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ORIGINAL ARTICLE

INVESTIGATION OF THE N-TERMINAL CODING REGION OF *MUC7* ALTERATIONS IN DENTISTRY STUDENTS WITH AND WITHOUT CARIES

Koç Öztürk L[1], Yarat A[1], Akyuz S[3], Furuncuoglu H[3], Ulucan K[1,2,*]

***Corresponding Author:** Dr. Korkut Ulucan, Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Üsküdar University, Haluk Turksoy Sok. No. 14, Üsküdar, İstanbul 34662, Turkey. Tel: +90-216-400-2222-2409. Fax: +90-216-400-2222; Mobile: +90-532-692-1922.
E-Mail: korkutulucan@hotmail.com

ABSTRACT

Human low-molecular weight salivary mucin (MUC7) is a small, secreted glycoprotein coded by *MUC7*. In the oral cavity, they inhibit the colonization of oral bacteria, including cariogenic ones, by masking their surface adhesions, thus helping saliva to avoid dental caries. The N-terminal domain is important for low-molecular weight (MG2) mucins to contact with oral microorganisms. In this study, we aimed to identify the N-terminal coding region of the *MUC7* gene between individuals with and without caries. Forty-four healthy dental students were enrolled in this study; 24 of them were classified to have caries [decayed, missing, filled-teeth (DMFT) = 5.6] according to the World Health Organization (WHO) criteria, and 20 of them were caries-free (DMFT = 0). Simplified oral hygiene index (OHI-S) and gingival index (GI) were used to determine the oral hygiene and gingival conditions. Total protein levels and salivary total protein levels and salivary buffer capacity (SBC) were determined by Lowry and Ericsson methods. DNA was extracted from peripheral blood cells of all the participants and genotyping was carried out by a polymerase chain reaction (PCR)-sequencing method. No statistical differences were found between two groups in the terms of salivary parameters, oral hygiene and gingival conditions. We detected one common single nucleotide polymorphism (SNP) that leads to a change of asparagine to lysine at codon 80. This substitution was found in 29.0 and 40.0%, respectively, of the groups with and without caries. No other sequence variations were detected. The SNP found in this study may be a specific polymorphism affecting the Turkish population. Further studies with extended numbers are necessary in order to clarify this finding.

Keywords: Dental caries, oral hygine indicis, low molecular weight salivar mucin (MUC7).

INTRODUCTION

Dental caries is one of the most prevalent human infectious diseases affecting life style and genetic factors. The skewed distribution of caries in the Western populations and its weak association with traditional life style factors, *e.g.,* sugar intake and oral hygiene, suggest genetic components in caries development. Early studies have shown that there were large individual differences in caries development in spite of similar exposures to sugars [1].

Mucins are vital components of the mucous layers covering the epithelial surfaces of the human body. In the oral cavity, mucins are secreted by submandibular and sublingual glands, and various minor salivary glands, but not by the parotid glands. Mucins constitute the third most abundant group of proteins in saliva and form various complexes with other sali-

[1] Department of Basic Medical Sciences, Faculty of Dentistry, Marmara University, İstanbul, Turkey

[2] Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Üsküdar University, İstanbul, Turkey

[3] Department of Clinical Sciences, Faculty of Dentristry, Marmara University, İstanbul, Turkey

vary proteins, thereby modulating their activities. Two structurally distinct mucins have been identified in human saliva: high-molecular weight (MG1) and the low-molecular weight (MG2) mucins [2].

The human *MUC7* gene encodes a relatively small mucin glycoprotein, MUC7 (125 kD). It is also known as MG2. Low-molecular weight mucin is secreted mainly by human sublingual and submandibular glands. As a salivary glycoprotein, it is involved in mastication, speech, swallowing and lubrication of the oral cavity [3]. Early studies indicate that MG2 takes part in the human salivary nonimmune defense system and interacts with oral microorganisms, and it also appears that MG2 plays a role in mediating interactions between neutrophils and bacteria. The ability of MG2 to self-associate, creating larger assemblies through non covalent bonds, has also been proposed to contribute to the agglutinating and eliminating properties of the mucin [4].

The exploitation of mucin molecules for diagnostics is gaining increasing interest in a variety of disease conditions [3]. A recent study has suggested that genetic polymorphisms, which can alter mucin gene expression, have been associated with mucin-related diseases [5]. While no studies have been performed on the MG2 polymorphism for dental caries, the potential of these molecules about dental caries cannot be ignored and will likely be subjected to further exploration.

To date, the relationship between the *MUC7* N-terminal coding region and dental caries is still unclear. Thus, in the present study, we aim to identify the effects of N-terminal coding region *MUC7* polymorphisms and dental caries.

MATERIALS AND METHODS

Study Groups. The study included 24 dental students with caries aged between 18 to 23 years (mean age 20.2 ± 1.2) and 20 dental students without caries aged between 19 to 26 years (mean age 21.95 ± 2.2). Forty-four healthy dentistry students were enrolled in this study, 24 of them (aged between 18 to23 years, mean age 20.2 ± 1.2) were classified to have carries [decayed, missing, filled-teeth (DMFT)=5.6] according to the World Health Organization (WHO) criteria, and 20 of them (aged between 19 to 26, mean age 21.95 ± 2.2) were caries- free (DMFT=0). Inclusion criteria for subjects with caries and without caries were as follows; no oral complains, good oral hygiene, absence of smoking and drinking habits, no systemic diseases, no drug abuse. Exclusion criteria was unwillingness to participate in the study. The subjects signed an informed consent form to participate in this study.

Clinical Examination. One experienced dentist examined all subjects for their adherence to inclusion criteria. The WHO criteria were used for DMFT [6]. The oral hygiene and gingival status were assessed using the simplified oral hygiene index (OHI-S) and gingival index (GI) [7]. A structured questionnaire was used to collect data on oral hygiene habits (frequency of tooth brushing, use of dental floss), professional counseling on oral health and hygiene, and the presence of gingival bleeding. All subjects had good oral health and had regular dental care.

Collection of Saliva. Fasting unstimulated whole saliva samples were collected at the same time of day (between 08:30 and 11:00 am) for a 1-week period by the same researcher in all cases. Before saliva collection, the mouth was rinsed with distilled water. Subsequently, saliva was allowed to accumulate on the floor of the mouth and the subjects were instructed to spit into the test tubes. During the saliva collection, the participants stayed in a relaxed position with their heads bent forward. Each saliva collection period was 10 minutes long. Immediately after collection, saliva volume was measured and then salivary flow rate calculated as mL/min. Saliva samples were stored at –20 °C until used.

Salivary Analysis. The temperature of the saliva samples was raised from –24 °C to 4 °C and kept for an hour. After thawing, they were centrifuged and supernatants were used for salivary analysis. Total protein level was determined by the method of Lowry *et al.* [8]. Bovine serum albumin (BSA) was used as a standard, and absorbance evaluated at 500 nm. Total protein level was expressed as mg%. Salivary pH was directly measured with pH paper (pH indikatörpapier, Merck Neutrolit-5.5-9.0; Merck, Darmstadt, Germany). Salivary buffer capacity (SBC) was measured by Ericsson's method, modified by Menteş *et al.* [9]. Two hundred µL of saliva was mixed with 600 µL HCl (0.0033 M). After 10 min., the pH of the mixture was immediately measured with pH paper (pH indikatörpapier, Merck Neutrolit-5.5-9.0; Merck). Based on the color change of the indicator paper strip, the pH was assessed in comparison with a color chart. The corresponding value is taken as the pH of the mixture.

DNA Genotyping. The Marmara University Ethics Committee approved the study protocol, and written informed consent was obtained from all participants. DNA was extracted from peripheral blood cells by using High Pure polymerase chain reaction (PCR) Template Preparation Kit (Roche Diagnostics Deutschland GmbH, Mannheim, Germany) according to the manufacturer's instructions. Isolated DNA samples were kept at –20 °C if not studied on the same day. The *MUC7* N-terminal coding region was amplified using specific primers: 5'-GAA GGT CGA GAA AGG GAT CAT-3' and 5'-GTC TTG TGG AGC TGG GGA AT-3'. Polymerase chain reaction was performed in a final volume of 50 µL containing 2-10 ng DNA, 200 mM of each deoxynucleotide triphosphate (dNTP), 1.5 mM $MgCl_2$, 10 mM Tris/HCl, pH 8.3, 50 mM KCl, 10 pmole of each primer, and 0.1 U Taq polymerase. Thermocycle settings consisted of a denaturation step at 94 °C for 3 min., followed by 35 cycles at 94 °C for 60 seconds, 62 °C for 60 seconds, and 72 °C for 60 seconds and a final extension at 72 °C for 5 min. The 199 bp amplicons were sequenced with ABI PRISM™ 3100 Genetic Analyzer using DYNemic ET Terminator Cycle Sequencing Kit (GE Healthcare Life Sciences, Little Chalfont, Buck-inghamshire, UK).

Statistical Analysis. The significance in the statistical analyses between groups was assessed using the χ^2 test [Statistical Package for the Social Sciences (SPSS) 10.0 for Windows; SPSS Inc., Chicago, IL, USA]. Relationships yielding *p* values less than 0.05 were considered to be significant.

RESULTS

Simplified oral hygiene index and GI were not significantly different between the two groups. Salivary pH, flow rate, buffering capacity and total protein levels were also not significantly different between the young adults with caries (DMFT = 5.62) and without caries (DMFT = 0) (p >0.05). Table 1 lists the values of these markers.

No new variants were observed in 44 subjects in the N-terminal coding region of the *MUC7* gene. When we compared the two groups, we detected no significant difference. Moreover, we detected one common single nucleotide polymorphism (SNP), namely, a C>G transversion leading to an asparagine to lysine change at codon 80 (N80K) (Figure 1). This substitution was found to be 29.0 and 40.0%, respectively, in the groups with and without caries.

DISCUSSION

Mucins coat the gastrointestinal, genito-urinal and respiratory tracts, as well as the oral cavity. In the oral cavity, they have a variety of functions such as lubricating and protecting the oral cavity and providing a nonspecific immune defense [10].

In our cohort, OHI-S, GI, salivary pH, flow rate, buffering capacity and total protein levels were not significantly different between the two groups. These parameters have important effects on oral hygiene, and also for dental caries. But in our study cohort, all of these parameters did not affect dental caries formation.

Low-molecular weight mucin contains 377 amino acids. The first 20 of these are located in the N-terminal domain and are very hydrophobic. It contains a central part, made of six tandem repeats of 23 amino acids that are rich in proline and hydroxylated amino acids, and having approximately 90-100 O-linked chains. A histatin-like domain with a leucine-zipper segment, followed by a moderately glycosylated region, constitute the N-terminal domain; a heavily glycosylated domain, and a second

Table 1. Mean levels of all parameters, and *p* values for the examined groups.

Parameters	Group With No Caries (*n*=20)	Group With Caries (*n*=24)	*p* Value
Simplified oral hygiene index	0.14 ± 0.008	0.15 ± 0.011	0.605
Gingival index	0.017 ± 0.008	0.02 ± 0.009	0.507
pH	7.02 ± 0.41	7.17 ± 0.35	0.292
Salivary flow rate (mL/min.)	0.48 ± 0.22	0.52 ± 0.24	0.577
Salivary buffering capacity	1.63 ± 0.32	1.50 ± 0.29	0.174
Salivary total protein (mg/dL)	131.00 ± 48.00	132.00 ± 49.00	0.871

[a] Results are presented as mean ± SD (standard deviation).

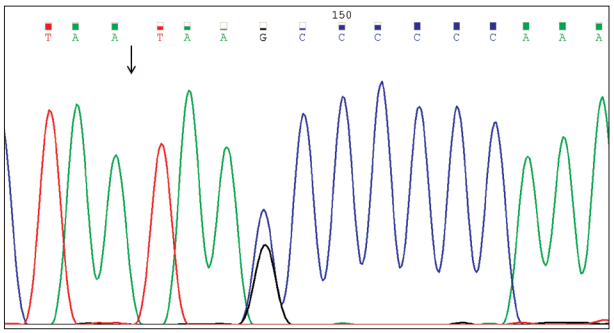

Figure 1. Chromatogram analysis showing the polymorphic site within the *MUC7* gene. The arrow indicate the SNP site. A C>G transversion at codon 80 leads to an asparagine to lysine change in the protein.

leucine-zipper segment for the C-terminal extremity. The distal regions of MUC7 do not exhibit any cysteine-rich domains, only two cysteine residues are present towards its N-terminal end. So far, MG2 has no sequence homology with any other mucins. The N-terminal region of the protein binds *Streptococcus mutans* (*S. mutans*). It also exhibits a candidacidal activity and, after cleavage, a subdomain of this region is microbicidal against a periodontal pathogen, *Actinobacillus actinomycetemcomitans*. Furthermore, a MUC7 20-mer displays potent killing activity against a variety of fungi and both gram-positive and gram-negative bacteria [4,11,12]. The two cysteine residues located in the N-terminal region of MUC7 seem to be directly implicated in these activities [12].

Low-molecular weight mucin has been reported to interact with several strains of streptococci by promoting their agglutination [13]. The N-terminal region of MG2 has a broad-spectrum fungicidal and bactericidal activity *in vitro* [14,15]. Low-molecular weight mucin peptides derived from the N-terminal of MG2 are the cationic anti-microbial peptides derived from the human low molecular weight salivary mucin, a component of innate immunity (the first-line of the host defense system against pathogens). Low-molecular weight mucin (357 amino acid residues in saliva), protects the oral cavity from microbial infections through more general protective mechanisms rather than the direct killing of microorganisms [16].

It was shown that a 20 kDa MG2 fragment, containing the N-terminal region, was present in the saliva and suggested that cleavage of MG2 *in vivo* may lead to the fragments with microbicidal properties [17]. The MG2 20-mer, sub-domain of N- terminal domain, has been shown to be able to cross the fungal cell membrane and accumulate inside the cells, suggesting a possibility that MG2 peptides may also exert killing activity against *S. mutans* [18]. In some conditions, rather than killing the bacteria directly, they disturb cariogenic and periodontal pathogen activity by interacting with them, binding the bacteria *via* their cysteine residues within their N-terminal domain [19,20]. The peptides derived from the N-terminal region of MG2 present somewhat preferential antimicrobial activity against *S. mutans*. They also have an effect on *in vitro* formation and reduction of the preformed *S. mutans* biofilm [15]. We did not detect any statistically significant polymorphisms between caries and caries-free groups in the present study. There was one common SNP, affecting the amino acid at position 80, changing the natural amino acid asparagine to lysine. This SNP seemed to have no effect on dental caries in individuals, but seems to be an important polymorphism in the examined Turkish subjects.

In conclusion, the SNP detected in this study may be a specific polymorphism effecting the Turkish population. Further studies with a larger number of individuals are necessary in order to clarify our findings.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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