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The Association of Salivary Mucin-7 Levels with Dental Caries in Adults

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Objectives: This study examines the potential relationship between the incidence of dental caries in adults and the level of mucin-7 glycoprotein, a specific parameter in saliva. **Methods:** In a case-control study, samples of whole unstimulated saliva from 40 volunteers in two different groups were classified according to the decayed, missing, filled teeth (DMFT) index. One group was caries-free (n = 17), and the other had severe carries (n = 23). We measured the level of mucin-7, salivary buffer system, and pH and examined for relationships to the DMFT index. **Results:** There was a statistically significant difference among the groups ($p < 0.001$) in the level of mucin-7, salivary buffer system, and pH. The DMFT index for case and control groups was calculated as 0 and 14.09 ± 3.78. There was a strong, negative relationship between the level of mucin-7 and the DMFT index. **Conclusion:** The development of dental caries is linked to a reduced concentration of mucin-7 in saliva. The level of mucin-7 may be clinically useful for evaluating dental caries risk.

Keywords: Dental Caries, Saliva, Oral Health, Mucins, Buffers

Introduction

Dental caries is a multifactorial infectious disease characterized by interactions between specific oral bacteria, their metabolic products, salivary constituents, and dietary carbohydrates [1, 8]. According to the National Survey of Oral Health Status in Mongolia, the prevalence of dental caries and mean Decayed, Missed, Filled Teeth (DMFT) were 97.8 % and 9.5 teeth among the 35-44 aged Mongolian population [13].

Human saliva, a biological fluid secreted by salivary glands, contains a mixture of organic and inorganic components and plays an essential role against oral pathogens, especially cariogenic bacteria, and oral health maintenance [3, 4, 10]. Its chemical characteristics, such as pH and buffering capacity, can

be evaluated. Salivary buffer systems can reverse the low pH in dental plaque and promote oral clearance of bacteria, thus preventing demineralization of dental hard tissue [4, 11].

Mucins are produced for the most part by mucous glands such as submandibular, sublingual, and minor glands. Currently, at least 20 structurally unique human mucins have been distinguished by the presence of MUC5B, MUC7, MUC19, MUC1, and MUC4 found in saliva [1]. It is known that human salivary glands make membrane-bound mucins, MUC1 and MUC4, in addition to the gel-forming mucins, mostly MUC7 and MUC5B. There are several mechanisms of interaction between mucins and salivary proteins. For instance, mucins bind other antimicrobial proteins to alter their localization and retention, which can lead to increased host defense of the oral cavity [10, 11].

MUC7 has a backbone of 357-amino-acid that lacks a terminal cysteine-rich domain. Salivary proline-rich proteins, statherins, and histatins bind the N-terminal domain on the MUC7 polypeptide backbone. Increasing their availability and duration in saliva could be beneficial against the development of dental caries [6, 7]. MUC7 is also known to protect against dental caries by binding to alpha-enolase protein, which leads to decreased colonization of Streptococcus mutans, the main cariogenic bacteria, on the surface of teeth [3, 6].

Recent studies reveal that salivary mucins protecting enamel may prevent dental caries [1, 3, 4, 10]. However, it remains to be determined whether the manifestation of dental caries in adults is associated with quantitative disturbance in mucins. To our knowledge, no study has established the level of salivary mucins in the Mongolian population. This study aimed to determine the relationship between indices of dental caries and the levels of MUC7, pH, and buffering capacity of saliva.

Materials and Methods

Study design and sampling

A case-control study total of 40 adult subjects, admitted at the "Tujdent" Dental Clinic during January and February 2020, selected based on history, oral examination, and the DMFT (Decayed, Missing, and Filled Teeth) index as calculated by the WHO (World Health Organization). Dental examinations were made using dental mirrors and probes according to 2013 WHO dental caries diagnostic criteria. Two research groups were identified: a group of 17 caries-free individuals (DMFT = 0) as a control group and another group that included 23 patients with severe caries (DMFT > 10) as a case group. Cases and controls were matched according to their age and education level. Inclusion criteria: older than 18 years of age with no general or chronic systemic diseases in anamnesis. Exclusion criteria: chronic systemic diseases, oral fungal infection, destructive periodontal diseases, and cigarette smoking.

Sample collection

Samples of saliva from patients were collected between 8:00 AM and 12:00 PM. All subjects abstained from eating and drinking for an hour before sampling. Unstimulated whole saliva was collected for 10 - 15 minutes by the spitting method. The volume of samples obtained was 2 ml. Saliva samples were homogenized by vigorous shaking with hands and clarified by centrifugation at 3000 g for 15 minutes at 4 °C. The samples of clarified supernatants were stored at - 80 °C for MUC7 measurement.

Estimation of salivary MUC7, buffer capacity, and pH

High sensitivity enzyme-linked immunosorbent sandwich assay (Human MUC7 ELISA Kit; Catalog No.: EH1942, Santai Santiple Tech, China) was used to determine the level of MUC7 in the saliva samples. Recombinant MUC7 was used as a standard (positive control) in the assay. The tests were achieved as recommended by the manufacturer. Absorbance values were read at the wavelength of A = 450 nm. Each estimation of the salivary MUC7 level was repeated twice, and the mean defined the individual result. Buffering capacity and pH were determined by qualitative test using indicator paper as recommended by the manufacturer (Saliva-Check BUFFER test; GC, Japan) (Table 1).

Statistical analysis

We used mean arithmetic, standard deviations, and median values to analyze the quantitative characteristics. We used the nonparametric Mann-Whitney U test to compare means and Fisher's Exact test to compare frequency distributions. The correlation between independent variables was examined using Spearman's correlation coefficient. Moreover, the receiver operating characteristic (ROC) curve analysis was tested by the DeLong method to define the relationship between the values of the studied mucins and demonstrated dental caries. A p-value of < 0.05 was used as the cut-off point for determining the

statistical significance. The data were analyzed using Statistical Packages for Social Sciences (SPSS) version 20 statistical software.

Ethical statement

The study was approved by the Research Ethics Committee of the Mongolian National University of Medical Sciences on June 13, 2019 (No. 2019 / 6 - 03). All patients signed an informed consent form before the clinical examination and sample collection.

Results

We identified 17 patients for the control group (caries-free) and 23 in the case group (severe caries). The mean age of all subjects was 33.5 ± 6.9 years. There were no significant differences regarding age and education level between both groups (Table 2).

Table 1. Evaluation of salivary buffering capacity.

Score	Buffering ability
0 - 5	Very low
6 - 9	Low
10 - 12	Normal

Characteristics of saliva and MUC7 status

Among the two groups, there was a statistically significant difference ($p < 0.001$) in levels of salivary pH, buffering capacity, and MUC7 (Table 3).

Twenty-one (52.5 %) of all patients included in the study had a normal rate of salivary buffering capacity (Table 4).

The cut-off point for MUC7 at a sensitivity of 100 % and a specificity of 82.6 % was 46.6 U/ml by the ROC curve analysis (Figure 1).

The results of the correlation analysis between variables showed the highest correlation with the concentration of MUC7 and the DMFT index ($r = -0.74$, $p < 0.001$), followed by buffering capacity ($r = - 0.73$, $p < 0.001$), and pH of saliva ($r = - 0.52$, $p < 0.001$) (Table 5).

Table 2. Demographic characteristics of patients.

Characteristics	Caries-free (n = 17) Mean ± SD	Severe caries (n = 23) Mean ± SD	Total (n = 40) Mean ± SD	p-value*
Age (years)	30.06 ± 5.24	36.04 ± 6.97	33.52 ± 6.95	0.058
Gender	**N (%)**	**N (%)**	**N (%)**	
Male	8 (47)	3 (13)	11 (27.5)	0.031
Female	9 (53)	20 (87)	29 (72.5)	
Education				
Middle	1 (5.9)	8 (34.8)	9 (22.5)	0.051
High	16 (94)	15 (65.2)	31 (77.5)	

*Mann-Whitney U test, Fisher's Exact test

Table 3. Caries status and salivary characteristics of patients.

Characteristics	Caries-free (n = 17) Mean ± SD	Severe caries (n = 23) Mean ± SD	Total (n = 40) Mean ± SD	p-value*
DMFT	-	14.09 ± 3.78	8.1 ± 7.53	0.001
Salivary pH	7.32 ± 0.22	6.97 ± 0.25	7.2 ± 0.29	0.046
Buffering capacity	11.47 ± 0.49	8 ± 1.79	9.25 ± 2.58	0.001
MUC7 (U/mL)	57.27 ± 8.33	39.45 ± 9.81	45.92 ± 13.37	0.001

*Mann-Whitney U test; DMFT - Decayed, Missing, Filled teeth; MUC7 - Mucin 7

Table 4. Salivary buffering capacity of patients.

Buffering capacity	Caries-free (n = 17) N (%)	Severe caries (n = 23) N (%)	Total (n = 40) N (%)
Very low	-	2 (8.7)	2 (5)
Low	-	17 (73.9)	17 (42.5)
Normal	17 (100)	4 (17.4)	21 (52.5)

Table 5. Correlation analysis between variables.

Variables	Age	DMFT	Salivary pH	Buffering capacity	MUC7
Age	1				
DMFT	0.38*	1			
Salivary pH	-0.18	-0.52***	1		
Buffering capacity	-0.27	-0.73***	0.69***	1	
MUC7	-0.35*	-0.74***	0.52***	0.69***	1

*** p < 0.001, ** p < 0.01, * p < 0.05; DMFT - Decayed, Missing, Filled teeth; MUC7 – Mucin-7

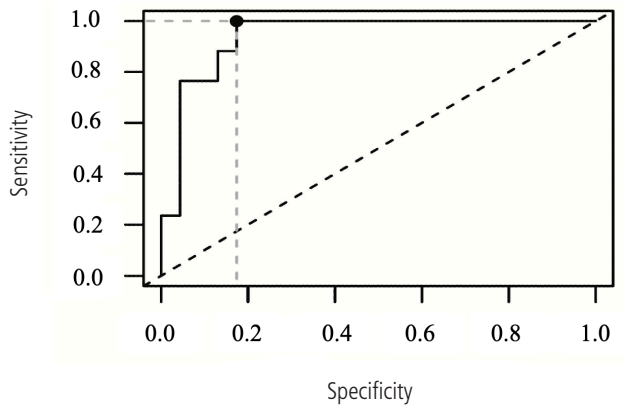

Figure 1. Receiver operating characteristics (ROC) curve of salivary levels of Mucin-7.

Discussion

The primary process in dental caries is a progressive loss of minerals through dissolution by plaque acid, also known as demineralization, during repeated cariogenic challenges [1, 4]. The Global Burden of Disease Study 2017 estimated that 2.3 billion adults and more than 530 million children suffer from permanent and primary teeth caries. Recent local studies have shown that caries prevalence and severity among Mongolians are high and increase with age [5, 9, 12]. Saliva is an important substance for food digestion, and it also plays a key role as a natural protective agent against carries [16]. Saliva is thought to function, in part, by forming pellicles on oral surfaces such as the tooth, the epithelium, and the plaque itself. Salivary protective mechanisms include bacterial clearance, direct antibacterial activity, buffers, and remineralization. The buffering capacity is determined primarily by the level of bicarbonate ions in saliva [3 - 6]. Several lines of evidence indicated that acids, products of bacterial metabolism in biofilm, may be neutralized by the buffering system of saliva [10]. Most hydrogen ions produced in the oral cavity react with saliva bicarbonate to form carbonic acid. The bicarbonate system can rapidly neutralize strong acids at pH < 6 and is more effective than phosphate [11]. One well-known salivary antibacterial protein is mucin. These glycoproteins that coat the oral tissue can form homotypic and heterotypic complexes with other salivary molecules, including secretory immunoglobulin A, lysozyme, and cystatins. There are several types of interactions between salivary mucin and bacteria. These interactions cause agglutination of bacteria, interactions that inhibit bacterial growth, interactions that foster bacteria adhesion to oral surfaces, and interactions that contribute to microbial nutrition [2 - 4]. MUC7 is a relatively small salivary glycoprotein produced by the mucous acinar cells of the salivary glands. Some studies show that Streptococcus sanguinis, S. sobrinus, and S. oralis bind to MUC7 but not MUC5. These studies indicate that MUC7 is important in preventing cavity formation [1, 3, 10].

In this study, we have compared the concentration of MUC7, level of buffering capacity, and pH in the saliva of caries-free persons and patients with severe dental caries. This study was conducted using high sensitivity ELISA tests to detect MUC7 and special buffer tests to detect buffering capacity and pH in the saliva samples. We analyzed two distinct groups of adults. The control group included healthy, caries-free individuals (DMFT = 0). The second group included patients with severe caries (DMFT > 14.09). Our study has identified significantly higher salivary MUC7, buffering capacity, and pH in the carries-free group compared to the severe-carries group. At the same time, the ROC curves showed a relationship between MUC7 values and dental caries. We have identified a high specificity of MUC7 levels in saliva in patients with severe caries, so mucin-glycoproteins may be helpful to evaluate the risk of dental caries. This conclusion is supported by the earlier studies of Szkaradkiewicz-Karpińska et al. who demonstrated significantly lower levels of MUC7 in 26 patients with high DMFTs compared to subjects without caries (1.39 ± 0.86 vs. 5.47 ± 1.18 ng/ml, $p < 0.001$) [1]. In Poland, one study included 8 adolescents with DMFT = 3 (control group) and 27 patients with DMFT > 11 (research group). In this study, MUC7 levels were slightly reduced in research group subjects, but this was statistically insignificant ($p = 0.918$) [4]. Since the control group included only eight subjects with DMFT = 3, it would be difficult to compare those results with the results presented in our study. In summary, we conclude that the reduced concentration of MUC7 in the saliva is associated with the development of dental caries. Some limitations of our study were the smaller number of subjects in the control group than in the case group, and we did not evaluate the saliva flow rate. We were concerned that other environmental factors could affect the results. Therefore, in further prospective studies, it would be advisable to investigate factors such as oral hygiene and intake of carbohydrates to improve report outcome accuracy. Studies on larger groups of subjects should be done in the future.

Conflict of Interest

The authors state no conflict of interest.

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