plaque before and after oral sucrose Rinse

Groups	Saliva/plaque samples	Pre-rinse Mean $\pm$ SD	Post rinse Mean $\pm$ SD	P value
Caries free	Saliva	1.25 $\pm$ .366	1.66 $\pm$ .440	<0.001
	Plaque	1.29 $\pm$ 1.30	1.51 $\pm$ .373	0.372
Caries active	Saliva	.609 $\pm$ .446	.813 $\pm$ .511	<0.001
	Plaque	.602 $\pm$ .408	.782 $\pm$ .501	<0.001

**Table 3:** Comparison of the pre rinse urease values of saliva and Plaque between caries active and caries free group

Urease activity		Min	Max	Mean	SD	Mean Difference	P value
Saliva	Caries free	0.40	.22	1.25	.366	.641	<0.001
	Caries active	0	1.4	0.609	.466		
Plaque	Caries free	.30	8.0	1.29	1.30	.680	0.008
	Caries active	.10	1.5	.602	.408		

**Table 4:** Comparison of the post rinse urease values of saliva and Plaque between caries active and caries free group

Urease activity		Min	Max	Mean	SD	Mean Difference	P value
Saliva	Caries free	.84	2.64	1.66	.440	.847	<0.001
	Caries active	.12	1.80	.813	.511		
Plaque	Caries free	.50	2.60	1.51	.373	.731	<0.001
	Caries active	.16	1.76	.782	.501		

## 5. Conclusion

In our study caries-free subjects had a higher urease activity compared to caries active groups, and in both groups urease activity increased after oral carbohydrate rinse for both saliva and plaque samples. According to the results, the presented hypothesis is accepted.

Oral urease level measurement is economical and can be done in laboratory using routine equipment's. Urease level activity in children could be considered as parameter for caries risk assessment in children. The findings of this research provide contribution for the expansion of knowledge about the novel methods of prevention of dental caries which disproportionately affects large number of children.

## 6. Conflict of Interest

None

## 7. Source of Funding

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### Author biography

Vinod Kumar, Professor and HOD

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