Original Article

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Dentistry Section

Estimation of Levels of Salivary Mucins MUC5B, MUC7 in Subjects with Dental Caries: A Case-control Study

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ABSTRACT

Introduction: Dental caries is a chronic, biofilm-mediated disease that results in the loss of tooth structure. It is considered multifactorial, and one of the contributing factors is mucin. Therefore, this study was conducted to investigate whether there is a correlation between the levels of salivary mucins MUC5B and MUC7 and dental caries.

Aim: To estimate the levels of salivary mucins MUC5B and MUC7 in subjects with dental caries.

Materials and Methods: A case-control study was conducted at Meenakshi Ammal Dental College and Hospital, Chennai, Tamil Nadu, India, over a period of three months (July 2022 - September 2022). Matching based on age in a ratio of 1:1 was performed, and approximately 66 samples were collected from participants aged 18 to 25 years. Of these, 33 saliva samples were collected from participants with a Decayed, Missing, and Filled Teeth (DMFT) index of over 10, forming the experimental group. Another 33 saliva samples were collected from participants with a DMFT index of less than 3, serving as the control group. The levels of MUC5B and MUC7 were assessed among both groups, and means and standard deviations were calculated. An independent sample t-test was utilised to determine whether the difference between the means of the two groups was statistically significant at a 95% confidence interval.

Results: The mean concentration of MUC5B in the experimental group (24.34±9.16 µg/L) was higher than in the control group (9.39±10.44 µg/L), demonstrating a statistically significant difference ($p<0.05$). There was also a significant statistical difference ($p<0.05$) in the levels of MUC7, which were higher in the experimental group (10.27±3.21 µg/L) compared to the control group (4.81±3.96 µg/L).

Conclusion: The study showed that the levels of both salivary mucins were elevated in participants with high caries. The demand for restorative treatment in developing countries exceeds the available resources. Thus, mucin levels can serve as an indicator of caries susceptibility.

Keywords: Biofilms, Dental caries, Mucins, Saliva

INTRODUCTION

In recent times, altered food habits have led to a significant increase in oral health problems. One of the major and most common global oral health issues faced today is dental caries [1]. According to a systematic review and meta-analysis conducted by Kazeminia M et al., in 2020, worldwide, 46.2% of the population has dental caries in their primary teeth, and 53.8% have dental caries in their permanent teeth [2]. Dental caries is a chronic, biofilm-mediated disease that results in the loss of tooth structure [3]. It is considered multifactorial, with one of the factors being mucin [4].

Mucin is the chief component of mucous, abundant in saliva, which bestows it with unparalleled lubricating properties [5]. Aside from the oral cavity, mucins are also present in other systems, such as the respiratory and gastrointestinal tracts. Among the 22 salivary mucins, MUC5B and MUC7 are recognised as playing significant roles in caries pathogenesis [6-8].

MUC5B is a high molecular weight, polymeric mucin and is a primary gel-forming mucin secreted by mucus cells in the submandibular, sublingual, palatine, and labial salivary glands. In earlier literature, this high molecular weight mucin was referred to as MG1 [9]. MUC7 is the major type of low molecular weight mucin (MG2) [9]. Dental biofilm significantly influences the formation of dental caries [10]. Generally, biofilms are aggregates of microorganisms that are in contact with one another within a polymeric matrix and are attached to surfaces [11]. Their formation increases when they exist in a flow system with a continuous supply of nutrients. In this context, dental plaque is also considered a biofilm. The continuous supply and alteration of nutrients in the oral cavity impact the composition of the microorganisms present in dental biofilm [11].

The two mucins play a crucial role in protecting the tooth surface from pathogens, although their mechanisms of protection differ. There are, however, studies indicating that these two mucins significantly contribute to caries pathogenesis. Contradictory reports exist regarding the levels of salivary mucins in caries-affected patients [6,7]. Despite the well-established role of biofilm in dental caries, few studies have attributed the levels of salivary mucins to caries susceptibility. Mucin, an important salivary glycoprotein, plays a vital role in dental biofilm formation [12].

Additionally, dietary factors can alter the characteristics of mucins. For example, the green tea polyphenol epigallocatechin gallate can modify the properties of MUC5B, the gel forming mucin, and MUC7, the non gel forming mucin [13]. Thus, it can be concluded that dental caries occurs whenever there is an imbalance between pathogens and mucins. Therefore, it can be hypothesised that differences in the levels of salivary mucins MUC5B and MUC7 can correlate with the severity of dental caries. The null hypothesis states that there is no correlation between the differences in the levels of salivary mucins MUC5B and MUC7 and the severity of dental caries.

Most caries-related studies have failed to demonstrate the role of mucins in caries pathogenesis. Generally, four factors are considered to play an important role in the development of dental caries: tooth structure, time, food, and microorganisms [14]. However, a mucin-rich environment may also be essential in the pathogenesis of caries. The process of dental caries begins when dental biofilm covers the surface of the tooth. The microorganisms in the biofilm gradually adapt to reduced pH levels, leading to dental enamel demineralisation [15]. Biofilms are microcolonies of bacteria that form in flow systems where microorganisms receive adequate nutrient supply and an environment conducive to multiplication and

survival. Consequently, dental plaque can also be considered a biofilm. Mucins promote the formation and growth of biofilm when present with sucrose. In the absence of sugar, mucins can extend the survival period and maintain certain microorganisms, especially *Streptococcus mutans*, in a stationary phase by providing galactose via the tagatose pathway [16].

Given this background, the study aims to estimate the levels of salivary mucins MUC5B and MUC7 in subjects with dental caries. The study intends to quantify the levels of salivary mucins MUC5B and MUC7 in the experimental group with a DMFT index greater than 10 and in the control group with a DMFT index less than 3, as well as to correlate the levels of these mucins with caries severity in both groups.

MATERIALS AND METHODS

The present case-control study was conducted at Meenakshi Ammal Dental College and Hospital, Chennai, Tamil Nadu, India, over a period of three months from July 2022 to September 2022. The study population comprised dental students and patients. The study complied with STROBE guidelines. The procedures followed adhered to the Helsinki Declaration of 1975, as revised in 2000. Approval for the study was obtained from the Institutional Ethical Committee (MADC/IEC-II/72/2022), and informed written consent was acquired from all patients.

Inclusion criteria:

- Participants aged between 18 and 25.
- Participants who volunteered for the study.

Exclusion criteria:

- Any medical illness.
- Use of any medications.
- History of tobacco use.
- History of alcohol consumption.

Sample size calculation: Based on a study conducted by Gabryel-Porowska H [6], the effect size for MUC5B levels was found to be 0.65. Using G Power software with an effect size of d = 0.65, an alpha error of 0.05, power of 0.8, and an allocation ratio of 1, the prior sample size was calculated at 33 for each group [6]. Thus, 66 saliva samples were collected for the study from participants aged 18 to 25 years, with 33 saliva samples collected from participants with a DMFT index greater than 10, forming the experimental group. Another 33 saliva samples were collected from participants with a DMFT index of less than 3, which served as the control group. A DMFT index of less than 3 is considered indicative of low caries severity [6]. A DMFT greater than 10 in the experimental group represents individuals with severe dental caries, allowing for a more distinct comparison with controls having a DMFT of less than 3.

The study was designed as a matched case-control study in which participants were matched based on age in a ratio of 1:1 between the experimental and control groups, as age is a significant determining factor for dental caries.

Study Procedure

Salivary sample collection: Unstimulated saliva was collected from participants over a duration of 10 minutes. Participants had their last meal at least two hours before collecting the sample. The saliva samples were homogenised by vigorous shaking using a vortex mixer. Subsequently, the samples were centrifuged at 3000 × g for 15 minutes. The aliquots of the clarified supernatants were then stored at -80°C for MUC5B and MUC7 estimation.

Estimation of salivary mucin MUC5B levels by (Enzyme-Linked Immunosorbent Assay) ELISA [6]: High-sensitivity ELISA kits (Abbkine) were used to estimate the levels of MUC5B (Human Mucin-5 subtype B (MUC5B) ELISA Kit (96T) - KTE61452) and MUC7 (Human Mucin-7 (MUC7) ELISA Kit (96T) - KTE61449).

- The microtiter plates provided in the kits were precoated with antibodies specific to Human MUC5B. Standards (solutions with known concentrations of the proteins being analysed) and samples were added to the microtiter plate wells and incubated for one hour at 37°C.
- The contents of each well were aspirated and washed, repeating the process four times for a total of five washes, each lasting 1-3 minutes.
- Each well was filled with wash buffer during the washing process, ensuring the complete removal of liquid at each step.
- After washing away any unbound substances, avidin conjugated to horseradish peroxidase was added to each microtiter plate well and incubated.
- The plates were covered with a plate cover and incubated for 30 minutes at 37°C.
- Following another aspiration and washing step, 50 µL of chromogen solution A and 50 µL of chromogen solution B were added to each well. The contents were gently mixed and incubated for 15 minutes at 37°C, protecting them from light exposure.
- The enzyme substrate reaction was terminated by adding a sulfuric acid solution, and the colour change was measured at a wavelength of 450 nm using a spectrophotometer.
- The assay was performed in duplicate, and the concentration of MUC5B in the samples was then determined by comparing the Optical Density (OD) of the samples to the standard curve.

Procedure for evaluation of salivary mucin MUC7 levels by ELISA [6]

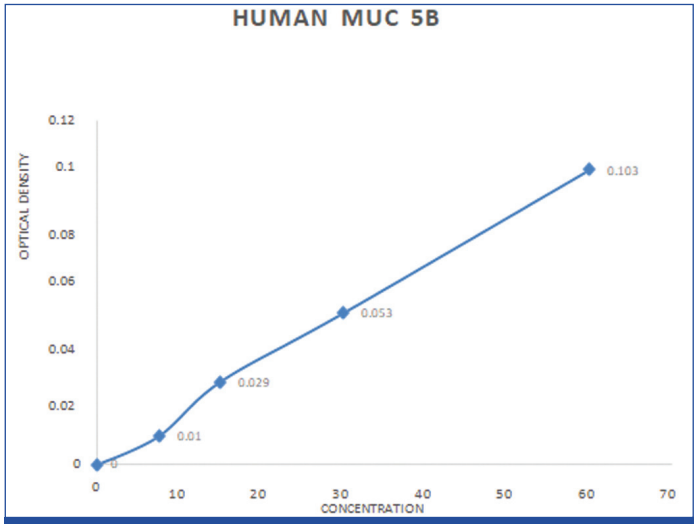
- The microtiter plates provided in the kits were precoated with antibodies specific to Human MUC7.
- Standards and samples were added to the microtiter plate wells and incubated for one hour at 37°C.
- The contents of each well were aspirated and washed, repeating the process four times for a total of five washes, each lasting 1-3 minutes.
- Each well was filled with wash buffer during the washing process, ensuring the complete removal of liquid at each step.
- After washing away any unbound substances, avidin conjugated to horseradish peroxidase was added to each microtiter plate well and incubated.
- The plates were covered with a plate cover and incubated for 30 minutes at 37°C.
- After another aspiration and washing step, 50 µL of chromogen solution A and 50 µL of chromogen solution B were added to each well.
- The contents were gently mixed and incubated for 15 minutes at 37°C, protecting them from light exposure.
- The enzyme substrate reaction was terminated by adding a sulfuric acid solution, and the colour change was measured at a wavelength of 450 nm using a spectrophotometer.
- The assay was performed in duplicate, and the concentration of MUC7 in the samples was then determined by comparing the OD of the samples to the standard curve.

STATISTICAL ANALYSIS

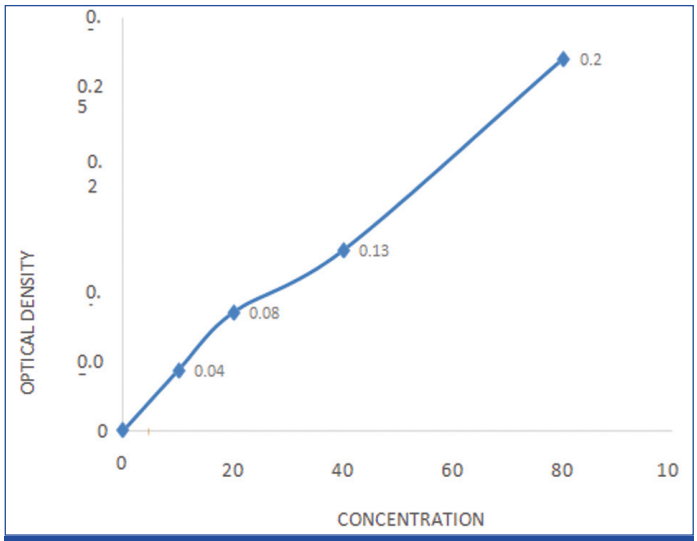
The data collected was entered into Microsoft Excel and analysed using Statistical Package for the Social Sciences (SPSS) version 23.0. Mean and standard deviation were calculated. An independent samples t-test was used to check whether the difference between the means of both groups was statistically significant at a 95% confidence interval. A p-value of <0.05 was considered statistically significant.

RESULTS

The standard curves were prepared by reducing the data using computer software capable of generating a four-Parameter Logistic (4-PL) curve fit. The mean absorbance for each standard was plotted on the X-axis against the concentration for each standard on the Y-axis. [Table/Fig-1,2] show standard curve graphs for MUC5B and MUC7, respectively. [Table/Fig-3] presents the mean concentration of MUC5B in the experimental group (24.34±9.16 µg/L) compared to the control group (9.39±10.44 µg/L), demonstrating a statistically significant difference ($p<0.05$). It also indicates the mean concentration of MUC7 in the experimental group (10.27±3.21 µg/L) compared to the control group (4.81±3.96 µg/L), which also showed a statistically significant difference ($p<0.05$). The results indicate that the null hypothesis was rejected.



[Table/Fig-1]: The standard curve graph for MUC5B.
The standard curve graph for MUC5B. The concentration plotted on the X-axis against the mean absorbance on the Y-axis.



[Table/Fig-2]: The standard curve graph for MUC7.
The standard curve graph for MUC7. The concentration was plotted on the X-axis against the mean absorbance on the Y-axis.

Character	Experimental group (n=33) Mean±SD (µg/L)	Control group (n=33) Mean±SD (µg/L)	t-test	p-value
MUC5B	24.34±9.16	9.39±10.44	6.18	0.01*
MUC7	10.27±3.21	4.81±3.96	6.15	0.02*

[Table/Fig-3]: Mean concentration of MUC5B and MUC7 in experimental group and control group.
*p-value<0.05 is considered statistically significant

[Table/Fig-4] explains the association between MUC5B and MUC7 levels with the study groups by categorising the participants into low and high mucin groups based on the median values of MUC5B and MUC7. The median value of MUC5B in the experimental group was 22.641, while in the control group, it was 4.528. The median value of MUC7 in the experimental group was 10.381 compared to 3.053 in the control group. The Odds Ratio (OR) of 1.131 suggests that individuals with high MUC5B levels have 1.131 times higher odds of being in the experimental group compared to those with low MUC5B levels. Similarly, the OR of 1.129 suggests that individuals with high MUC7 levels have 1.129 times increased odds of being in the experimental group compared to those with low MUC7 levels; however, these findings were not statistically significant.

Character		Experimental group (n=33)	Control group (n=33)	Odds Ratio (OR) (95% Ci)	p-value
MUC5B	Low	19	18	1.131 (0.426-2.991)	0.062
	High	14	15		
MUC7	Low	18	17	1.129 (0.429-2.971)	0.060
	High	15	16		

[Table/Fig-4]: Association between MUC5B and MUC7 levels with the study groups.

DISCUSSION

The results indicated that the levels of mucins MUC5B and MUC7 were higher in the experimental group than in the control group, and these results were statistically significant. This finding suggests that a greater number of dental caries in an individual correlates with higher levels of salivary mucins, MUC5B and MUC7. Similar to the present study, research by Gabryel-Porowska et al., showed elevated levels of mucins MUC5B and MUC7 in individuals with dental caries [6]. However, mucin levels were reduced in the study conducted by Szkaradkiewicz et al., wherein the mean levels of MUC5B and MUC7 were lower in subjects with a DMFT score above 13.9 compared to caries-free individuals. The probable reason for this discrepancy may be attributed to differences in dietary habits and ethnic variation among subjects. Additionally, the former study involved participants aged 25 to 40, whereas the present study focused on younger adults between 18 and 25 years of age [17]. Notably, the study by Banderas-Tarabay et al., reported significantly reduced or absent levels of both salivary mucins in individuals with higher DMFT scores [18]. The reasons for these differences could be related to the stages of dental caries when the mucins were measured. Furthermore, in the study conducted by Hertel et al., both mucins showed no significant difference between children with and without caries [19].

Dental caries is a major global health problem and the most common microbial disease of the oral cavity. The levels of salivary mucins are important for maintaining a healthy oral environment. MUC5B and MUC7 were chosen for this study because they are the predominant types of mucins present in the oral cavity [20]. Participants with a DMFT score greater than 10 constituted the experimental group, while those with a DMFT score less than 3 formed the control group. The participants were aged 18 to 25 years and had no history of adverse habits such as tobacco or alcohol use. The mucin levels were detected using ELISA.

This study is the first of its kind conducted in a South Indian population. In this study, the levels of MUC5B and MUC7 were estimated in saliva only. By assessing the levels of salivary mucins MUC5B and MUC7 in individuals, we can predict their susceptibility to caries and implement necessary prophylactic measures. Future studies should assess the levels of MUC5B and MUC7 in dental biofilms to determine the definitive etiological role of these mucins in the development of dental caries.

While mucins can provide protection for the tooth surface, they are also major constituents of biofilms and dental plaque. MUC5B and MUC7 can serve as a nutrient source, potentially increasing the

longevity of oral microbes. Future research is required to explore the mechanisms of interaction between caries causing pathogens and salivary mucins. Furthermore, low socio-economic status, a low literacy rate, and the non availability of dental insurance are contributing factors to the lack of oral health maintenance in India. The demand for restorative treatment in developing countries often exceeds the available resources. Therefore, early detection of caries is critical, and mucin levels may serve as an indicator of susceptibility to caries.

Limitation(s)

This study did not assess and correlate the plaque index with the DMFT score and mucin levels. Additionally, the effects of a cariogenic diet were not explored. Since the participants were recruited solely from a hospital, they may not be representative of the general population. Factors such as oral hygiene practices and saliva flow rate may also influence caries severity, but these were not assessed in this study. Given that some studies have shown conflicting levels of mucins in relation to caries, it is recommended that future studies involve a larger and more diverse sample population spanning various age groups.

CONCLUSION(S)

The findings of the present study concluded that the levels of salivary mucins MUC5B and MUC7 are higher in the experimental group with dental caries compared to those in the control group. Individuals with high levels of MUC5B have 1.131 times higher odds of being in the experimental group compared to those with low MUC5B levels. Similarly, the odds ratio (OR) of 1.129 suggests that individuals with high levels of MUC7 have 1.129 times increased odds of being in the experimental group compared to those with low MUC7 levels, although these findings were not statistically significant.

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