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Short Communication

Clinical and oral microbiome pattern of halitosis patients with periodontitis and gingivitis

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Abstract

Halitosis is caused by a bacterial proteolytic process that induces the production of volatile sulfur compounds, odor-causing gases. The aim of this study was to determine the clinical oral hygiene state and oral microbiome pattern of halitosis patients with periodontitis and gingivitis. The oral hygiene state of halitosis patients with periodontitis and gingivitis was assessed using the oral hygiene index simplified (OHI-S), decay missing filled teeth (DMFT), and tongue biofilm. The dorsum of the tongue and subgingival swabs were cultured for bacteria, and bacterial morphology was evaluated using Gram staining. Evaluation of the bacterial genus using the Bergey's systematic bacteriology diagram as a guide. A total of ten patients with periodontitis and gingivitis were included. Our data indicated that the scores of OHI-S and DMFT were different significantly between halitosis patients with periodontitis and gingivitis (both had $p<0.001$) while tongue biofilm score was not different between groups. On the dorsum of the tongue, periodontitis patients had a significant higher oral microbiome population ($85.65x10^6$ CFU/mL) compared to those with gingivitis ($0.047x10^6$ CFU/mL) with $p=0.002$. In contrast, the number of microbiomes in the subgingival had no significant different between periodontitis and gingivitis. On the dorsum of the tongue, six bacterial genera were isolated from periodontitis cases and seven genera were detected from gingivitis patients. On subgingival, 10 and 15 genera were identified from periodontitis and gingivitis, respectively. Fusobacterium, Propionibacterium, Eubacterium and Lactobacillus were the most prevalent among periodontitis cases while Porphyromonas was the most prevalent in gingivitis patients. In conclusion, although OHI-S and DMFT are different between periodontitis and gingivitis, overlapping of bacterial genera was detected between periodontitis and gingivitis cases.

Keywords: Halitosis, oral microbiome, periodontitis, gingivitis, oral malodour

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Introduction

Halitosis is unpleasant breath caused by the type of food consumed, improper dental hygiene, disease, or an unhealthy lifestyle [1]. Volatile sulfur compounds (VSCs) gases such as methyl

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mercaptan (CH_3SH), hydrogen sulfide (H_2S), and dimethyl sulfide (CH_3SCH_3) are responsible for the odor of halitosis. VSCs gas is created by a bacterial proteolytic process that degrades proteins into peptides and amino acid components, resulting in an odor[2].

Individuals with halitosis whose condition is exacerbated by periodontal diseases such as gingivitis and periodontitis will produce significantly higher levels of VSCs than non-halitosis patients due to increased epithelial cell death and bleeding in the gingiva and sulcus fluid [3]. A study found that the individuals who had gingival pocket with 3 mm are likely to have higher VSCs production than those with 3 mm pockets [4]. A gingival pocket measuring less than 3 mm defined as periodontitis. Patients with periodontitis are 1.8 times more likely to have poor breath than those without periodontitis [5]. In addition, halitosis can be aggravated by the tongue's anatomical shape and the gingival sulcus. The tongue has an uneven physical shape due to the presence of tongue papillae and tongue fissures, resulting in a rough surface [6]. Due to its rugged and uneven texture, leave over foods (bacteria substrates for VSCs production) are difficult to clean, particularly in the third posterior of the dorsum of the tongue, where the circumvallate papillae have numerous apertures [7]. In addition, the gingival sulcus, a tiny and deep area, is also an ideal habitat for oral bacteria [8]. This is the main characteristic of individuals with gingivitis.

Porphyromonas gingivalis, *Tannerella forsythya*, *Fusobacterium nucleatum*, *Provotella intermedia*, *Treponema denticola*, and *Solobacterium moorei* are typically engaged in proteolytic processes. Bacteroides, Eubacterium, Fusobacterium, Peptostreptococcus, Porphyromonas, Selenomonas, *Tannerella forsythia*, and Veillonella, produce hydrogen sulfide from L-cysteine to produce several VSCs gases. Some bacteria such as Bacteroides, Eubacterium, Fusobacterium, Porphyromonas, and *Treponema denticola* create methyl mercaptan from L-methionine [9]. The presence of Gram-positive and Gram-negative bacteria in halitosis patients is associated with oral hygiene status indicators such as the oral hygiene index simplified (OHI-S), decay missing filled teeth (DMFT), and tongue biofilm. It is important to investigate the variety of the oral microbiome in halitosis patients with periodontitis and gingivitis in order to be able to treat appropriately. The aim of this study was to determine and compare the oral hygiene indicators and the oral microbiome pattern of halitosis patients with periodontitis and gingivitis.

Methods

Study setting and patients

A cross-sectional study was conducted between June 2019 and January 2022. Patients diagnosed with halitosis aggravated by periodontal infection and gingivitis at the Dental Hospital of Universitas Syiah Kuala, Banda Aceh, Indonesia were recruited. Individuals suffering from halitosis, periodontal disease, not using scented products two hours before the examination, not eating or chewing gum two hours before the test, and not brushing their teeth or rinsing their mouth two hours before the investigation were set as criteria to be eligible in this study. Those who used of removable partial or complete dentures; having upper and lower respiratory tract infections, systemic diseases, and metabolic problems; having antibiotic therapy for one month; and had or having alcohol consumption were excluded. Clinical examinations were conducted to halitosis-suspected patients including an evaluation of the depth of attachment loss or clinical attachment loss (CAL), Calculus Index (CI), OHI-S, DMFT, and tongue biofilms.

Halitosis assessment

The patients were asked not to eat or drink, to stop using anti-aging medications and breath fresheners, and refrain from brushing their teeth and mouth at least 2 hours before the test. The patients were required to complete a questionnaire and provide informed consent. Halitosis was evaluated by analyzing organoleptic characteristics and breath odor checker [10].

Organoleptic assessment was performed face-to-face, with the organoleptic equipment positioned between the examiner and the subject. The patient was asked to hold the breath for 30 seconds and exhale via an organoleptic tube. The scores as follow: 0 = no odor, 1 = faint odor, 2 = subtle odor, 3 = moderate odor, 4 = strong odor, and 5 = severe stench. The breath odor checker

was measured directly and the scores were similar with organoleptic evaluation. Patients were diagnosed with halitosis if the cumulative score more than more than 2.

Decay missing filled teeth (DMFT) assessment

The DMFT examination was conducted utilizing a mouth mirror and a sonde. A mouth mirror was used to pull the corners of the mouth to gain a clear view of the oral cavity, while a sonde was used to check teeth damaged by caries, teeth with extraction indications, and teeth that have been treated. Beginning with the region I (top right), the dentist examined region II (top left), III (bottom left), and IV (bottom right). Each tooth with cavitation, restoration, and caries-related loss was noted. Calculating the DMFT index involved assigning codes to each tooth element (D (decay) refers to cavities; M (missing) for a tooth that has been removed or has remaining roots; F (filling) refers to tooth fillings. Each finding was given a score of one. For each patient, all teeth were examined for all three components (D, M and F) and the final DMFT score were summed using formula explained previously [11]. The DMFT then was classified as good (score 0.0–2.6), moderate (score 2.7–4.4) and poor (score >4.4).

Oral hygiene index simplified (OHI-S) assessment

Oral hygiene status was assessed using calculating the OHI-S by evaluating the Debris Index (DI) and Calculus Index (CI). DI and CI were measure using six teeth from each section of the oral cavity to represent all posterior and anterior teeth: the buccal surfaces of teeth 16, 26, the labial surfaces of teeth 11 and 31, and the lingual surfaces of teeth 36 and 46. OHI-S score, cumulative of CI and DI scores, ranged between 0 and 6 and were classified as: good OHI-S score (0 to 1.2); intermediate (1.3 to 3); and poor (3.1 to 6) [12].

Assessment of tongue biofilm

The evaluation of biofilm on the tongue was based on observations of the tongue's dorsum. The six sections of the dorsum tongue were separated into three sections posteriorly and three sections anteriorly. Each sextant of tongue covering was evaluated as 0 (no biofilm), 1 (mild biofilm), or 2 (heavy biofilm) as previously described [12]. Therefore, the biofilm score ranged between 0 to 12.

Bacteria count and identification

Isolated bacteria from gingival sulcus plaque and the dorsum of the tongue were cultivated for 24 hours at 37^{o}C in a brain heart infusion broth (BHIB) medium. The bacterial suspension from the gingival sulcus and dorsum of the tongue was recultivated on blood agar. A total of 0.1 mL of diluted bacterial solution was dispersed on prepared sterile agar media. Next, the suspension was leveled and incubated at 37^{o}C for three days. The number of bacteria growing on the surface of the media was calculated using the total plate count (TPC) method. Gram staining was conducted to all isolated bacteria; the color and morphology of the microorganisms observed were recorded. The Bergey's Manual of Determinative Bacteriology to identify the bacterial genus [13].

Statistical analyses

Data on the oral hygiene statuses (OHI-S, DMFT, and tongue biofilm) and oral microbiome were compared between individuals with periodontitis and gingivitis using the Mann-Whitney test. Wilcoxon test was used to compare the oral microbiome between different part (dorsum of tongue and sublingual) within periodontitis or gingivitis group. The correlations of halitosis score with OHI-S score, DMFT score, and tongue biofilm score were determined the Spearman rho correlation. All analyses were conducted using SPSS for Windows (IBM SPSS Statistics version 20.0 for Windows, IBM Corporation, Armonk, NY, USA).

Results

Patients' characteristics

The characteristics of halitosis patients with periodontitis and gingivitis are presented in **Table 1**. Based on OHI-S status, 80% and 20% of the periodontitis and gingivitis had poor score. All

periodontitis patients had poor DMFT status and poor tongue biofilm. Although all gingivitis patients had poor tongue biofilm, 50% of them had good DMFT status (**Table 1**).

Table 1. Distribution of epidemiological data on research subjects based on clinical examination

Type	Variable	Frequency	Mean	Standard deviation	Frequency
Periodontitis	Sex				
	Male	5	-	-	50%
	Female	5	-	-	50%
	Ages (year)				
	Male (ranges: 28-49)	5	33	8.90	50%
	Female (ranges: 44-57)	5	52	5.54	50%
	Oral hygiene index simplified (OHI-S) score				
	Good	0	-	-	0
	Moderate	2	4.50	0.70	20%
	Poor	8	5.25	1.28	80%
	Decay missing filled teeth (DMFT)				
	Good	0	-	-	0
	Moderate	0	-	-	0
	Poor	10	10.9	2.42	100%
	Tongue biofilm	0	-	-	0
	Good	0	-	-	0
	Intermediate	0	-	-	0
	Poor	10	9.1	1.72	100%
Gingivitis	Sex				
	Male	4	-	-	40%
	Female	6	-	-	60%
	Ages (year)				
	Male (ranges: 20-21)	4	21	0.50	40%
	Female (ranges: 21-22)	6	21	0.52	60%
	Oral hygiene index simplified (OHI-S) score				
	Good	3	2.33	0.00	30%
	Moderate	5	2.80	0.44	50%
	Bad	2	2.50	0.70	20%
	Decay missing filled teeth (DMFT)				
	Good	5	0.6	0.54	50%
	Moderate	4	2.75	0.50	40%
	Poor	1	5	0.00	10%
	Tongue biofilm				
	Good	0	-	-	0
	Intermediate	0	-	-	0
	Poor	10	8.7	2.05	100%

Comparation of oral hygiene between periodontitis and gingivitis

The comparation of oral status of halitosis individuals with periodontitis and gingivitis is presented in **Table 2**. Our data indicated that the scores of OHI-S and DMFT were different significantly between halitosis patients with periodontitis and gingivitis (both had $p<0.001$). In contrast, the state of the tongue biofilm was not significantly different periodontitis and gingivitis cases ($p=0.640$).

Table 2. Comparation of oral status of halitosis individuals with periodontitis and gingivitis

Oral status	Origin	n	Mean	Standard deviation	*p*-value
Oral hygiene index simplified (OHI-S) score	Periodontitis	10	2.2	0.63	<0.001[a]
	Gingivitis	10	1.2	0.66	
Decay missing filled teeth (DMFT) score	Periodontitis	10	11.0	2.42	<0.001[a]
	Gingivitis	10	1.5	1.59	
Tongue biofilm score	Periodontitis	10	9.5	1.72	0.640[a]
	Gingivitis	10	9.0	2.05	

[a] Analyzed using Mann-Whitney test

Comparation of oral microbiome pattern between periodontitis and gingivitis

The community of oral bacteria isolated from halitosis patients with periodontitis and gingivitis is presented in **Table 3**. The number of oral microbiomes on the dorsum of tongue of periodontitis patients was significantly higher than that of gingivitis patients (85.65x10^6 CFU/mL vs. 0.047x10^6 CFU/mL, p=0.002). In contrast, in the subgingival region, the number of microbiome population in gingivitis patients had no significant different than those with periodontitis 0.445x10^6 CFU/mL vs. 0.047x10^6 CFU/mL, p=0.306.

Table 3. Oral microbiome pattern of halitosis individuals with periodontitis and gingivitis

Case	n	Bacteria growth (CFU/mL)				*p*-value
		Dorsum of tongue		Subgingival		
		Mean	SD	Mean	SD	
Periodontitis	10	85.65 x 10^6	339.32	0.047 x 10^6	922.86	0.002[a]
Gingivitis	10	0.047 x 10^6	0.12	0.445 x 10^6	483.01	0.306[a]
p-value		0.048[b]		0.036[b]		

[a] Analyzed using Wilcoxon test
[b] Analyzed using Mann-Whitney U

Table 4. Distribution and population frequency of bacterial genera from halitosis subjects with a history of periodontitis and gingivitis from the tongue dorsum and subgingival

Sample origin	Dorsum of tongue				Subgingival			
	Group (Gram)	Bacteria genus	%	TPC (10^6)	Group (Gram)	Bacteria genus	%	TPC (10^6)
Periodontitis	-	Neisseria	33	0.004	-	Fusobacterium	30	0.0008
	+	Solobacterium	33	0.004	+	Propionibacterium	30	0.0008
	-	Capnocytophaga	33	0.004	+	Eubacterium	30	0.0008
	+	Staphylococcus	67	0.008	+	Lactobacillus	30	0.0008
	+	Solobacterium	67	0.008	-	Neisseria	10	0.0003
	-	Veillonella	33	0.004	+	Micrococcus	10	0.0003
					-	Prevotella	10	0.0003
					-	Porphyromonas	10	0.0003
					-	Tannerella,	10	0.0003
					-	Veillonella	10	0.0003
Gingivitis	-	Enterobacter	71	15.198	-	Capnocytophaga	7	0.1498
	-	Pseudomonas	71	15.198	-	Enterobacter	7	0.1498
	+	Solobacterium	29	6.079	-	Eikenella	13	0.2996
	-	Corynebacteria	29	6.079	-	Porphyromonas	27	0.5993
	+	Lactobacili	29	6.079	-	Fusobacterium	13	0.2996
	+	Streptococcus	57	12.158	-	Prevotella	13	0.2996
	+	Staphylococcus	57	12.158	-	Tannerella	13	0.2996
					-	Treponema	13	0.2996
					-	Neisseriaceae	7	0.1498
					-	Veilonella	7	0.1498
					+	Streptococcus	7	0.1498
					+	Lactobacili	7	0.1498
					+	Propionibacteria	7	0.1498
					+	Actinomyces	7	0.1498
					-	Bacteriodes	7	0.1498

TPC: total plate count

The locations and frequency of bacterial genera isolated from halitosis patients with periodontitis and gingivitis of the tongue dorsum and subgingival are presented in **Table 4**. On the dorsum of the tongue of periodontitis cases, six genera (50% Gram-positive and 50% Gram-negative) were isolated and seven genera from gingivitis patients (57% Gram-positive and 43% Gram-negative) were identified. Ten genera (40% Gram-positive and 60% Gram-negative) from subgingival of periodontitis cases and 15 genera (27% Gram-positive and 73% Gram-negative) from gingivitis patients were identified. Overlapping bacterial genera were detected not only on the dorsum of the tongue but also on the subgingival portion between group.

Correlation between halitosis score and with oral status scores

The correlations between halitosis score with OHI-S status, DMFT, and tongue biofilm score are depicted in **Table 5**. Halitosis score was correlated positively with OHI-S and DMFT scores of which it had a stronger correlation with OHI-S score than DMFT (Spearman rho correlation 0.60 and 0.51, respectively). This suggested that halitosis had a relatively moderate correlation with OHI-S and DMFT score.

Table 5. Spearman's correlation between halitosis score and with oral status scores

Correlation	correlation coefficient	*p*-value
Halitosis score – Oral hygiene index simplified (OHI-S) score	0.60	0.005
Halitosis score – Decay missing filled teeth (DMFT) score	0.51	0.021
Halitosis score – Tongue score	0.10	0.668

Discussion

The findings in this present study suggest that OHI-S and DMFT status had the most vital link with halitosis occurrence, but tongue biofilms have a less relationship. Changes in DMFT status in patients with gingivitis and periodontitis and halitosis status can be explained by the fact that halitosis encourages the formation of gram-positive and negative bacterial species, hence intensifying DMFT and OHI-S status. All oral status indicators differ significantly between patients with halitosis and periodontitis or gingivitis (**Table 2**). Tongue biofilms are referred as the primary and most prevalent cause of halitosis because the tongue coating hinders the physical access of taste buds to taste pores and prevents their attachment to taste receptors [14]. This is in line with a previous study reporting that the products derived from bacterial proteolytic process was strongly linked with halitosis at $p<0.05$ [15].

The results of the present study revealed that the tongue biofilm score in individuals with periodontitis and gingivitis ranged from 6 to 12, indicating that a thin-to-thick tongue biofilm. This suggests that tongue biofilm should still be considered as a contributing factor of halitosis. In a previous study, a correlation between halitosis and tongue biofilm is attributed to the rising number of bacterial contents on the dorsum of the tongue of patients with periodontal tissue injury [16]. Another study reported that the dorsum of a patient's tongue with halitosis has 100 germs attached to a single epithelial cell. Therefore, though a patient has good OHI-S, the bad breath might still occur [9].

Herein, we also reported that population of the oral microbiome on the dorsum of the tongue of periodontitis patients is much larger than that of gingivitis patients. As a cause and trigger of halitosis, the dorsal surface of the tongue makes a substantial contribution to bacterial colonization, where fissures, crypts, and mucous papillae are high, thereby favoring the formation of anaerobic microbiota, which can produce VSC [17]. Moreover, its location as a connection between the oral cavity and the pharynx gives access to numerous nutrients, products, and microorganisms [18]. In addition, the findings in this present study suggest that periodontitis associated halitosis has a significantly higher bacterial count than that associated with gingivitis. This present study further found that, on the of dorsum of the tongue of gingivitis patients, the majority is gram-positive than gram-negative. On the subgingival area, gram-negative bacteria appear to be more dominant than gram-positive bacteria. The bacteria are believed to belong to the genera Haemophilus, Aggregatibacter, Eikenella, Porphyromonas, Tannerella, and Prevotella. Gram-negative bacteria were significantly more prevalent with coccus and bacilli only present in the subgingival and not on the dorsum. Gram-negative bacteria have been found to be frequently predominant in halitosis cases [19]. Active bacterial contamination can provoke an inflammatory response in the gingiva and lead to periodontal degeneration [20]. A study suggested an association between increased VSCs in patients with CAL>3 mm and the inflammatory process in periodontal tissue [4].

The findings of this study are crucial in depicting the biodiversity of bacteria causing halitosis in patients with gingivitis and periodontitis. This research was conducted in a simple manner, indicating the need for further studies capable of detecting bacterial biodiversity down to species and with more accurate quantification.

Conclusion

The study reveals a linear relationship between Halitosis, OHI-S, DMFT, and tongue biofilm status, but its impact on the increase in halitosis among individuals with a history of periodontitis and gingivitis is minimal. The oral microbiome population on the dorsum of the tongue is significantly higher in halitosis patients with a history of periodontitis compared to those with gingivitis. Conversely, in the subgingival region, individuals with a history of gingivitis have a larger oral microbiome population than those with a history of periodontitis. Halitosis patients with periodontitis show six genera on the dorsum of the tongue, while those with gingivitis show seven genera. In the subgingival region, periodontitis patients have ten genera, whereas gingivitis patients have fifteen genera. Gram-positive bacteria constitute a higher proportion on the dorsum of the tongue, while Gram-negative bacteria dominate the gingival source.

Ethics approval

This research has been approved by the Ethical Committee of Faculty of Dentistry's Universitas Syiah Kuala, Banda Aceh, Indonesia No. 178/KE/FKF/2019.

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Competing interests

The authors declare that there is no conflict of interest.

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