ORIGINAL ARTICLE

The Relation Between Decay Missing Filled-Teeth (DMF-t), Body Mass Index (BMI) with Salivary Human Beta-3 (HBD-3) Secretion in Children with Caries and Free Caries

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ABSTRACT

Introduction: Dental caries in children is a major problem of mouth disease throughout the world, so too is there currently an increase in health problems in children due to obesity. Human Beta defensing(HBD) has been found in saliva and from several studies stated that HBD aside from being a broad-spectrum antimicrobial can act as an immunomodulator. The purpose of this study is to reveal whether there is a relationship between obesity and HBD-3 salivary concentration in caries patients and caries-free patients. **Methods:** This cross-sectional observational study was involved 62 children with caries and caries-free, aged 9-11 years, students at Qommarudin Islamic Boarding School, Gresik, East Java Indonesia. dental caries examination, carried out in accordance with World Health Organization (WHO) diagnostic criteria. Body mass index (BMI) was measured from the height and weight of individuals, HBD-3 concentrations were tested with an ELISA kit from Bioassay Technology Laboratory (China) from saliva samples. Evaluate the results with the Kruskal Wallis test, followed by the Mann-Whitney test. The level of significance used in this statistical test was 0.05. **Results:** there was a relationship between BMI level and HBD-3 concentration in the caries group ($p < 0.05$, $p = 0.009$) with a moderate level of association. but there was no significant relationship in the caries-free group ($p > 0.05$, $p = 0.189$). **Conclusion:** There was an association between BMI and HBD-3 salivary concentration in caries patients but there was no relationship in the caries-free group.

Keywords: Human beta defensin-3 (HBD-3), Body mass index (BMI), Decay Missing Filled-Teeth (DMF-t), Saliva, Elisa

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INTRODUCTION

Body Mass Index (BMI) is a number that becomes a standard assessment to indicate nutritional status by classifying thin, normal, overweight, and obese. Body Mass Index can also predict the occurrence of a disease, including dental caries (1,2). Data from the World Health Organization (WHO) reveals that 60-90% of children suffer from cavities (3). Dental caries is tooth surface damage due to an imbalance in demineralization and re-mineralization due to multifactorial etiology, such as the presence of substrates (sucrose), agents (*Streptococcus mutans*), and host response (innate immunity) (4). Antimicrobial peptide (AMP), which is contained in saliva, is an innate immunity that provides broad-spectrum defense against pathogens (5,6).

Defensins are included in AMP, specifically components of the innate immune system against pathogenic bacteria and opportunistic pathogens that can cause caries, viral infections or fungal infections, and act against periodontal disease. In humans, there are three defensins subfamily identified and structurally classified: α-defensins, β-defensins, θ-defensins (7). Human beta-defensins (HBD) have three sub-families, namely HBD.-1, -2 and -3. HBD has two different roles, HBD -1 will be expressed continuously to prevent normal flora from turning opportunistic, while HBD. -3 -3 is expressed in response to lipopolysaccharide (LPS), peptidoglycan, and lepoteichoic acidic bacteria, acting as a proinflammatory mediator, as well as interferon. The mechanism of action possessed by HBD is effective against almost all pathogens (8,9).

BMI scores reflect the amount and structure of body fat tissue. Obesity is a chronic, low-grade inflammatory status that expresses various cytokines, complements chemokine proteins, and proinflammation. Fat cells too

produce cytokines and chemokines such as TNF-α, IL-6, IL-1b, IL-8 and other factors. This substance is increased by the expression of various inflammatory genes that are activated in the presence of fat tissue. This then influences the innate immune response to produce AMP including HBD-3 (10,11).

From the description above, the purpose of this study is to reveal whether there is a relationship between obesity and HBD-3 salivary concentration in caries patients and caries-free patients.

MATERIALS AND METHODS

Sample

This study was approved by the Health Research Ethics Committee (KEPK) Faculty of Dentistry, Airlangga University, Indonesia (Number: 150/HRECC.FODM / VIII / 2017). This observational cross-sectional study involved 62 children in Qommarudin Islamic Boarding School, Gresik. Children were asked to sign informed consent and fill out questionnaires, then measure height using GEA Medical HT721 wireless body height meters (Korean brand), measure body weight using digital weight scales (KrisBow brand made in China), and dental caries screening. Sample criteria for this study were children aged 9 to 10 years, DMF-T caries sample ≥ 3 and caries free, physically fit, and currently not undergoing immunosuppressive treatment; while exclusion criteria are ill when saliva samples are taken, take drugs that suppress the immune response (antibiotics), using fixed orthodontic equipment.

Saliva collection

Samples were taken from the refrigerator to room temperature, then centrifuged at 2400g for 10 minutes at -4°C (Oregon Nesco - China digital centrifuge LC-04C Plus). The supernatant was then stored in a freezer at -30 ° C (Sanyo - Japan) until testing. HBD-3 levels in the sample were measured by ELISA readers using the ELISA HBD-3 kit from Bioassay Technology Laboratory (China). Analysis of HBD-3 levels was carried out at the Institute of Tropical Disease at Airirlangga University.

Statistic analysis

The data collected was then analyzed using the Kruskal Wallis test, followed by the Mann-Whitney test. The level of significance used in this statistical test was 0.05.

RESULTS

Calculation of Body Mass Index (BMI) according to the Ministry of Health WHO is done by dividing body weight in kilograms by height squared in meters. At the age of children, the relationship between economic status and BMI is almost invisible, but will be seen in adulthood. (3) BMI scores describe the condition of adipose tissue and the structure of blood vessels that produce immune cell counts and cytokine production that affect the immune system and HBD-3 secretion.HBD-3 levels were tested with ELISA kit Bioassay Technology Laboratory from salivary samples because the presence of antimicrobial peptides (HBD-3) in saliva can be a biological factor that contributes to susceptibility or resistance to caries. The results of the HBD-3 ELISA in all the samples studied can be seen in Table I.

Table 1: Mean and standard deviation of HBD-3 levels for each groups

Groups		HBD-3 Levels X ± SD (ng/ml)
Free Caries	BMI ≤ 15kg / m2	3.366 ± 0.892
	BMI ≥ 16 kg / m2	2.941 ± 0.772
Caries	BMI ≤ 15kg / m2	3.262 ± 0.742
	BMI ≥ 16 kg / m2	2.579 ± 0.636

Homogeneity test results showed that the data variance was homogeneous so that the data analysis fulfilled the requirements for further independent sample t tests to distinguish HBD-3 levels between the BMI caries-free group ≤ 15 kg / m^2 and the BMI ≥16 kg / m^2, and between the BMI caries group ≤ 15 kg / m^2 and BMI ≥16 kg / m^2. Significant results from independent sample t tests (Table II). Obtained p = 0.189 so that p> 0.05, means that there is no significant difference between HBD-3 levels in the

Table II: The results of the significance of the independent sample t test

Groups		Sig. (p)
Free caries	BMI≤ 15 kg/m^2	0.189
	BMI≥16 kg/m^2	
Caries	BMI≤ 15 kg/m^2	0.007
	BMI≥16 kg/m^2	

caries-free BMI group ≤ 15 kg/m^2 and BMI ≥16 kg/m^2. In the caries group BMI ≤ 15 kg/m^2 and BMI ≥ 16 kg/m^2 obtained p value = 0.007 so that p <0.05, which means that there are significant differences in these two groups. BMI can be used as an indicator to predict the incidence of a person's illness and death (1). Dental caries is one of the incidences of disease associated with BMI (2). After a series of analyzes with normality testing using the Komogorov-Smirnov One-Sample test, and an independent sample t test to distinguish the difference in HBD-3 levels between the caries group and the caries-free group, then to see the relationship between the BMI score and the HBD level - 3 in each sample group, then the correlation test was performed with the Spearman test. The results of the correlation test analysis with the Spearman test can be seen in Table III. The caries-free group showed a significance value of p = 0.199 so that p> 0.05, meaning that there was no correlation between BMI and HBD-3. level score. The significance value in the caries group was p = 0.009, so that p <0.05, which means that there is a correlation between the BMI score and the HBD-3 level with the correlation coefficient

Table III: Significance results and correlation coefficients of Spearman's test BMI scores with HBD-3 levels in free caries and caries-groups

Sample group	Sig. (p)	Correlation Coefficient (r)
Free caries	0.199	-0.250
Caries	0.009	-0.443

r = -0.444, which shows an inverse relationship with strength, a moderate relationship.

DISCUSSION

The results of this study found that HBD-3 was also secreted in saliva in the caries group and caries-free group. This is in accordance with several studies which state that peptides are secreted in saliva response to innate immunity in maintaining the balance condition of the oral cavity microbiome (12). Human Beta Defensin-3 (HBD-3) is a peptide that has a wide distribution and spectrum of various microorganisms including Gram-positive bacteria, Gram-negative bacteria, fungi, parasites and viruses (13,14). HBD-3 can be found in the oral epithelium, gingival crevicular fluid and salivary glands (15). Some researchers claim that the majority of HBD-2 andHBD-3 are mostly contained in the oral cavity at the infected site and effectively prevent the spread of bacterial infections. HBD-1 has a role to prevent local commensal bacteria in the oral cavity to become pathogenic bacteria, while HBD-2 and HBD-3 levels can be increased if there is microbial stimulation and act effectively as an antimicrobial (4, 5).

This study measured HBD-3 levels in two sample groups with a BMI score ≤ 15 kg/m^2 and ≥ 16kg/m^2 based on data collected. BMI scores ≤ 15 kg/m^2 for children aged 9-10 years are included in the normal category, while for BMI scores ≥ 16 kg/m^2 are included in the obesity category (3). The results of the research that have been done, show that there are differences between the 2 levels of BMI in the carious-free BMI group ≤ 15 kg/m^2 and the carious-free BMI group ≥ 16 kg/m^2, the average level of HBD -3 in the BMI group ≤ 15 kg/m^2 higher than BMI ≥ 16 kg/m^2. However, there was no significant difference. This shows that HBD-3 secretion still occurs in caries-free conditions, this is supported by the opinion of Dhople who reported that analysis using real-time reverse transcription-polymerase chain reaction (RT-PCR) detected the presence of HBD-3 in the oral mucosa and salivary glands, with inflammatory or non-inflammatory conditions. The presence of HBD-3 levels is also caused by stimulation of local commensal bacteria (16).

In the BMI caries group ≤ 15 kg/m^2 and BMI ≥ 16 kg /m^2 there was a significant difference in HBD-3 levels, the average level of HBD-3 in the BMI group ≤ 15 kg/m^2 was higher than the BMI group ≥ 16 kg/m^2. *Streptococcus mutans* induces TLR-2 cell surfaces together with TLR-1 or TLR-6 in oral epithelium, due to the presence of Peptidoglycan and lepotheicoic acid in bacterial cell walls, then activates NF-κB to encourage them to produce TNF-α proinflammation. cytokines, IL-1β, IFN-γ, IL-6, IL-8, which activate the innate immune system and secrete AMP, one of which is HBD-3 (17,18). From the results of the study it can be seen that the average level of saliva HBD-3 in the caries-free group is higher than the caries group. The characteristic of AMP is that it consists of various amino acids, making it easy to make changes to the structure and surface conditions that interfere with AMP. Peptides which are arranged on the AMP surface will react if they interact with the target cell membrane (19). When there are many types of AMP bacterial infections expressed in the oral cavity such as cathelicidin, LL-37, HNP 1-4, HBD 1-3 and histatin (5,20). The range of AMP in the oral cavity works together against bacteria by performing microbial killing mechanisms. Cations as characters possessed by AMP bind negative poles of the phospholipid microbial membrane and cause damage to the cell membrane (21). The bond between lepotheicoic acid and peptidoglycan S.mutans bacteria with TLR-2 increases the delivery signal to increase AMP secretion. AMP which is more sensitive to the surface structure of Peptidoglycan bacteria and lepotheicoic acid directly performs the mechanism of microbial killing and its effect on the level of HBD-3 secretion. It is suspected that HBD-3 responds to S. mutans so caries does not occur.

The mean level of HBD-3 in the group with a BMI ≤ 15 kg/m^2 was greater than the BMI group ≥ 16 kg/m^2, in caries and non caries conditions. This shows that a person's BMI influences HBD-3 secretion. Test results showed no correlation between BMI and HBD-3 levels in the caries-free group, but there was a relationship with a moderate level of relationship between BMI scores and HBD-3 levels in the caries group. Several studies have shown that BMI is closely related to cell numbers namely leukocytes, monocytes, neutrophils and total immune system modulation parameters (10). Individuals with high BMI experience changes in hormone levels and supply of nutrients, such as glucose and lipids. Immune cells that circulate in peripheral tissues exposed to high-energy environments, that is, the concentration of hormones change so that they affect the metabolic function of immune cells (22).

Changes in hormone levels that occur can be influenced by the sex of the individual. In this study saliva samples from boys and girls aged 9 to 10 years were criteria for women who had never menstruated. According to fromUnicef data girls in Indonesia experience their first menstruation most often at around 13 years of age. Research conducted by Batubara stated that in 4,145 young women from all regions of Indonesia experienced their first menstrual period at the age of 12-14 years. Another study states that between 12 and 13 years, a

woman experiences her first menstruation (23,24). The general status for each child in this study also looked at health data from each boarding school belonging to the child's health unit. Daily food consumption was also asked in the questionnaire because nutrition affected BMI (25). Every child in this study is a student boarding school so that the level of consumption is considered to have the same or homogeneous eating patterns. Sex differences that affect hormones also affect the growth process of individuals. Sex affects the effects arising from sex hormones and interactions on adipose tissue, because the distribution of adipose tissue in the body varies according to sex (26). From various studies have shown that cells of the immune system and humoral are more active in women than in men, one of which is a steroid sex hormone that acts as a regulator of the inflammatory process. The difference is when someone has entered adulthood or has experienced puberty (27,28).

A constant ratio between stromal cell counts seen from adipose and adipocyte tissue independently by an individual BMI. Based on several studies which state that the most sensitive period of adipose tissue development occurs at the age of children (29). Genes and the environment have an effect on a person's BMI value, high BMI is associated with many metabolic diseases, several studies have shown that immune cell responses in adipose tissue have an important role in regulating homeostasis (30).

Adipose tissue not only functions as a fat storage mass, but is also known as a tissue that secretes a lot of adipokines, cytokines and chemokines. The cytokines produced include proinflammatory cytokines including TNF-α, IL-1β, IL-6 and IL-8 (11). Increasing the amount of fat will increase the production of cytokines which will trigger a series of inflammatory processes namely pathophysiology. Individuals with high BMI are associated with low-level chronic inflammation due to signals of abnormal production processes and inflammatory mediators. Inflammation that occurs spreads in several parts of the body, such as the liver, pancreas, and muscles. An inflammatory process is involved when neutrophils, eosinophils, monocytes and lymphocytes penetrate into adipose tissue. Increased chemokine production and proinflammation of stitokines can trigger local effects on the endothelium which cause an increase in vascular cells and intracellular adhesion, and vascular permeability. This allows cells such as polymorphonuclear and mononuclear phagocytes (monocytes) to travel extravascularly. The accumulation of macrophages in adipose tissue which also results in molecular proinflammation and the release of inflammatory processes, maintains more and more chemoattractants that occur so that the inflammation that occurs is chronic. Increased production of cytokines by adipose tissue that occurs continuously causes metabolic and immunological disorders (31).

Supported by the opinion of several researchers who revealedthat TLR can be induced in adipose tissue and is associated with TNF-α and NF-κB activation, as well as effects on the cytokine release mechanism. Excess TNF-α, IL-6 and IL-1β produced in adipose tissue can be excreted in the blood and potentially have side effects on vascular permeability. can reduce the sensitivity of immune cells when there is infection (22,33).

CONCLUSION

There was an association between BMI and HBD-3 salivary concentration in caries patients but there was no relationship in the caries-free. In addition to directing antimicrobial activity, some AMPs (HBD-3) can also inhibit the formation of biofilms and disrupt existing biofilms, so it is advisable to know how the mechanism of these obstacles.

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