# Salivary Human Beta Defensin-1 Level and Oral Health Status of Tobacco Smokers

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**Abstract**

Salivary human beta defensin-1 (SHBD-1) is an important component of innate immune defense against attacking oral microbes. Tobacco smoking causes many changes to the oral cavity, including changes to salivary components, but its effects on SHBD-1 have not been widely studied.

We evaluated the effects of tobacco smoking exposure on SHBD-1 level.

A total of 68 male study patients were divided into 2 groups: 44 smokers and 24 nonsmokers. Data were collected from anamnesis, clinical (oral, dental, and saliva status), and laboratory examinations. Saliva samples were stored at −80 °C until they were tested for SHBD-1 via enzyme-linked immunosorbent assay.

Significant differences ($P < 0.05$) were observed in the amount of SHBD-1 between smokers and nonsmokers, which had median (minimum–maximum) SHBD-1 levels of 5.65 (0.07–45.02) and 2.34 (0.04–16.76) pg/mL, respectively.

Tobacco smoking exposure adversely affects saliva components, including SHBD-1 level, regardless of cigarette type, smoking duration, and smoking frequency.

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## Introduction

Indonesia is a developing country with a population of >250 million and ranks as the 5th largest tobacco-consuming country with an estimated consumption of 215 billion cigarettes per year.[1] On the basis of the Indonesian National Health Survey in 2013, the highest prevalence of smokers were seen in male subjects, people with low incomes, senior high school students, farmers, fishermen, and laborers.[2]

Smoking is a common addiction that has been implicated as an etiologic agent for many chronic diseases, such as infections, cancers, and heart and lung diseases. Tobacco smoking negatively affects the oral cavity (e.g., the oral mucosa), dental health status, and salivary profile.[3] Regarding oral mucosal status, smoking habit may cause various oral diseases, including nicotine stomatitis, smokers melanosis, hairy tongue, black hairy tongue, median rhomboid glossitis, precancerous lesions (leukoplakia, erythroleukoplakia, and oral lichen planus), and oral cancer.[3] Regarding dental health status, the changes in tooth structure caused by smoking include staining and abrasion. For salivary profile, constant tobacco smoking exposure disrupts the secretory functions of the salivary glands, taste receptors, and other components, thus inhibiting food digestion, protection, lubrication, and speech.[3]

Salivary human beta defensin-1 (SHBD-1) is an important salivary component of the innate immune defense against an invasion by oral microbes. It provides signaling for the adaptive immune system and has an important role in pulling immature dendritic cells and T cells by binding chemokine receptors. It also maintains the homeostasis of microbial pathogens by preventing bacterial colonization and viral infection. Previous studies showed that patients with various oral diseases, such as lichen planus, leukoplakia, glossitis, glossodynia, and oral discomfort, have increased defensin-1 levels.[4,5]

We evaluated SHBD-1 levels in smokers and nonsmokers by using saliva samples and

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provided a brief overview of SHBD-1 level in association with tobacco smoking habit.

## Methods

In this cross-sectional study, 44 male subjects with smoking habit and 24 male subjects without smoking habit (age, 20–55 years) underwent clinical examination (Oral Hygiene Index (OHIS); Diseased, Missing, and Filled Teeth (DMFT); and oral lesions) and salivary sampling. Further examinations of the saliva samples were performed in the laboratory of oral biology. This procedure was performed in accordance with the guidelines on the ethical approval of the Ethics Committee, Faculty of Dentistry, Universitas Indonesia.

### Enzyme-Linked Immunosorbent Assay Protocol

Approximately 68 saliva samples were selected by consecutive sampling. The BDEF-1 enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, Bethesda, MD, USA) was used. Before measuring the SHBD-1 saliva level, we calculated the total protein content in each sample by using the Bradford test to measure the total protein levels in saliva with the Coomassie Plus Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Approximately 20 μL of each sample was divided into 10 μL for each well (duplo).

After obtaining the total salivary protein data in each sample, equating was performed by dilution so that all samples were equal to the total amount of protein. Thereafter, the SHBD-1 saliva level was immediately measured after the following stages.

The assay procedure began by adding 100 μL standard solution and sample to each well and incubating for 90 minutes at 37 °C. Thereafter, the liquid was removed, and 100 μL Biotinylated Detection Ab were added and incubated for 60 minutes at 37 °C. After aspiration and washing 3 times, 100 μL horseradish peroxidase conjugate was added and incubated for 30 minutes at 37 °C. After aspiration and washing 5 times, a 90 μL substrate reagent was added and incubated for 15 minutes at 37 °C. The final step was the addition of a 50 μL stop solution. The results were read immediately by using an ELISA reader at 450 nm wavelength.

### Statistical Analysis

Data were collected and analyzed using SPSS version 22 (SPSS, Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to test normality. For normally distributed data, the independent *t*-test was applied; otherwise, the Mann–Whitney *U* test was selected.

## Results

The 68 men studied (age, 20–55 years) were divided into 44 smokers and 24 nonsmokers (Table 1). The saliva profile was measured via an unstimulated salivary flow rate and degree of acidity. Oral health status in the OHIS and DMFT were also measured. In both groups, the average OHIS was 0–4 (moderate), and the unstimulated salivary flow rate was 1–2 mL/min (normal). The acidity level (pH) in both groups was also normal.

| | Salivary Profile and OHIS | | |
|---|---|---|---|
| | *n* | Mean ± SD | Min–Max |
| **pH** | | | |
| Smokers | 44 | 6.89 ± 0.62 | 6–8 |
| Nonsmokers | 24 | 7.25 ± 0.53 | 6–8 |
| **Salivary Flow Rate** | | | |
| Smokers | 44 | 0.80 ± 0.82 | 0.16–4.25 |
| Nonsmokers | 24 | 0.51 ± 0.26 | 0.10–1.10 |
| **OHIS** | | | |
| Smokers | 44 | 2.26 ± 0.87 | 0–4 |
| Nonsmokers | 24 | 1.94 ± 1.03 | 1–4 |
| **DMFT** | | | |
| Smokers | 44 | 6.41 ± 3.6 | 0–15 |
| Nonsmokers | 24 | 5.22 ± 4.62 | 0–15 |

**Table 1.** Salivary Profile and OHIS

Table 2 shows the ratio of SHBD-1 between the smokers and nonsmokers; the difference was significant ($P = 0.01$).

| | SHBD-1 Level (pg/mL) | | | |
|---|---|---|---|---|
| | *n* | Median (min–max) | Mean ± SD | *P* |
| **Smoking status** | | | | |
| Smokers | 44 | 5.65 (0.07–45.02) | 10.38±11.11 | 0.01* |
| Nonsmokers | 24 | 2.34 (0.04–16.76) | 4.66±4.73 | |

Mann–Whitney *U* test; $P < 0.05$, significant difference; SD, standard deviation.

**Table 2.** Salivary SHBD-1 level of smokers and nonsmokers.

The $\chi^2$ test on SHBD-1 level showed that no significant relationship existed between cigarette type, cigarette filter, smoking duration, and smoking frequency (Table 3).

| | SBDH-1 Level (pg/mL) | | | |
|---|---|---|---|---|
| | n | Median (min–max) | Mean ± SD | *P* |
| **Type of cigarette** | | | | |
| Kretek | 38 | 5.93 (1.10–45.02) | 11.28±11.63 | 0.22 |
| Nonkretek | 6 | 3.43 (0.07–11.57) | 4.70 ± 4.08 | |
| **Duration of Smoking** | | | | |
| 5–10 years | 34 | 6.13 (1.01–45.02) | 10.40±10.38 | 0.40 |
| >10 years | 10 | 3.97 (0.06–38.78) | 10.33±13.97 | |
| **Frequency of Smoking** | | | | |
| 1–5 cigarette/day | 14 | 7.75 (2.37–45.02) | 14.66±14.43 | 0.11 |
| 5–10 cigarettes/day | 19 | 6.01 (1.25–28.91) | 9.25 ± 7.18 | |
| 10–20 cigarettes/day | 10 | 2.21 (0.07–38.78) | 7.33 ± 11.75 | |
| >20 cigarettes/day | 1 | 2.56 | | |

Kruskal–Wallis test; $P < 0.05$ significant difference; SD, standard deviation.

**Table 3.** Salivary SBDH-1 level based on smoking habit.

## Discussion

The unstimulated subject salivary flow rates were higher in the smokers than in the nonsmokers. Rad et.al.,(2010) found that smoking has a strong influence on saliva flow rate [6], but other studies stated that the average saliva flow rate in smokers was not affected by the amount of cigarette consumption.[7] The nicotine content in cigarettes has a parasympathomimetic effect and acts as a cholinergic receptor that can increases the parasympathetic response; therefore, in the early stages of smoking, the saliva flow rate increases in a short time. However, long-term use does not affect saliva flow rate. Other studies suggest that long-term smoking may decrease salivary flow rates. In our study, salivary flow rates were normal in smokers and nonsmokers. This result may be due to the subject being scheduled for saliva sampling and clinical examination after not smoking for 2 hours before salivary sampling. Several studies have suggested a decrease in the salivary flow rate, but many other studies have concluded that there is no significant difference between smokers and nonsmokers. The influencing factors of observed and investigated smoking activity can cause these differences. Rad et.al.,(2010) found a significant difference between salivary flow rate and pH between smokers and nonsmokers.[6] The decrease in salivary flow rate in smokers will affect salivary pH because of the decreased salivary flow rate and will decrease the salivary bicarbonate level, thus decreasing pH. The decreased salivary flow rate and pH will further affect the condition of the oral cavity and may also lead to incidences in the teeth and soft tissue mucosa. The salivary pH levels in the current study indicated that there was no significant difference between smokers and nonsmokers. This result can be attributed to the normal saliva flow path of the research subjects. In another study, Rehan et.al.,(2016) stated that salivary pH was clearly different between smokers and nonsmokers.[7]

The oral health profile of this study was represented by OHIS and DMFT values. The comparison of outcomes between the two subject groups was not significantly different. However, data on oral smokers and nonsmokers in this study showed that the OHIS and DMFT values tended to be higher in smokers than in nonsmokers (Table 2). There were no significant differences in subjects with dental fillings and tooth loss according to the DMFT scores. The results of other studies on oral health profiles in smokers varied. Hagh et.al.,(2013) found no significant difference between OHIS and DMFT values in smokers, but the results may have been affected by the small sample size.[8] The decreased salivary levels that may occur in smokers and the decreased pH may increase the risk of caries and periodontal tissue abnormalities in smokers compared with nonsmokers.

There was a significant difference in the level of SHBD-1 between smokers and nonsmokers (Table 2). SHBD-1 may act as an antimicrobial agent in wound healing as part of the innate immune system and also has a specific role in the regulation of tumor development and metastasis in oral carcinoma.[9] Defensin can be identified in biological fluids, such as urine, bronchial fluid, nasal secretions,

saliva, and gingival crevicular fluid.[10] In smoking activity, oral tissues are initially exposed to various toxins contained in cigarette smoke, thus allowing the toxins to spread to other organs in the body.[11] In the case of increased levels of SHBD-1, the role of SHBD-1 is appropriate as a first-line defense peptide agent in the oral cavity.

Our study also correlated the relationship between SHBD-1 and cigarette type, smoking duration, and smoking frequency and demonstrated that no significant differences existed between them. The association between SHBD-1 levels and the presence of various lesions in the oral cavity associated with smoking activity did not show any significant difference. However, exposure to cigarette smoke may modulate expression and trigger beta defensin secretion. Further investigations should be performed on the relationship between cigarette smoke and SHBD-1 level abnormalities. These results may provide a new perspective for investigating localized smoking resistance and oral lesions.[12]

SHBD-1 is predicted to be derived from oral mucosal keratinocytes rather than from salivary glands and specific salivary duct cells. Defensin may play a role in periodontal disease by preventing periodontitis and caries. SHBD-1 level may change in the presence of oral disease. Therefore, assessing salivary-based risk in the future may be useful.[13]

The weakness of this study is the location of the clinical-based sampling and consecutive sampling. This limitation did not allow the sample to fully describe the actual population, thus possibly leading to selective bias. The number of samples must also be improved in subsequent studies.

## Conclussion

There was a difference between salivary SHBD-1 levels in smokers and nonsmokers ($P = 0.01$). We found no difference in salivary flow rate and salivary pH between smokers and nonsmokers, and no significant difference was found in salivary SHBD-1 on the basis of smoking type, duration, and frequency. Furthermore, there was no significant difference in salivary SHBD-1 levels according to the salivary and oral health profiles.

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## Conflict of Interest

The authors have no conflicts of interest to declare.

## References

1. Amtha R, Razak IA, Basuki B, et al. Tobacco (Kretek ) Smoking , Betel Quid Chewing and Risk of Oral Cancer in a Selected Jakarta Population. Asian Pac J Cancer Prev 2014;15(20):8673–8.
2. Kementerian Kesehatan Republik Indonesia. RISET KESEHATAN DASAR. 2013.
3. Meraw SJ, Mustapha IZ, Rogers RS. Cigarette Smoking and Oral Lesions Other Than Cancer. Clin Dermatol 1998;16(25):625–31.
4. Mizukawa N, Sugiyama K, Ueno T, Mishima K, Takagi S, Sugahara T. Levels of Human Defensin-1, An Antimicrobial Peptide, in Saliva of Patients with Oral Inflammation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;87(5):539–43.
5. Pierson T, Pierson SL, Pinto D, Hoek ML. Cigarette Smoke Extract Induces Differential Expression Levels of Beta-Defensin Peptides in Human Alveolar Epithelial Cells Cigarette Smoke Extract Induces Differential Expression Levels of Beta-Defensin Peptides in Human Alveolar Epithelial Cells. Tob Induc Dis 2013; 11(1):10.
6. Rad M, Kakoie S, Brojeni FN, Pourdamghan N. Effect of Long-term Smoking on Whole-mouth Salivary Flow Rate and Oral Health. J Dent Res Den Clin Den Prospects 2010; 4(4):110–14.
7. Rehan F, Khan RS, Khurshid Z, Memon MS, Naqvi S, Zafar MS. Analysis of Resting Mouth Salivary Flow Rate and Salivary pH of Tobacco Chewers and Smokers. J Pak Dent Assoc 2016;25(4):158–63.
8. Golpasand Hagh L, Zakavi F, Ansarifar S, Ghasemzadeh O, Solgi G. Association of Dental Caries and Salivary Siga with Tobacco Smoking. Aust Dent J 2013;58(2):219–23.
9. G Diamond, LK Ryan. Beta-Defensins: What are They REALLY Doing in the Oral Cavity? Oral Dis 2011;17(7):628–35.
10. Dale BA, Krisanaprakornkit S. Defensin Antimicrobial Peptides in the Oral Cavity. J Oral Pathol Med 2001;30(6):321–7.
11. Ahmed A, Thliveris JA, Shaw A, Sowa M, Gilchrist J, Scott JE. Caspase 3 Activity in Isolated Fetal Rat Lung Fibroblasts and Rat Periodontal Ligament Fibroblasts: Cigarette Smoke Induced Alterations. Tob Induc Dis 2013;11(1):25.
12. Wang W, Ye P, Qian Y, et al. Effects of Whole Cigarette Smoke on Human Beta Defensins Expression and Secretion by Oral Mucosal Epithelial Cells. Tob Induc Dis 2015;13(1):3. Bhagawati S. Role of Defensins in Oral Diseases: An Overview. J Indian Acad Oral Med Radiol 2012;24(2):142–5