

Increased Levels of Human B Defensins in Children with Dental Caries

Retno Indrawati Roestamadji^{a*}, Dian Agustin Wahjuningrum^b, Mohammat Lutfi^a, Tantiana^a, Agung Soesiawan^c and Prof. Seno Pradopo^d

^aDepartment of Oral Biology Faculty of Dental Medicine, Universitas Airlangga, Surabaya,

^bDepartment of Endodontics Faculty of Dental Medicine, Universitas Airlangga, Surabaya

^cDepartment of Public Health Faculty of Dental Medicine, Universitas Airlangga, Surabaya

^dDepartment of Paediatric Dentistry Faculty of Dental Medicine Universitas Airlangga, Surabaya

* Corresponding : retnoindrawati@fkg.unair.ac.id

ABSTRACT

Background: Human Beta Defensin peptide in saliva plays a role as innate immunity to dental caries. The purpose of this study is to determine whether peptide secretion of hBD1,2 and 3 saliva can be used as biological immune response to dental caries. **Methods:** Unstimulated whole saliva was collected for 55 elementary school students (6-10 years old) at Pondok Pesantren Qomaruddin Gresik, for levels of hBD1,2 and 3 were assessed by ELISA. Salivary pH was measured using digital pH meter. **Results:** The median hBD1, hBD2 and hBD3 saliva levels for the caries-free group (n = 22) were 0.86 µg / ml, 1.76 µg / ml and 1.88 µg / ml respectively and for the caries group (n = 33) were 5.26 µg / ml, 4.25 µg / ml and 4.85 µg / ml. The result proves that the Levels of hBD1, hBD2 and hBD3 has a significant correlation with caries experience (p <0.01). The saliva pH in the caries group patient is also more acidic (6.0 - 6.5) than the caries-free group pH (6.9 - 7.0). **Conclusion:** From the results of this study it can be concluded that the high saliva levels secretion hBD1,2 and 3, can represent the immune response to dental caries and can be used as a new way to prevent dental caries and caries risk assessment

INTRODUCTION

WHO data shows that the prevalence of dental caries is 60-90% in elementary school-children and nearly 100% in adults (WHO, 2012). According to the US National Health and Nutrition Examination Survey (NHANES) in 1999-2004 children aged 6-8 years had a prevalence of dental caries and permanent tooth of 56.12% and the prevalence of untreated and untreated permanent tooth by 37.38% (WHO, 2012; Ghazal *et al.*, 2016).

External and internal factors affect each other's condition in the oral cavity. Diet or nutrition as well as mechanical activity (toothbrushing) are examples of external factors, while saliva (including pH and saliva flow rate), DMF levels, number of *Streptococcus mutans*, and oral immune systems are some examples of internal factors in maintaining oral conditions, because a balanced oral cavity condition will maintain normal flora in the oral cavity, whereas if conditions change, normal flora may turn into opportunistic pathogens, one of which is characterized by dental caries (Beverly *et al.*, 2005).

Dental caries is an infection with multifactorial causes that one of them may be affected by host factors such as salivary antimicrobial peptides. AMP is a natural antimicrobial which is the first line of defense against a wide spectrum of pathogens. Human beta defensin as part of the AMP may be very important in the oral cavity, where members of the microbial flora present at all times in high quantities (Beverly *et al.*, 2006).

Human Beta Defensin (HBD) is a natural antimicrobial that is found in saliva. It plays a few roles in maintaining a balance between health and disease and as part of the host's innate immune response. The secretion of hBD2 in saliva is induced by Commensal bacterial community of the oral cavity, this suggesting that the commensal bacterial community acts in a favorable way in preparing the innate

immune response in the oral cavity, and this may have major significance for understanding the complex defenses in the oral environment (Winter *et al.*, 2012; Gosh *et al.*, 2007).

Human Beta Defensin-1 (hBD-1) is constitutive, and serves to preventing commensal bacteria from becoming opportunistic, whereas expression of hBD-2,3 is induced by proinflammatory mediators such as IL-1 β , TNF- α , IFN- γ , lipopolysaccharide (LPS) and are more effective against almost all pathogens. These peptide has a special function in maintaining the level of commensal bacteria. (Beverly *et al.*, 2005). Human beta defensin also acts as chemoattractants for T cells, immature dendritic cells, B cells, neutrophils and macrophages in enhancing adaptive immunity, so that the expression of saliva human beta defensin could have a role in protecting the tooth structure from caries as well as protecting oral mucosa (Beverly *et al.*, 2006; Gosh *et al.*, 2007).

MATERIALS AND METHOD

We conducted research on elementary school children aged between 6 and 10 years old in Pondok Pesantren Qomaruddin Gresik. First, the subjects must meet the criteria of inclusion and exclusion of simple random sampling technique. Fifty five children participated in the study. Health history surveys are completed by the parent / guardian of the subject. The data gathered and recorded in this study include: student identity, physical criteria, BMI index, OHI index, DMF index, saliva pH, saliva flow rate, oral condition and family economic status. The data obtained are used for the next research correlation. The subject's informed consent was obtained through a protocol approved by the medical research ethics committee of the Dental faculty of Airlangga University.

5ml unstimulated saliva was collected in saliva collection tube (Salimetrics, USA) and then added with the NonI-P-40 (Sigma, St. Louis, MO) detergent 0.1%, and stored in 80°C for further analysis.

For the analysis of level hBD1, hBD2 and hBD3, saliva diluted and then centrifuged twice at 15,000 rpm for 10 min. Saliva samples were tested by ELISA (Salimetrics, USA) tests as per manufacturer's instructions (Winter et al., 2012). Saliva sampling was performed in the morning (9-10 am). pH saliva were analyzed using a digital pH meter (Shenzhen Youfu Cina). (Janet et al., 2005).

RESULTS

The results of hBD-1,2 and 3 caries and caries-free clusters were tested for One Sample Kolmogorov-Smirnov Normality Test, to see if the data obtained were normally distributed. In each sample group (Dental Caries n = 33 and Caries Free n = 22), the significance value $p > 0.05$ then all groups of research data is normally distributed.

The median hBD1, hBD2 and hBD3 saliva levels for the caries-free group (n = 22) were 0.86 $\mu\text{g} / \text{ml}$, 1.76 $\mu\text{g} / \text{ml}$ and 1.88 $\mu\text{g} / \text{ml}$ respectively and for the caries group (n = 33) were 5.26 $\mu\text{g} / \text{ml}$, 4.25 $\mu\text{g} / \text{ml}$ and 4.85 $\mu\text{g} / \text{ml}$. This means that the Levels of hBD1, hBD2 and hBD3 has a strong correlation with caries experience significantly ($p < 0.01$). The saliva pH in the caries group patient was more acidic (6.0 - 6.5) than the caries-free group pH (6.9 - 7.0)

DISCUSSION

Research on the relationship of dental caries with hBD-1 and hBD-2 has been successfully completed, but in combination with hBD-2 and hBD-3 is still ongoing. This study involved elementary school students in Qomaruddin Gresik boarding school with minimal dental care facility. (Gosh et al., 2007).

Streptococcus mutans is bacteria that causes dental caries and can stimulate the cytokine. The release of proinflammatory cytokines (IL-1 β , IFN- γ , TNF- α , IL-6, IL-8), will stimulate the secretion of hBD-2 and hBD-3 saliva. Furthermore, the peptide hBD-2 and hBD-3 will perform antimicrobial action in fight against oral bacteria. Sophie Joly et al (2004) used Radial diffusion to test activities hBD-2 and hBD-3 against oral bacteria. The result hBD-3 demonstrated greater antimicrobial activity and is effective according to the results of this study, the mean saliva levels of hBD3 were 1.88 $\mu\text{g} / \text{ml}$ for the caries-free group and 4.85 $\mu\text{g} / \text{ml}$ for all caries-proof subjects, the highest compared with hBD-2. (Seethalakshmi et al., 2016; Anton et al., 2002; Sophie et al., 2004).

Human Beta Defensin-1 (hBD-1) is secreted in saliva to inhibit normal flora such as Streptococcus mutans from becoming opportunistic pathogens. This is in agreement with the results found in this study in which hBD-1 increases to a higher level in the caries group (0.86 $\mu\text{g}/\text{ml}$, to 5.26 $\mu\text{g}/\text{ml}$), because it is responsible to ensure that microorganism that cause dental caries will not become pathogen / virulent (Hahn et al., 2000).

When bacteria colonize within the oral cavity and cause inflammatory processes, the lesions induce innate and adaptive immune responses. Cells in periapical lesions are macrophages, neutrophils, T and B lymphocytes, osteoclasts, osteoblasts, and fibroblasts, in which they express a large number of proinflammatory cytokines, including IL-6, IL-4, IL-1 β , IL-1 α , TNF- A, IL-12, IFN- γ . The components of the cytokine TNF- α , IL-8, and IL-6 in saliva are elevated in dental caries state compared with caries-free. hBD-2 and hBD-3 are peptides that appear when induced by microorganisms or components of proinflammatory cytokines TNF- α , IFN- γ , and IL-1 β . This causes the levels of hBD-2 and hBD-3 to increase in the caries group compared with the caries-free group (Hahn et al., 2007; Joly et al., 2004).

Human β -defensins 1, 2 and 3 (hBD-1, hBD-2 and hBD-3) are inducible peptides present at sites of infection in the oral cavity. A few studies have reported broad-spectrum antimicrobial activity for that three peptides. Sophie Joly et al (2004) used Radial diffusion to test activities hBD-2 and hBD-3 against Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum, Porphyromonas

gingivalis, Peptostreptococcus micros, Actinomyces naeslundii, Actinomyces israelii, Streptococcus sanguis, Streptococcus mutans, Candida tropicalis, Candida parapsilosis, Candida krusei, Candida glabrata, and Candida albicans, The result hBD-3 demonstrated greater antimicrobial activity and is effective According to the results of this study, the mean saliva levels of hBD3 were 1.88 $\mu\text{g} / \text{ml}$ for the caries-free group and 4.85 $\mu\text{g} / \text{ml}$ for all caries-proof subjects, the highest compared with hBD-2. (Janet et al., 2005; Seethalakshmi et al., 2016; Anton et al., 2002).

pH of saliva in patients with dental caries is also more acidic than pH of saliva of the control group. This can be explained in accordance with the study conducted by Takahashi et al (1999), that the microorganism which are responsible for caries have a favourable environment for growth in an acidic pH such as Streptococcus mutans grows at a pH of 5.5-7.0 (Joly et al., 2004; Anderson, et al., 2001).

From these results it is furthermore necessary to know the antimicrobial proteins of the beta-defici in human peptide. This will open a new perspective to design natural antimicrobials and open up new perspective for the application of peptides against multiresistant microorganisms

CONCLUSION

From the results of this study it can be concluded that the high saliva levels secretion hBD1,2 and 3, can represent the immune response to dental caries. It also can be used as a new way to prevent dental caries and caries risk assessment

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