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Comparing salivary antibacterial peptides in children with and without Caries

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Abstract

Background: salivary proteins have, today, gained special importance in studies of the role of saliva in tooth decay. Among the peptides, histatins and defenses play a more important role. The aim of this study was to determine the concentration of salivary antibacterial peptides in children with early childhood caries (ECC, SECC) compared to children without caries.

Methods: This comparative-case study was conducted on 48 young children (under 6 years of age) with milk teeth referred to a private pediatric dentistry center. The participants were divided into three groups of 16 with early childhood caries (ECC, SECC) and without decay. After collecting children's saliva, the samples were sent to the laboratory to obtain peptide concentration in salivia using ELISA and the results were analyzed using SPSS 22 software.

Results: The average index of histatin-5 in the mild caries group was significantly lower than those in the moderate caries ($p<0.001$) and severe caries groups ($p<0.001$). The average index of beta-defensin-1 in the mild caries group was significantly higher than those in the moderate caries ($p<0.001$) and severe caries groups ($p<0.001$). The average beta-defensin-2 index in the mild caries group was significantly higher than those in the moderate caries group ($p<0.001$); and in the severe caries group, it was significantly higher than that in the moderate caries group ($p<0.001$).

Conclusion: With the increase of salivary HST-5, the progress of caries increased. Also, the progress of caries was associated with a decrease in the amount of β-defensin-1. No correlation was observed between the caries process and the amount of β-defensin-2.

Key Words: Anti-Bacterial Agents, Dental Care for Children, Dental Caries, peptides, Saliva.

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1- INTRODUCTION

Caries chronic diseases are a global problem affecting many children in all countries. Regular dental care is not accessible and affordable for all children around the world (1). Therefore, finding scientific and practical solutions to prevent dental caries, especially in children, is an important research priority (2). Premature tooth decay in childhood is a common type of decay in the teeth of infants and children, which can (3) lead to pain, infection, and interference with eating, because increased risks of new decay in milk teeth, and finally have destructive effects on the growth of permanent teeth (4). Like other dental caries, the factors that cause premature caries in children include carious microorganisms, fermentable carbohydrates, and susceptible hosts (5). Saliva is the main part of the caries process and is of fundamental importance in providing dental health during caries (6). Having sufficient knowledge of the composition of saliva, including the amount of salivary peptides, can play an important role in examining the existing condition of the mouth and controlling the activity of bacteria as well as the mineral content of teeth (7). Human saliva contains different forms of antimicrobial polypeptides that play a vital role in innate immunity and fight against invading foreign pathogens (7), wound healing (8) and apoptosis (9). Histatins (HST) secreted in parotid and sublingual salivary glands are one of the main natural antimicrobial proteins of saliva. 85% of the total content of salivary proteins including HST-1, HST-3 and HST-5, and HST-3 and HST-5 have the strongest antimicrobial activity and are components of the acquired enamel pellicle. Histatins destabilize the bacterial cell membrane by absorbing its surface and lead to bacterial cell damage. In addition, histatins reduce the release of pro-inflammatory cytokines (interleukins, TNFα, arachidonic metabolites and other acids) that occurs as an organism's response to stimulation of the cell wall of gram-negative bacteria with lipopolysaccharide (10). HST-5 is the only protein from the group of HSTs that has antiviral activity. HST-5 plays an undeniable role in preventing periodontitis by inhibiting the activity of proteases and clostripins (Clostridiopeptidase B, Clostridium histolithium proteinase B) (11). Saliva contains alpha-defensin and beta-defensin. Beta-defensins (1 and 2) are expressed in salivary duct cells and their role is still not fully understood (12). Human beta-defensin-1 (hBD-1), which is expressed in salivary glands and oral tissues, has antibacterial properties against gram-negative bacteria (13). hBD-1 shows antimicrobial activity against Gram-positive and negative bacteria as well as adenovirus (14). hBD-2 is more functionally interesting than hBD-1 and is active against gram-negative bacteria and fungus (Candida albicans), but not against gram-positive bacteria (15). The level of expression of different hBD-1 and hBD-2 genes can be determined when comparing diseased and healthy gums (13). Increasing information on the mentioned proteins can be useful for the prevention and treatment of caries. Considering the few studies and conflicting results in the field of salivary proteins(16), this study aimed to determine the concentration of salivary antibacterial peptides in children with early childhood caries (ECC), with Severe early childhood caries (SECC) and without caries with the practical purpose of helping to better determine the prognosis of caries diseases in children.

2- MATERIALS AND METHODS

This comparative-case study was conducted in a private pediatric dentistry center (Tabassom) in Zanjan, Iran. The participants included 48 under 6-year-old children with S-ECC or ECC, or without caries. They were included in the study in case their parents agreed and signed a

written consent form. The required sample size was calculated based on the studies by Munther (17) and Lips (18) according to the formula of comparing the average of two populations. The study power was 90% and the study error was 5%. According to the formula, 15 samples were needed in each group (ECC, SECC, and comparative), which, considering the 0.10 percentage drop, 16 samples were, finally, examined in each group. The used sampling method was convenient method and after receiving the code of ethics from Zanjan University of Medical Sciences (IR.ZUMS.REC.1401.248), the researcher went to the clinic on certain days and the patients who were eligible to enter the study were examined until the desired sample size was reached.

Children who had any type of oral diseases other than caries, any systemic, acute or chronic diseases in their medical history, had a history of allergies, stress and anxiety, respiratory infection, headache, sore throat, fever, or were taking medication and had taken antibiotics and painkillers in the last 2 weeks, were excluded from the study. Only children who visited the dentist for the first time were examined, and children who had a history of tooth extraction due to caries were excluded from the study. Although it was not possible to control the nutritional details of children, parents were asked about breastfeeding. Most children brushed their teeth without toothpaste or with baby toothpaste (rarely) once or twice a week. According to the parents, none of the children used mouthwash. The researcher tried to match the three studied groups in terms of gender and age.

The information of the children participating in the study such as age and gender and questions related to the DMFT index (the number of filled, decayed and fallen teeth) (19) were entered in the relevant checklist. Then, using a sterilized vial, an about one-cc unstimulated saliva was collected for each case, 10 minutes after the oral examination, and poured into sterile tubes. Children with DMFT > 6, 6<DMFT<13 and 13<DMFT were placed in mild (comparative), moderate (ECC) and severe (SECC) caries groups, respectively. Salivia samples were collected in the same way from all three groups and children were forbidden to eat and drink for 2 hours before the sample collection. All samples were sealed in sterile test tubes to prevent access to oxygen and were kept in the refrigerator of the clinic to be sent to the laboratory by travel refrigerators. In the laboratory, saliva samples were centrifuged at 1500 x g for 10 minutes. Supernatants were collected and stored at -20°C. Then salivary β-defensin-2 and HST-5 levels were measured by ELISA method (BIO-RAD, Hercules, CA, USA) and reported in $(\mu g/ml)^{-1}$ units. After recording the data in SPSS software version 22 and GraphPad Prism version 9, the data were analyzed and the results were presented in the form of graphs and tables.

3- RESULTS

We analyzed the distribution of gender and age among the three study groups (mild, moderate, severe) through chi-square test and ANOVA. The results showed that the subjects are similar in terms of sex and age distribution, and the groups were matched with regard to these variables (**Table 1**).

In the mild caries group, half of the children were girls. In the moderate caries and severe caries groups, respectively, 56% and 53% of them were girls. The average age of children in all three study groups was close to each other and between 4.40 and 4.56 years. The three investigated groups did not have a significant difference in terms of feeding with breast milk and brushing teeth (**Table 2**).

Table-1: Distribution of gender and age among study groups

Factor	DMFT groups	Girl	Boy	mean ± Standard deviation	P-Value
Sex	Mild (DMFT<6)	8(50)	8(50)	-	0.939*
	moderate (6<DMFT<13)	7(44)	9(56)	-	
	Severe (13<DMFT)	7(47)	8(53)	-	
Age	Mild (DMFT<6)	-	-	4.44 ± 1.20	0.916**
	moderate (6<DMFT<13)	-	-	4.56 ± 1.09	
	Severe (13<DMFT)	-	-	4.40 ± 1.12	

*: Chi-square Test
**: ANOVA

Table-2: Distribution of breastfed and dental hygiene among study groups

Factor	DMFT groups	Mild (DMFT<6)	moderate (6<DMFT<13)	Severe (13<DMFT)	P-Value
Brestfed	Yes (at least 6 month as main course)	5(31)	6(38)	5(31)	0.966*
	Causally (not as main course)	4(36)	3(28)	4(36)	
	No (fewer than one month)	5(29)	7(42)	5(29)	
Brushing times	Three times per day	0(0)	0(0)	0(0)	0.382*
	Twice per day	0(0)	0(0)	1(100)	
	Once per day	5(50)	3(30)	2(20)	
	3-4 time per week	2(25)	2(25)	4(50)	
	1-2 time per week	5(21)	11(47)	7(32)	

*: Chi-square Test

Therefore, we examined the average of the study indices (beta-defensin-1, beta-defensin-2 and histatin-5) among the DMFT groups without considering the confounding variable, using Kruskal-Wallis non-parametric test.

The averages of beta-defensin-1 ($p<0.001$), beta-defensin-2 ($p<0.001$) and histatin-5 ($p<0.001$) were significantly different among different DMFT groups (**Table 3**).

Table3: Average peptides among different DMFT groups

Factor	DMFT groups	mean ± Standard deviation	P-Value
Beta-defensin-1	Mild (DMFT<6)	1.255 ± 0.299	<0.001*
	moderate (6<DMFT<13)	0.108± 0.348	
	Severe (13<DMFT)	0.067 ± 0.195	
Beta-defensin-2	Mild (DMFT<6)	0.148 ± 2.130	<0.001*
	moderate (6<DMFT<13)	0.136 ± 1.725	
	Severe (13<DMFT)	0.103 ± 2.093	
Histatin-5	Mild (DMFT<6)	1.187 ± 16.510	<0.001*
	moderate (6<DMFT<13)	0.354 ± 42.268	
	Severe (13<DMFT)	0.081 ± 42.848	

*: Kruskal-wallis test

Therefore, we used multiple comparisons tests to examine the pairs in each of these groups (**Fig. 1**). The average beta-defensin-1 index in the mild caries group was significantly higher than that in the moderate caries ($p<0.001$) and severe caries groups ($p<0.001$) (**Fig. 1-a**). The average beta-defensin-2 index in the mild caries group was significantly higher than that in the moderate caries ($p<0.001$) and severe caries groups ($p<0.001$). As demonstrated in the graph, there was no clear relationship between defensin levels and caries (**Fig. 1-b**). The average index of histatin-5 in the mild caries group was significantly lower than those in the moderate caries ($p<0.001$) and severe caries groups ($p<0.001$) (**Fig. 1-c**).

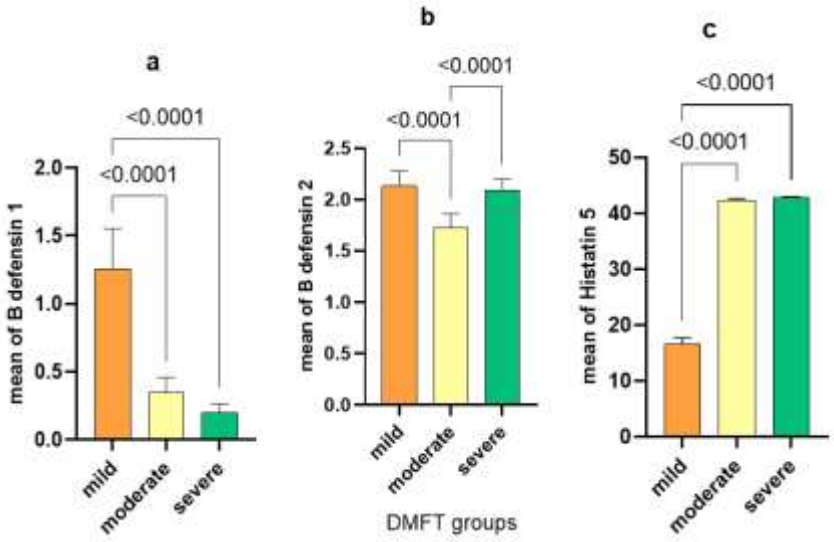

Fig. 1: Results of multiple comparisons test on the pairs of each group

4- DISCUSSION

The obtained results indicated that the level of HST-5 was higher in the saliva of children with a higher caries level. On the other hand, the average level of hBD-1in children's saliva was lower in children with high levels of caries in comparison to those with lower levels of caries. Also, no clear trend was observed between hBD-2 level in saliva and caries. The results of a study investigating the behavior of HST-5 in the unstimulated saliva of 18-year-old adolescents were also in line with those of the present study (20). Jurczak et al., who examined 82 children with an average age of 5 years with mild, moderate and severe caries, also reported that HST-5 increased along with the progression of decay (21). On the other hand, a study in which stimulated saliva was used showed that HST-5 among children without caries is significantly higher than that among those with moderate and severe caries (22). The use of stimulated saliva probably affects the amount and volume of peptides secretion. HST-5 has antibacterial activity against several species of oral bacteria, including S. mutans, by destabilizing the bacterial cell membrane and degrading their surfaces and damaging the bacterial cell. Therefore, its increase, at the same time as the progress of caries, may be one of the body's defense mechanisms (23).

In another study investigating the concentration of 9 salivary peptides by chromatography in the unstimulated saliva of 106 children aged 10 to 71 months, no relationship was found between hBD-2 and hBD-1 with caries (24). Yet another study examining the unstimulated saliva of

149 teenagers did not observe a significant relationship between caries and hBD-1 (25). Similarly, in the study by Phattarataratip et al., who examined 13-year-old children, no relationship was observed between hBD-2 and caries (26). It was also indicated in a review article that there is no direct relationship between hBD-1 and hBD-2 levels and caries (27). The increase of hBD-2 in oral keratinocytes can occur via rapid (within 2 h) or slow (10-12 h) pathways. Cycloheximide, as a protein synthesis inhibitor, prevented the rapid upregulation of hBD-2 by commensal bacterial extracts, indicating the need for new protein synthesis. Therefore, understanding the regulation of hBD-2 expression in the oral epithelium requires more experimental approaches (28). The complexity of hBD-2's behavior may be related to its highest activity against gram-negative bacteria and caries-causing species like S. mutans and S. sobrinus (29). On the other hand, hBD-1 is ineffective on Streptococcus mutans and it seems that during the process of negative self-regulation, the amount of this peptide decreases with increasing caries (30). Another difference between these two peptides is their place of expression. In normal gingival tissue, hBD-2 and hBD-1 are found in the spinous layer of the tissue and hBD-1 is constitutively expressed in oral keratinocytes, while hBD-2 is regulated by bacterial and proinflammatory stimuli (31).

Examining the relationship between peptides and caries can help peptidomic methods to identify new candidate biomarkers for the occurrence and development of caries. The identification of proteins for carious-related conditions may help in understanding the mechanisms of actions, predicting the risks, and advancing in caries control or anti-caries approaches (32).

5- CONCLUSION

Based on the results of the present study, it can be said that HST-5 and hBD-1 have a specific behavior towards caries. With increasing caries, HST-5 increases and hBD-1 decreases. With more extensive studies and increased information about salivary peptides, these peptides can be used in the prevention and treatment of caries. It is suggested that researchers interested in peptides and caries investigate more details of the relationship between HST-5 and hBD-1 in order to extract diagnostic and therapeutic biomarkers.

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