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Correlation Between DMF-T/def-t and The Secretion of Salivary HBD-1 in Carious and Non-Carious Group of Children

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ABSTRACT

Background: DMF-T/def-t (Decay⁹ Missing Filled Teeth) is an index for assessing dental and oral health status, in this case, dental caries. Dental caries is a multifactorial disease with factors namely teeth and saliva, microorganisms, dietary, and time that affecting each other. Human Beta Defensins-1 (HBD-1) is an Antimicrobial Peptides (AMPs) that has a role as an adaptive immune system in oral health, is a factor that affects the susceptibility and development of dental caries that exhibits activity in *Streptococcus mutans* bacteria.

Objective: To prove the relationship between DMF-T/def-t and the levels of HBD-1 saliva secretion in the carious and non-carious groups of children.

Method: This study was an observational analytic with cross-sectional study design, with the division of 3 groups namely: group 1 (DMF-T/def-t = 0, caries-free), group 2 (DMF-T/def-t = 1-3, low caries), and group 3 (DMF-T/def-t > 6, high caries).

Result: DMF-T/def-t index (1,000) obtained HBD-1 concentration (-0.451). Low DMF-T/def-t index has high HBD-1 concentration, whereas high DMF-T/def-t index has low HBD-1 concentration. The value of $p < (0.05)$ showed there was a significant correlation between the DMF-T/def-t index with salivary HBD-1 concentrations.

Conclusion: There was a relationship between DMF-T/def-t and the level of HBD-1 saliva secretion in the carious and non-carious groups of children.

Keywords: DMF-T/def-t, Saliva, *Streptococcus mutans*, Dental Caries, Human Beta Defensins-1

Introduction

Dental caries is the most common dental and oral disease found in the community, especially in children. Based on RISKESDAS 2013 data, caries

prevalence in Indonesia reached 53.02%. Dental caries in Indonesia tends to increase over years. Dental caries is a multifactorial disease with causative factors, namely teeth and saliva, microorganism, dietary, and time that affecting each other. The main group that suffered dental caries was the age group of 6-14 years old with DMF-T index of 2.21.

DMF-T (Decay Missing Filled Teeth) is an index to assess the oral and dental health status, in this case is dental caries. D value is the number of cavities or decayed teeth due to dental caries, M value is the number of extracted teeth due to dental caries, F value is the number of filled or treated teeth due to caries and in good condition. The DMF-T value is the total of D, M, and F scores.^{1,2}

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To assess the health status of deciduous teeth, commonly used def-t index (decay exfoliated filling teeth). Def-t value is an index that shows the number of deciduous teeth with caries in 9-12 years old children. D value is the number cavities or decayed teeth due to dental caries, e value is the number of deciduous teeth loss due to premature loss or extraction that caused by dental caries, and f value is the number of filled or treated deciduous teeth due to caries. The def-t value is the total of d, e, and f scores.³

The bacteria of the oral cavity can cause inflammatory processes, inducing an innate or adaptive immune system response to the host. Cytokines regulate many aspects of the immune response. In leukocyte circulation, cytokines have been shown to affect the expression of associated chemokine adhesion. In the innate immune response system, pathogenic microbes of the oral cavity have a molecular bond form with similar forms of receptors on host cells, including dendritic cells that then activate the inflammatory response together with the release of proinflammatory cytokines, such as IL-1 β , IL-6, IL-8 and TNF- α .⁴

So far several ways have been conducted to prevent the dental caries, among others by brushing your teeth properly, giving topical fluoride application and using mouthwash, but not showing maximum results to prevent dental caries. Some studies have shown that Antimicrobial Peptides (AMPs) are antibodies that provide a defense against pathogenic bacteria. This peptide is very important in the oral cavity and acts as an antimicrobial. Antimicrobial peptides (AMPs) in saliva are important contributors to maintaining a balance between health and illness as part of the host's innate immune response. AMP is considered to affect the susceptibility and development of dental caries in oral health.⁵

Human beta-defensins (HBDs) are in a family of AMP, which have a role as an immune response. Human Beta Defensin-1 (HBD-1) is expressed in the oral cavity tissue and secreted in saliva. HBD-1 as innate immunity, as well as chemoattractants for T cells and dendritic cells in microorganisms. The expression of HBD-1 is constitutive, and physiological salivation is not known. This study aimed to investigate the relationship between DMF-def-t with salivary HBD-1 secretion as a new way to prevent caries and biomarker candidates for caries risk assessment.⁶

Materials and Method

This study was an observational analytic with cross-sectional study design, with the division of 3 groups namely; group 1 (DMF-T/def-t = 0, caries-free), group 2 (DMF-T/def-t = 1-3, low caries), group 3 (DMF-T/def-t > 6, caries high). The research sample was the saliva that taken from elementary school students who were caries-free and carious at Qomaruddin Gresik Islamic Boarding School that qualified the inclusion and exclusion criteria using purposive random sampling technique.

The research materials used saliva samples, Human Beta-defensin 1 ELISA Bioassay Technology Laboratory kit (anti HBD-1 antibody; streptavidin-HRP; wash buffer; substrate solution A; substrate solution B; stop solution). Research tool used; sterile conical cup, 15 ml cryovial tube, 1.5 ml eppendorf, refrigerator with temperature -30°C, ice box, mask, mouthpiece, straight round, handpiece, handscoon, centrifuge with a temperature of -4°C, micropipette, elisa microplate, microplate wash (Bio-Rad), ELISA reader (Bio-Rad microplate reader) and stopwatch.

The students who became research samples were asked to fill out the questionnaire. The questionnaires contained student identity, criteria for measuring DMF-T/def-t index in oral condition. Students were instructed not to eat about 1 hour before saliva collecting (allowed to drink water) and avoid to intake of sweet foods and those containing caffeine. Before the saliva collecting was conducted, the students were asked to rinse for 1 minute with clean and distilled water, after gargling, they had to wait for approximately 5 minutes. Saliva collecting was conducted at 8-10 am with the method of passive droll, which by bowing the head and saliva that collected in the mouth was taken out into the cryovial tube. Saliva samples that had been collected should be processed in the laboratory in less than 2 hours.^{7,8}

The saliva sample was collected until reached 5 ml. The samples were stored in an ice box, then then stored in the refrigerator with a temperature of -30°C, so the sample could survive for long periods of time.⁹

Sample Preparation: The cryovial tubes were removed from the refrigerator and kept in the area at a room temperature, then centrifuged at 2400 g for 10 minutes, -4°C. The centrifugation was carried out in order to separate the supernatant in saliva, the supernatant would form clearly after centrifuge, separate from cells that contained in whole saliva.⁹ The supernatant was put into the eppendorf tube and stored in a refrigerator at a temperature of -30°C until the ELISA test time was conducted.

ELISA Sandwich Procedure: Salivary supernatant samples were removed from the refrigerator until they reached room temperature. The ELISA kit was removed from the refrigerator for approximately 30 minutes until all reagents reached at room temperature. A sample of 40 µl of salivary supernatant was put into the well, added 10 µl of anti HBD-1 antibody, then added 50 µl of streptavidin-HRP. Samples of saliva supernatant and reagent in plate were mixed using micromixer. The plate was covered with a sealer and incubated in a dark room at temperature of 37°C for 60 minutes. The wash buffer dissolved was prepared in distilled water in a ratio of 10ml : 290ml, then put into a microplate wash tube. The sealer was removed from the plate and the microplate was put into a microplate wash. The plate was washed 5 times, then dried by tapping lightly to dry.

The substrate solution A and substrate solution B were added to each well. When adding the substrate solution, the researcher must avoid the sunlight exposure directly. The plate was covered again with a sealer then incubated in a dark room at a temperature of 37°C. A 50 µl stop solution was put in each well. ELISA readings was with OD results and the concentration levels was used a microplate reader with a wavelength of 450 nm.

Result

¹³ The results of this study showed the levels of HBD-1 in carious and caries-free children were obtained from 3 groups; namely the DMF-T/def-t group = 0 (free caries), DMF-T/def-t = 1-3 (low caries), DMF-T/def-t => 6 (high caries). Before examination was conducted, all groups performed oral cavity examination first, to obtain DMF-T/def-t index. Then 5 ml of saliva was taken and divided according to caries-free criteria, low DMF-T/def-t index, and high DMF/def-t. The data was collected in 9 times visit on September 11st - November 15th, 2017 at Pondok Pesantren Qomaruddin.

Table 1: Mean of DMF-T/def-t and salivary HBD-1 concentrations in each group

SAMPLE	DMF-T/def-t	HBD-1 Concentration
	Mean ± SD	Mean ± SD
Group 1 (caries free)	0	1000.8 ± 339.38
Group 2 (low caries)	1.86 ± 0.83	1036.8 ± 366.9
Group 3 (high caries)	7.80 ± 2.68	709.93 ± 243.30

The Table 1 showed the caries-free group as a control resulted the mean of DMF-T/def-t score of 0 (caries-free) and mean of salivary HBD-1 concentration was 1000.8 ± 339.38 pg/ml. The low caries group with low DMF-T/def-t resulted the mean of DMF-T/def-t score of 1-3 (low caries) was 1.86 ± 0.83 and the mean of HBD-1 concentration was 1036.8 ± 366.9 pg/ml. The high caries group with high of DMF/def-t score more than 6 (high caries) resulted the mean of DMF-T/def-t of 7.80 ± 2.68 and the mean of HBD-1 concentration was 709.93 ± 243.30 pg/ml.

Before salivary HBD-1 levels test was conducted, the data was firstly analyzed for its normality and homogeneity of data. Normality test was performed by Kolmogorov-Smirnov test, showed the probability of each treatment groups. The group 1 was with alpha value of 0.5 so the data distribution of DMF-T/def-t was normal. While the group 2 and group 3 were with alpha value more than 0.5, so the data distribution were normal concentrate.

Table 2: Spearman's test results

Spearman's Test	DMF-T/def-t	Concentration	p
DMF	1.000	-0.451	0.002
Concentration	-0.451	1.000	

Table 2 contains the results of Spearman's test that showed p value of 0.002, which the alpha value is less than 0.05, that means there was a significant correlation between the DMF-T/def-t scores and the salivary HBD-1 concentration. The value of r < 0 indicates that the correlation between DMF-T/def-t scores and the salivary HBD-1 concentration was inversely related. That means low DMF-T/def-t was associated with high levels concentration of salivary HBD-1, whereas high DMF-T/def-t was associated with low levels concentration of salivary HBD-1.

Discussion

¹ This study was conducted to determine the relationship between DMF-T/def-t and salivary Human Beta Defensin-1 (HBD-1) secretion in carious and caries-free children groups. Before sample grouping based on the DMF-T/def-t scores was conducted, the samples were examined their oral cavity using DMF-T/

def-t index. The 5 ml saliva sample was placed in the centrifuge tube and divided into two groups, namely low DMF/def-t and high DMF/def-t. The results of this study showed there were a different levels of salivary HBD-1 in the low DMF/def-t group and high DMF/def-t group. This research involved elementary students of Qomaruddin Gresik Islamic Boarding School, which provides a dental treatment facilities.

The results of mean HBD-1 concentration in caries-free condition was 1000.8 ± 339.38 pg/ml. Salivary HBD-1 levels in the oral cavity can be measured in normal conditions because the peptides can be detected. Salivary HBD-1 is produced when inflammation occurs, in the salivary epithelial tissue and the gingival crevicular fluid.¹⁰

HBD-1 has the role of preventing commensal bacteria in the oral cavity differentiate into pathogenic bacteria. While salivary HBD-2 and HBD-3 are the antimicrobials, which are inducible, the levels may increase because of the stimulation of microbes, and have a major role against pathogenic bacteria.¹¹

In the low caries group was with the mean of DMF-T/def-t scores of 1.86 ± 0.83 and the salivary HBD-1 concentration was 1036.8 ± 366.9 pg/ml. The presence of low frequency caries is because the pathogenic bacteria is inhibited by the activity of HBD-1. While the high caries group with the mean of DMF-T/def-t scores of 7.80 ± 2.68 and the salivary HBD-1 concentration was 709.93 ± 243.30 pg/ml. This is because HBD-1 is unable to inhibit the pathogenicity of cariogenic bacteria, then it is replaced with HBD-2 which strengthen the antimicrobial activity against the invasion of pathogenic bacteria, resulting in decreasing the levels of HBD-1.¹⁰

HBD-2 is produced when there is a chronic inflammation and the inflammatory mediator that can increase HBD-2 production, namely IL-1 β , IFN- γ , TNF- α , IL-6, IL-8.⁴ From the results of this study, it is known that DMF-T/def-t and the salivary HBD-1 concentration are the new biomarker candidates for dental caries risk assessment to prevent dental caries. *Streptococcus mutans* is a dental caries etiology factor and has been studied extensively on the relationship between dental caries and *S. mutans*. Several studies also assessed the association between the development of carious lesions with immunocompetent cell responses. Cytokines are an important mediator during inflammation and infection,

12
in addition to the role of controlling the inflammatory response to bacterial infections. The host's active cells secrete these molecules as paracrine or autocrine signals to recruit immune system cells (chemokine), produce inflammation (proinflammatory cytokines) or regulate inflammatory responses (anti-inflammatory cytokines). The role of cytokines in dental caries pathogenesis is not clearly, but the production of proinflammatory cytokines can be induced by *S. mutant* components.^{12,13}

Conclusion

Based on the results of this study, we can conclude that there was a relationship between DMF-T/def-t and the level of HBD-1 saliva secretion in the carious and non-carious groups of children. The correlation between DMF-T/def-t scores and the salivary HBD-1 concentration was inversely related.

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14
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Ethical Clearance: This study had received a letter of approval for ethical clearance on human subjects from the Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia, with number 132/HRECC.FODM/VIII/2017.

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