Research Article

Knowledge, awareness and perception about salivary markers in dental caries – A survey

Preetha Parthasarathy[1], V. Vishnu Priya[2], R. Gayathri[2]*

ABSTRACT

Introduction: Dental caries is characterized by the destruction of tooth tissues by synergistic complex effects among acids generated from the fermentation of dietary carbohydrates by bacteria and susceptible host factors, such as teeth and saliva. Cavities are most commonly seen in patients with a lower salivary flow due to a decrease in the antibacterial, buffering and cleansing functions. The aim of this study is to evaluate the knowledge, awareness and perception among dental students on salivary biomarkers in dental caries. **Materials and Methods:** The study participants answered a structured questionnaire to determine their knowledge and awareness regarding the biomarkers used in caries detection. Result: From this survey, 94% of the participants opted that saliva can be used in early diagnosis of dental caries. 62% of the students are aware that the predominantly prevalent caries associated species in humans to be Streptococcus mutans. **Conclusion:** From the survey conducted, it can be concluded that awareness among dental students about salivary markers in caries detection is fair and there is the need for continuous education and for formal inclusion of the methods used in detection of caries, in the students' curriculum.

KEY WORDS: Saliva, dental caries, salivary biomarkers

INTRODUCTION

Dental caries are one of the most common chronic infectious diseases of school-going children characterized by the destruction of tooth tissues by synergistic complex effects among acids generated from the fermentation of dietary carbohydrates by bacteria and susceptible host factors such as teeth and saliva.[1]

Early childhood caries (ECC) is a term used to describe dental caries in children aged 6 years or younger. Streptococci, such as *Streptococcus mutans* and *Streptococcus sobrinus*, are considered to be the key agents in producing dental caries in children. Other bacteria, such as *Prevotella* spp. and *Lactobacillus* spp., and fungus, that is, *Candida albicans*, are related to the development and progression of ECC.[2]

Biomolecules in saliva, mainly proteins, affect the survival of oral microorganisms by multiple innate defensive mechanisms, thus regulating the oral microflora. Therefore, the protein composition of saliva can be a sensitive indicator for dental health.

Human saliva is considered as a clear, slightly acidic (pH 6.0–7.0) heterogeneous fluid that is composed of 98% water and 2% other compounds such as electrolytes, mucus, antibacterial compounds, and various enzymes.[3,4] Saliva is also a complex mixture of oral fluids which is composed of salivary gland secretions, gingival crevicular fluid, expectorated bronchial and nasal secretions, serum and blood derivatives from oral wounds, bacteria and bacterial products, viruses, fungi, desquamated epithelial cells, other cellular components, as well as food debris.[5,6]

Salivary proteins are believed to protect oral cavity by protecting against caries by a film of salivary mucins and proline-rich glycoprotein, early pellicle proteins, proline-rich proteins, and statherin. They also promote remineralization of the enamel by attracting calcium ions, retarding demineralization by the pellicle proteins, in concert with calcium and phosphate ions in saliva.[7] Salivary glycol proteins

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[1]Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India, [2]Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India

***Corresponding author:** V. Vishnu Priya, Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai - 600 077, Tamil Nadu, India. E-mail: drvishnupriyav@gmail.com

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prevent the adherence of oral microorganisms to the enamel pellicle and inhibit their growth.

The development of dental caries may be related by the disparity between free radicals levels and antioxidants in saliva. Antioxidants combat the adverse effects of any reaction that causes excessive oxidations by neutralizing the toxicity of free radicals and cytokines, and reduction in antioxidant levels leads to oxidative stress.[8,9] It has been shown that total protein and total antioxidant in saliva increased with caries activity. Moreover, a higher total antioxidant capacity (TAC) was observed with dental caries in deciduous but not in permanent teeth and TAC increased with the age of the children.[10]

The salivary flow is important in the prevention of caries, and there is a high risk of caries in individuals with a low unstimulated salivary flow.[11]

Cavities are most commonly seen in patients with a lower salivary flow due to a decrease in the antibacterial, buffering, and cleansing functions. The salivary flow helps in dilution of the substances, cleans the oral cavity off carbohydrates, non-adherent bacteria, desquamated epithelial cells, and food debris. This phenomenon is essential for decreasing the availability of sugars for the biofilm.[12] The salivary viscosity reduces the hydration capacity of saliva and consequently raises the caries risk.

The biomarkers of dental caries are as follows:

I. Functional properties of saliva as biomarkers of caries
    1. Salivary flow rate
    2. Salivary pH and buffering capacity
II. Microorganisms
III. Salivary electrolytes
IV. Salivary proteins (Proteomes)
    1. Immunoglobulins (Igs)
    2. Acidic proline-rich proteins
    3. Mucins 1 e2 (mucous glycoprotein [MGs])
    4. Agglutinins
    5. Lactoferrin and lysozyme
    6. Cystatin S and statherin
    7. Defensin
    8. CD14
    9. Glucosyltransferases (GTFs)
    10. Amylase.

The main responsible bacteria for this disease are the endogenous strains *S. mutans*, *S. sobrinus,* and *Lactobacillus* species present in the biofilm. A key role of *mutans* streptococci in the initiation of caries has been well established due to the formation of extracellular polysaccharides from sucrose which is responsible for their firm attachment to teeth and promotes tight cell clustering, rapid fermentation of carbohydrates to acids, and tolerance to low pH.[13]

Among salivary electrolytes, fluoride, calcium, phosphate, and bicarbonate are considered of particular importance for protecting teeth from caries. The presence of fluoride in saliva is very important because it reduces acid production in the biofilm.[14] Fluoride, calcium, and phosphate ions keep saliva supersaturated with respect to hydroxyapatite and offer a reparative and protective environment for maintaining the integrity of dental tissues.

The Igs in saliva primarily belong to the IgA subclass (>85%) and to some extent, to the IgG and IgM subclasses. Altogether, Igs make up 5–15% of total salivary proteins. Salivary antibodies are the first line of immune defense against antigens present in saliva.[15]

MGs (mucins) constitute a family with two members, namely high-molecular-weight mucins (MG1) and low-molecular-weight mucins (MG2). There is inverse relationship between mucins 1 and 2 levels and the prevalence of dental caries. The mucins of saliva form a seromucosal cover that protects, lubricates, prevents the dehydration, and maintains the viscoelasticity of saliva.[14]

Salivary agglutinin is a mucin-like glycoprotein that is known to mediate the aggregation of many oral bacteria. Agglutinins interact with unattached bacteria, resulting in clumping of bacteria into large aggregates, which are more easily swallowed or flushed away.[16]

Lactoferrin competes with various microorganisms in binding to free iron; this competition mechanism has a bacteriostatic and bactericidal effect on various microorganisms that depend on this ion to survive.[14]

Saliva presents seven different cystatins; cystatin A, cystatin B, cystatin C, cystatin D, cystatin S, cystatin SA, and cystatin SN. These proteins are cysteine protease inhibitors and are mainly present in submandibular saliva. The capacity of oral protection of cystatins is thought to be related to the inhibition of cysteine proteases. Cystatin S can be phosphorylated in five sites. Statherin has many functions, the most important being the inhibition of precipitation in supersaturated solutions of calcium.[17] Therefore, it is the primary regulator of mineralization in the oral cavity.

Defensins are small, cationic proteins with antimicrobial activity. The bacterial charge is an important factor for the susceptibility of bacteria to cationic peptides. These peptides are able to kill a variety of Gram-positive and Gram-negative bacteria, fungi, and enveloped viruses.[18] Defensins can be divided in two subfamilies including α-defensins and β-defensins. Higher salivary a-defensins (HNP1, 2, and 3) have been detected in caries-free children than in children affected by caries.

CD14 acts as an important anticariogenic factor. An inverse relationship is seen between the presence of sCD14 in saliva and caries lesions. It helps in the binding between the epithelial cells and bacteria and activates the production of cytokines for the recruitment of phagocytes.

The dental caries is an infectious disease and studies indicate that can be preventable with mucosal immunization. GTFs from *S. mutans* are a candidate for the production of dental caries vaccine.[19]

Hence, this survey aimed at evaluating the knowledge and awareness among dental students regarding whether salivary microbes can be utilized as biomarkers for ECC risk assessment,[2] the influence of salivary proteins on oral microorganisms and caries occurrence and[3] whether salivary proteins can be used as biomarkers to predict ECC susceptibility and outcomes.

MATERIALS AND METHODS

The study has adopted a cross-sectional study design with a sample size of 100 dental students and interns. The students belonged to the third, fourth, and internship, which are the clinical years of the course.

All participants answered a questionnaire to determine their knowledge and awareness regarding the biomarkers used in caries detection. A structured questionnaire was designed and developed on the basis of thorough literature review. It included various sections such as age, gender, level of education, and awareness on the different types of markers in caries risk assessment, culture methods, and other diagnostic methods.[20]

Questionnaire

1. Can saliva be used in early detection and diagnosis of dental caries? Yes/No.
2. Presence of low levels of soluble IgA in saliva possesses high risk of caries progression. True/false.
3. Presence of high levels of mucin glycoprotein 1 and 2 in saliva can help reduce the risk of dental caries. Yes/No.
4. Are you aware that statherin and cystatin S are used as salivary biomarkers in caries assessment? Yes/No.
5. Predominantly prevalent caries associated species in humans are (a) *S. mutans* (b) *S. aureus* (c) *S. sobrinus*.
6. The *S. mutans* are most susceptible to (a) hBD1 (b) hBD2 (c) hBD3.
7. Which one of the following is a candidate of *S. mutans* for the production of dental caries vaccine (a) GTF (b) Hyaluronidase (c) Phosphofructokinase.
8. Presence of low levels of alpha-defensins HNP1-3 can contribute to caries susceptibility in children. Yes/No.
9. Can polymerase chain reaction (PCR)-based bacterial identification be used to detect the array of microorganisms in saliva? Yes/No.
10. Which of the following technique is more sensitive for enumeration for *S. mutans* in saliva? (a) PCR (b) Quantitative PCR (qPCR) (c) Culture methods
11. Does salivary buffering capacity protects the tooth from dental caries? Yes/No.
12. High risk of caries susceptibility is associated when the unstimulated salivary flow rate is lower than (a) 0.5 mL/min (b) 1 mL/min (c) 0.3 mL/min.
13. High risk of caries susceptibility is associated when the stimulated salivary flow rate is lower than (a) 0.3 mL/min (b) 0.5 mL/min (c) 0.7 mL/min.
14. Currently available commercial kit for detection of salivary lactobacilli is based on (a) Rogosa's medium (b) Mitis salivarius-bacitracin (MSB) medium (c) MSB broth medium.

RESULTS

From the survey conducted, 94% of the participants agree that saliva can be used in early diagnosis of dental caries. 62% of the students are aware that the predominantly prevalent caries associated species in humans to be *S. mutans*. About 46% of the participants are aware that *S. mutans* is susceptible to Hbd2. 88% of the survey population are aware of the usage of PCR in detection of microorganisms in saliva. About 72% of the participants find Rogosa's medium to be the kit used in detection of lactobacillus in saliva.

DISCUSSION

From the present study, the awareness on salivary markers in caries risk assessment was found to be fair.

From the research conducted by Lihong *et al*., which states that dental caries-associated oral streptococci are called the *mutans* streptococci with *S. mutans* (*S. mutans*) and *S. sobrinus* (*S. sobrinus*) being the predominantly prevalent caries-associated species in humans. Furthermore, it is considered a potential risk factor when the unstimulated salivary flow rate is lower than 0.30 mL/min and the stimulated salivary flow is lower than 0.7 ml/min. PCR-based bacterial identification can detect a large array of microorganisms in saliva and provides accurate measurements of the known cariogenic species in saliva. The real-time qPCR technique was found to be more sensitive for enumeration of *S. mutans* in saliva compared to the traditional culture-based methods.

According to El-Kwatchy *et al*., the study shows that *S. mutans* and lactobacilli play an important role in the pathogenesis of dental caries. The number of

children with *S. mutans* or lactobacilli ≥105 cfu/ml was significantly higher in caries affected children compared to caries-free children. Children with caries experience had low levels of α-defensin and salivary pH and greater numbers of *S. mutans* and lactobacilli.[8]

CONCLUSION

From the survey conducted, it can be concluded that awareness among dental students about salivary markers in caries detection is fair and there is the need for continuous education and for formal inclusion of the methods used in detection of caries, in the students' curriculum. The dental caries affect the salivary proteome. Consequently, saliva appears to be a potential source of biomarkers for dental caries. Further studies are needed to define whether the individual has an increased risk of caries

REFERENCES

1. Hemadi AS, Huang R, Zhou Y, Zou J. Salivary proteins and microbiota as biomarkers for early childhood caries risk assessment. Int J Oral Sci 2017;9:e1.
2. Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancet 2007;369:51-9.
3. Van Nieuw Amerongen A, Bolscher JG, Veerman EC. Salivary proteins: Protective and diagnostic value in cariology? Caries Res 2004;38:247-53.
4. Zalewska A, Zwierz K, Zółkowski K, Gindzieński A. Structure and biosynthesis of human salivary mucins. Acta Biochim Pol 2000;47:1067-79.
5. Mandel ID, Wotman S. The salivary secretions in health and disease. Oral Sci Rev 1976;8:25-47.
6. Kaufman E, Lamster IB. The diagnostic applications of saliva a review. Crit Rev Oral Biol Med 2002;13:197-212.
7. Nireeksha S, Hegde MN, Kumari NS, Ullal H. Salivary proteins as biomarkers in dental caries: In vivo study. Dent Oral Craniofac Res 2017;3:1-7.
8. El-kwatehy WM, Youssef AR. Salivary biomarkers in caries affected and caries free children. Int J Dent Oral Sci 2016;3:348-52.
9. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990;186:421-31.
10. Tulunoglu Ö, Demirtas S, Tulunoglu I. Total antioxidant levels of saliva in children related to caries, age, and gender. Int J Paediatr Dent 2006;16:186-91.
11. Llena-Puy C. The rôle of saliva in maintaining oral health and as an aid to diagnosis. Med Oral Patol Oral Cir Bucal 2006;11:E449-55.
12. Navazesh M, Christensen C, Brightman V. Clinical criteria for the diagnosis of salivary gland hypofunction. J Dent Res 1992;71:1363-9.
13. Zaura E, Nicu EA, Krom BP, Keijser BJ. Acquiring and maintaining a normal oral microbiome: Current perspective. Front Cell Infect Microbiol 2014;4:85.
14. García-Godoy F, Hicks MJ. Maintaining the integrity of the enamel surface: The role of dental biofilm, saliva and preventive agents in enamel demineralization and remineralization. J Am Dent Assoc 2008;139:25S-34S.
15. De Almeida Pdel V, Grégio AM, Machado MA, de Lima AA, Azevedo LR. Saliva composition and functions: A comprehensive review. J Contemp Dent Pract 2008;9:72-80.
16. Bansode PV, Pathak SD, Wavdhane MB, Birage PP. Salivary biomarkers of dental caries a review article. IOSR J Dent Med Sci (IOSR-JDMS) 2019;18:52-8.
17. Vitorino R, Lobo MJ, Ferrer-Correira AJ, Dubin JR, Tomer KB, Domingues PM, *et al.* Identification of human whole saliva protein components using proteomics. Proteomics 2004;4:1109-15.
18. Fábián TK, Hermann P, Beck A, Fejérdy P, Fábián G. Salivary defense proteins: Their network and role in innate and acquired oral immunity. Int J Mol Sci 2012;13:4295-320.
19. Chia JS, You CM, Hu CY, Chiang BL, Chen JY. Human T-cell responses to the glucosyltransferases of streptococcus mutans. Clin Diagn Lab Immunol 2001;8:441-5.
20. Guo L, Shi W. Salivary biomarkers for caries risk assessment. J Calif Dent Assoc 2013;41:107-9, 112-8.

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