Dental Research Journal

Original Article

Association between early childhood caries, *streptococcus mutans* level and genetic sensitivity levels to the bitter taste of, 6-N propylthiouracil among the children below 71 months of age

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Received: January 2012
Accepted: June 2012

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ABSTRACT

Background: Oral health is an integral component of pre-school health and well-being. Unfortunately, many children are afflicted with dental caries at an early age, even those as young as 12 months of age. The purpose of this study is to determine the association between Early Childhood Caries (ECC), *Streptococcus mutans* and genetic sensitivity levels to the bitter taste of, PROP among the children below 71 months of age.

Materials and Methods: Total of 119 children belonging to the age group of 36 to 71 months of both sexes, were recruited from A. J. Institute of Dental Sciences, Mangalore (Karnataka). PROP sensitivity test was carried out to determine the inherent genetic ability to taste a bitter or sweet substance. One who tasted bitter as taster and one who was not able to differentiate/tasted like paper as non-tasters. Facial expression was observed during the tasting to support the verbal response. Estimation of *S. mutans* level and caries experience was recorded. The results were statistically analyzed using Mann'Whiteney-U Test and Kruskal value test.

Results: In the total of 119 children, the mean DMFS was definitely higher in non-taster children compared to tasters and also had a high *S. mutans* level. Tasters had low ECC experience, low *S. mutans* level. The tasters had a mean DMFS value of 9.5120 (S.D. 7.0543) and non-tasters had a value of 7.7250 (S.D. 8.33147), which was statistically significant.

Conclusion: Children who had higher level *S. mutans* had ECC and were non tasters. The PROP sensitivity test (filter paper test) proved to be a useful diagnostic tool in determining the genetic sensitivity levels of bitter taste. Age and low socio-economic status of pre-school children suggest a complex multifactorial relationship between *S. mutans* colonization, ECC and taste perception.

Key Words: Early childhood caries, propylthiouracil, *S. mutans*, taste

INTRODUCTION

Oral health is an integral component of pre school health and well-being. Unfortunately, many children are afflicted with dental caries at an early age, even those as young as 12 months of age. The presence of one or more decayed (non cavitated or cavitated), missing (due to caries), or filled tooth surface in any primary tooth in a child 71 months of age or younger is termed as ECC (Early childhood caries).[1] It is a chronic, transmissible and infectious disease with a complex and multi-factorial etiology and is strongly influenced by excessive bottle feeding with sugar contained liquids; breastfeeding on demand, nursing beyond recommended age for weaning, increase in intake of sugar-rich foods and unbalanced diet.[2,3] Other factors associated with ECC include: Genetic predisposition, parental education, nutritional, environmental, socio-economic, and parental style

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factors.[1] Those affected, often suffer from a reduced oral health-related quality of life when compared with their caries-free peers.

Although, the rapid secular rise is due to significant food intake in promoting caries risk, genes presumably interact along with food intake conferring difference of caries risk in individuals. One such genetic factor is bitter taste perception, which appears to be a large part mediated by the TAS2R38 gene. The insensitivity to bitter compounds like 6-n-propylthiouracil (PROP) is mediated by this gene.[4] PROP, a pharmacological drug used in treatment of graves' disease, proved to be useful tool in determining the genetic sensitivity levels to bitter and sweet taste. Inherited behavior and taste thresholds may play an important role in the frequency of carbohydrate intake. Genetic sensitivity to taste may be associated with the preference for or rejection of some foods by children.[5]

Most of the studies on taste and caries have been conducted on school going children and adults. There is a relative paucity of studies in the dental literature with respect to the relationship between ECC and taste. A child's preferences for sugar may lead to increase in dental caries and a compromised state of health.[2,6-8]

Streptococcus mutans (*S. mutans*) is the main microbial etiologic factor in dental caries. Increased salivary levels of *S. mutans* are associated with an increased risk for dental caries. The early colonization of *S. mutans* in children is associated with dental caries.[9,10]

The purpose of this study is to determine the association between ECC, *S. mutans* and genetic sensitivity levels to the bitter taste of, PROP among the children below 71 months of age.

MATERIALS AND METHODS

Source of data

A total number of 119 children belonging to the age group of 36 to 71 months of both sexes, who reported to the department of Pedodontics and Preventive Dentistry, were the part of this study.

Exclusion criteria

1. Children whose parents do not give their consent for the examination.
2. Children under any medication and anti-biotics three months before the study.
3. Children who did not fall under American Society of Anesthesiologists (ASA) physical status of class I.

Methodology

Preparation of the prop strips

The pure sample of PROP was obtained from the pharmaceuticals and the PROP strips were prepared in the department of pharmaceutics, N.G.S.M Institute of Pharmaceutical Sciences, Mangalore. Whattman filter paper was cut into 2 × 2 cm size and sterilized in an autoclave at 121°C for 15 min. The sterilized strips was weighed and stored in the desiccator till they were used for further preparation, 6-n-propylthiouracil (10 mg/ml) was dissolved in 5 ml of ethyl alcohol in a beaker, 10 previously cut and sterilized Whattman filter paper strips were soaked in the above solution for 1-h for the complete absorption of the drug. The strips were removed and were allowed to dry at room temperature. The weight of the strips after the drying was determined and recorded. The difference between the initial weight of the filter paper and the weight after the impregnation of the drug gives the actual amount of drug impregnated on each strips. An average amount of the drug impregnated on each strip was approximately 1.6 mg.

PROP sensitivity test

119 children were classified as taster and non-taster by performing PROP sensitivity test. PROP sensitivity test was carried out by placing a filter paper containing approximately 1.6 mg of 6-n-propylthiouracil on the dorsal surface of the subject's tongue for 30 seconds; to determine the inherent genetic ability to taste a bitter or sweet substances. After tasting the paper they were asked, "Do you taste anything?" If their response was no, they were asked whether it tasted like paper. If they responded that it had no taste and tasted like paper, they were classified as non-tasters. If they responded that it tasted yucky or bad they were classified as tasters. If they responded that they did not know what it tasted like or provided an ambiguous response, they were retested at a later time. Facial expressions were observed during the tasting to support the verbal response and to identify any ambiguous or conflicting responses.[11,12]

Caries experience

The caries experience (DMFS index) was recorded using visible light, mouth mirror and CPI probe. All the teeth were examined for coronal surface caries and restorations. The number of decayed, missing and filled surfaces (DMFS) in the coronal portion of each tooth was determined.[13,14]

Estimation of streptococcus mutans level

Paraffin wax stimulated whole saliva was collected between 9.30-11.30 am. The subjects were asked to refrain from eating for one hour before collection. 2 ml of stimulated saliva was collected in a sterile bottle and transported to the microbiology laboratory. By means of a sterile disposable syringe 1 ml aliquot of saliva was transferred from the bottle to the previously labeled sterile tube containing 4 ml of broth (thioglycolate broth). The saliva sample was vortexed, to uniformly mix the saliva and the broth using a cyclomixer. Using an inoculation loop (4 mm inner diameter) 10 ul of the vortexed 1:5 dilution sample was streaked in duplicate on Mitis salivarius bacitracin agar (MSB) selective for *S. mutans*. The MSB agar plates were incubated anaerobically for 24 to 48 h at 37°C in 5% CO 2 in nitrogen. Following incubation, counts was done for colonies with morphological characteristic for *S. mutans* on the MSB agar. Identification for *S. mutans* was confirmed by biochemical tests like manitol fermentation and gram staining catalyst test. Colony counting was done with a magnifying glass and the count of *S. mutans* was expressed as the number of colony forming units per milliliter (cfu/ml) of saliva.

Semiquantitation of the number of colonies was done by multiplying the actual colony count with 1 × 103 because of the part that the saliva sample diluted one thousand times (1:5 dilution).[8,15-18] The results were subjected to statistical analysis

RESULTS

In the total of 119 children the mean dmfs was more in non-taster children compared to tasters. The tasters had a mean dmfs value of 9.5120 (S.D 7.0543) and non-tasters had a value of 7.7250 (S.D 8.33147), which was statistically significant [Table 1, Figure 1].

The mean dmfs in the low *S. mutans* level group was 6.5652 (S.D 9.2038), moderate *S. mutans* level group the value was 7.5676 (S.D 5.09386), and in the high *S. mutans* level group of children had a value of 17.3182 (S.D 10.19984). This was found to be statistically highly significant [Table 2, Figure 2].

The mean *Streptococcus mutans* count was found to be 1.21 × 10^6 in 65 tasters and 53 non tasters had mean Streptococcus Count of 1.39 × 10^6, which was also found to be statistically significant [Table 3].

Table 1: Multiple comparisons of the mean dmfs among tasters and non-tasters

Taster groups	N	Mean	Std. deviation
Taster	43	9.5120	7.0543
Non-taster	76	7.7250	8.33147

Z=2.195, *P*=0.046 sig

Table 2: *Streptococcus mutans* count with mean dmfs

S. mutans count	N	Mean	Std. deviation
Low	23	6.5652	9.20388
Moderate	74	7.5676	5.09386
High	22	17.3182	10.19984

H=32.858, *P*<0.001 vhs

Table 3: *Streptococcus mutans* count among tasters and non-tasters mutans vs. PROP

Taster group	N	Mean	Std. deviation
Tasters	65	121649.1	954713.3
Non-tasters	53	139492.2	695198.1

H=29.91, *P*<0.001 vhs

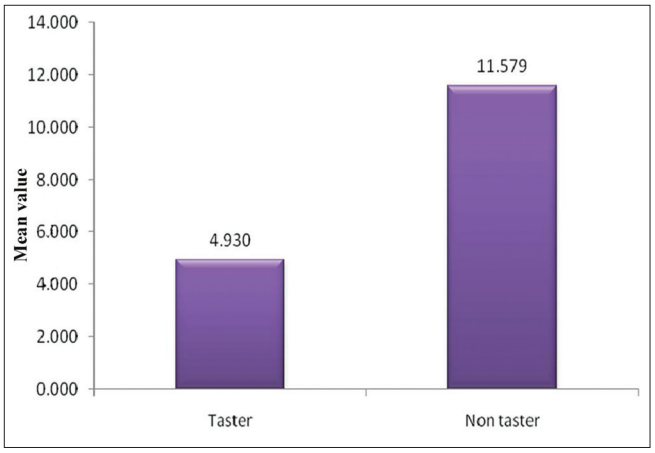

Figure 1: Bar diagram showing the mean dmfs among the taster group

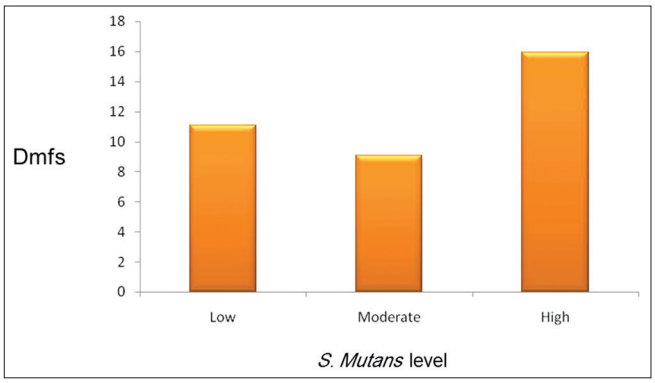

Figure 2: Bar diagram showing the mean dmfs vs. *Streptococcus mutans* level

DISCUSSION

ECC is the most common chronic disease in childhood, resulting in a severe problem for worldwide public health. It is a virulent form of dental caries that can destroy the primary dentition of toddlers and pre-school children. ECC is a multi-factorial disease and children's genetic taste perception is a common underlying factor in their causation. Hence, the present study was conducted to assess the relationship between caries experience, *S. mutans* and genetic taste sensitivity levels among children aged 36 to 71 months.

Of the 119 children of screened at Department of Pedodontics and Preventive Dentistry. 33.13% were tasters and 63.86% non-tasters. The children who were in taster and non-taster category could be identified easily as they would either find the taste of PROP extremely bitter or absolute tasteless respectively.

In this study, we used a much simpler scaling; PROP strip paper test; one who tasted bitter as taster and one who was not able to differentiate/tasted like paper as non-taster. Although, previous studies were conducted using PROP paper strip method in young children, their results were found to be inappropriate.[19] We found children at this young age are in a state of developing psyche and undergo behavioral changes. So, the use of LMS scales in children below 71 months of age would not produce appropriate results. Labeled Magnitude Scale (LMS) is a quasi logarithmic scale with label descriptors that is equivalent to magnitude estimation. Moreover, we modeled our methods in using facial expression to inform classification of children as PROP tasters.[20,21] Our experience in conducting this protocol suggested that facial expressions in these young children was a valid indicator of tasting bitter, but this question requires further investigation.

The caries experience (dmfs) was found to be more in non-tasters than in tasters with mutans streptococci counts. Majority of the non-tasters were sweet likers and preferred strong tasting food products, while majority of the tasters were sweet dislikers and preferred weak tastes. The data recorded in this study reveals that PROP non-tasters tended to be 'sweet-liker' with increased caries risk and PROP tasters tended to be 'sweet-dislikers' with decreased caries risk. Tasters or sweet dislikers might avoid sweet food because their oral sensations are too intense and thus, less pleasant to accept the intensely bitter, strong sweet substances thus making tasters less prone to decay. This could be also due to the number of distribution of fungiform papillae on the tongue, which are present in high numbers in anterior third, on the sides and tip of the tongue. The number of fungiform papillae is related to genetic variation in the ability to taste. Tasters have a higher growth of fungiform papillae as compared to non-tasters.[22]

Thus, the non-taster children may have a higher concentration and higher frequency of sugar intake compared to children who are tasters, and are therefore more susceptible to dental caries and *Streptococcus mutans* levels in their mouth.

Moreover, our result shows high levels of mutans Streptococcus in non-taster group. This may be due to high carbohydrate intake in non-tasters groups. However, differentiation made between taster and non-taster group in our study is purely genetical, by use of PROP strips. Therefore the oral colonization of Mutans streptococci may be due to genetic makeup of host related factors, including the nature of the oral biofilm.[22-24]

Thus, in this study a strong correlation was found between ECC, Microbiological and Genetic sensitivity levels to the bitter taste of PROP. Hence, the current study has been successful in gathering a wide spectrum of etiological factors that contribute to the development of ECC. A strong positive correlation was established in present study between genetic sensitivity levels of a pre-schooler with ECC experience.

It has generally been assumed in the past that ECC is mainly determined by environmental factors, and so most of the strategies for preventing or managing the disease have focussed on modifications to that environment, including oral hygiene or diet alteration. However, ECC continues to be a major public health issue, even in Asian countries like India.[1,3] There is a growing interest in the identification of risk factors that might predispose individuals to Early Childhood Caries and also in identifying factors that might provide individuals with protection. A greater understanding of the behaviour of cariogenic bacteria in the oral environment, together with improved knowledge of the nature of the interplay between a person's genetic makeup and their exposure to environmental factors, should lead to better methods for assessing caries risk and, in turn, establishing more effective prevention strategies.[25]

CONCLUSION

In conclusion, clinical applications of findings from our study focus on PROP strip technique as a diagnostic tool for early detection of early childhood caries. The current paradigm of disease treatment is not designed to account for multitude of genetic information known to impact our oral health. Knowledge of genetic predisposition to host bacteria associated with caries could allow for prophylactic treatment for patients. Within the study limitations, results from this analysis of a small sample of pre-school children suggest a complex multi-factorial relationship between taste perception, ECC and *S. mutans* levels

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How to cite this article: Pidamale R, Sowmya B, Thomas A, Jose T, Madhusudan KK, Prasad G. Association between early childhood caries, *streptococcus mutans* level and genetic sensitivity levels to the bitter taste of, 6-N propylthiouracil among the children below 71 months of age. Dent Res J 2012;9:730-4.

Source of Support: Nil. **Conflict of Interest:** None declared.