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Association between viral hepatitis B infection and halitosis

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Abstract

Objective. Oral malodor can be increased in breath of liver patients. However, no study has been performed for the association between volatile sulfur compounds (VSCs) and viral hepatitis. The aim of the present study was to determine the relationship between viral hepatitis and VSCs. ***Methods.*** This study analyzed 182 subjects and measured hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH) and dimethyl sulfide [$(CH_3)_2S$] using the OralChroma®. Hepatitis type B was evaluated. Periodontal health was assessed using the Community Periodontal Index (CPI) and bleeding on probing (BOP). Tongue coating score (TCS) was evaluated. Multiple logistic regression analyses were conducted to evaluate the relationship. ***Results.*** Viral hepatitis had an elevated odds of dimethyl sulfide defined halitosis (OR = 9.22, 95% CI = 2.08–40.95) after controlling for age, gender, alcohol consumption, current smoking, periodontitis, BOP, TCS and tongue brushing habit. The magnitude of the association between viral hepatitis and VSCs defined halitosis attenuated with adjustment of mediators (alcohol consumption, periodontitis, BOP, TCS and tongue brushing habit for hydrogen sulfide defined halitosis; periodontitis, TCS and tongue brushing habit for methyl mercaptan defined halitosis; tongue brushing habit for dimethyl sulfide defined halitosis). ***Conclusions.*** Findings of this study suggest that viral hepatitis may be associated with methyl mercaptan defined halitosis.

Key Words: *Gingivitis, halitosis, oral hygiene, periodontal disease, viral hepatitis*

Introduction

Halitosis is a general term used to define an unpleasant odor emanating from the breath and can be of oral or non-oral origin. The vast majority of pathologies causing halitosis lie within the oropharynx (tongue coating, gingivitis, periodontitis and tonsillitis) and bacterial formation of the odorous volatile sulfur compounds (VSCs) including hydrogen sulfide (H_2S) and methyl mercaptan (CH_3SH) within the oral cavity, especially in the tongue coating, play a predominant role [1]. In 10–15% of the patients, however, breath malodor has an extra-oral cause, mostly due to the presence of dimethyl sulfide [$(CH_3)_2S$] [2,3]. Examples include disturbances of the upper and lower respiratory tract, disorders of the gastrointestinal tract, some systemic diseases, metabolic disorders and carcinomas [2,4,5]. In contrast to oral malodor, less attention has been paid to extra-oral halitosis in dentistry [2,3,6,7]. Extra-oral halitosis could be a manifestation of a serious disease for which treatment is much more complicated. Physicians know that the odor of a patient's breath is associated with several diseases and may give an insight into physiological and pathophysiological processes in the body. For example, the sweet smell of acetone on the breath accompanies uncontrolled diabetes, a fishy smell is the result of an elevated level of trimethylamine due to a lack of the enzyme trimethylamine-oxidase in the liver and a urine-like smell is related to kidney failure [2,4,8,9]. Different studies dealing with this topic demonstrated that various diseases such as liver cirrhosis [10] and recent smoking behaviors [11] are associated with specific volatile organic compound profiles in human exhalations.

Liver disease is an important extra-oral cause of bad breath. Patients with various degrees of hepatocellular failure and portosystemic shunting of blood may acquire a sweet, musty or slightly fecal aroma of the breath, termed fetor hepaticus, which has been

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mainly attributed to sulfur compounds [12]. If the metabolizing function of the liver fails, the concentration of the metabolites, normally processed in the liver, will increase and they will enter again the systemic circulation. Part of them will then be exhaled.

The hepatitis B virus (HBV) causes chronic infection in ~ 400 million people in the world. Most carriers of chronic HBV, including Asians, Africans and a proportion of persons in Mediterranean countries, acquire the infection at birth or within the first 1–2 years after birth [13]. In Korea, the prevalence of HBV infection was 5.1% in males and 4.1% in females [14]. HBV infection may cause acute and/or chronic hepatitis and premature death from liver cirrhosis, liver failure or hepatocellular carcinoma [15]. There might be a biologically plausible possibility that HBV infection could be associated with oral malodor because dimethyl sulfide was increased in breath of liver patients [10].

It is of utmost importance that dental professionals can discriminate HBV infection from those with oral malodour, because HBV infection can be chronic for many years. Therefore, the hypothesis of this study is that HBV infection would be associated with VSCs defined halitosis and this association might be mediated by several potential mechanisms including periodontal health (periodontitis, gum bleeding), tongue coating and health behavior (tongue brushing habit, alcohol consumption and smoking). The aim of the present study was to investigate the relationship between HBV infection and VSCs defined halitosis.

Methods

Subject selection

From April to September 2008, 1851 subjects (1040 males and 811 females) aged 20 years or older visited the Health Promotion Center at the Pusan National University Hospital for a routine health checkup. The health screening program available at the Health Promotion Center includes anthropometric measurements, a laboratory blood test, oral/general health assessment by a dentist and a physician and a detailed clinical examination. All subjects were also asked to complete a questionnaire designed to assess sociodemographic factors, health-related behaviors and current or past medications. Among the 1851 subjects who visited the Health Promotion Center, the final number of subjects included was 182 and were comprised of 112 men and 70 women aged from 20–72 years, with a mean and standard deviation (SD) of 48.5 ± 10.1 years. Exclusion criteria were (i) subjects with a single missing value on the health assessment or questionnaires, (ii) subjects who don't want to join this study and (iii) subjects having < 20 natural teeth excluding wisdom teeth. The Institutional Review Board for Human Subjects at the Pusan National University Hospital approved all the procedures involved in sampling and data collection and written informed consent was obtained from all participants (approval number: 0740-155).

Measurement of sulfur compounds

A trained dental hygienist measured H_2S, CH_3SH and $(CH_3)_2S$ levels using a portable gas chromatograph, OralChroma^{TM} (Abilit Corporation, Osaka City, Japan), which was previously validated for clinical values [16,17]. Exhaled gas samples were collected with a disposable syringe (all-plastic syringes, 1 ml), which was inserted into the volunteers' oral cavity. Subjects had to close their mouth for 30 s before sample collection and 0.5 ml of mouth air was then exhaled into the measuring device. After 8 min, the process was completed and the concentrations of the three gases were displayed in either ng/10 ml or ppbv (nmol/mol).

All subjects received a letter with instructions before the examinations. One day before their appointment, the participants had to avoid garlic, onions and spicy food. Twelve hours before the measurements, the participants also had to refrain from drinking alcohol or coffee and from smoking. On the morning of the appointment, the use of chewing gum, mints, drops, scents and mouth rinses was forbidden. On the other hand, the participants could perform normal oral hygiene (tooth brushing) and have breakfast. All measurements were recorded in the morning between 8:30 and 11:30 (before lunch) and at least 2 h after eating or drinking and oral hygiene. The cut-offs for halitosis were 95 ppb for H_2S, 12 ppb for CH_3SH and 18 ppb for $(CH_3)_2S$, which are modified from a previous study [18]. Therefore, each of the three VSCs was categorized according to the individual cut-off level.

Assessment of liver diseases

Routine procedures of biochemical tests for liver and kidney function were applied. The participant's sera were tested for hepatitis B surface antigen (HBsAg). Samples positive for HBsAg were defined as HBV infection.

Assessment of covariates

Confounders in this study were age and gender. Mediators considered in the relationship between viral hepatitis and VSCs defined halitosis were health behaviors (tongue brushing habit, alcohol consumption and current smoking), periodontal health [periodontitis and bleeding on probing (BOP)], and tongue coating score (TCS).

A dentist assessed periodontitis, BOP and TSC. The subjects' periodontal conditions were based on

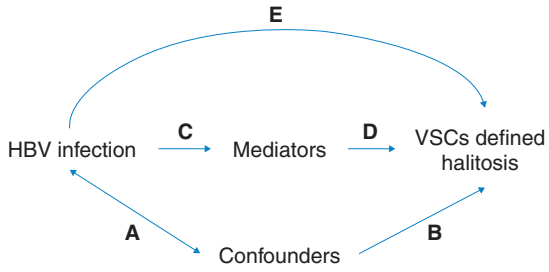

Figure 1. A model for the relationship between HBV infection and VSCs defined halitosis and the roles of confounders and mediators in the relationship. (A) is the association between HBV infection and confounders. (B) is the association of confounders with VSCs defined halitosis. The relationships of mediators with HBV infection and VSCs defined halitosis are shown in (C) and (D), respectively. Adjusted odds ratio of HB infection represents the un-confounded total impact of HBV infection on VSCs defined halitosis (E).

the World Health Organization (WHO) guidelines (Community Periodontal Index [CPI]); the WHO periodontal probe was used for the examination [19]. The five CPI scores used to evaluate the periodontal health status were as follows: normal (CPI 0), gingival bleeding (CPI 1), calculus (CPI 2), shallow periodontal pocket of 3.5–5.5 mm (CPI 3) and deep periodontal pocket of 5.5 mm or more (CPI 4). The measurements were performed using a CPI probe at six sites (mesiobuccal, midbuccal, distobuccal, distolingual, midlingual and mesiolingual) per tooth. Ten teeth were selected for the periodontal examination, the two molars in each posterior sextant and the upper right and lower left central incisors. If no index teeth or tooth was present in a qualifying sextant, the adjacent remaining teeth in that sextant were examined. Periodontitis was defined as the existence of CPI 3 or 4 and the number of sextants with periodontitis determined the extent of periodontitis.

BOP was also measured at the same teeth as the absence (code 0) or presence of mild (code 1), moderate (code 2) or severe (code 3) bleeding within 15 s after pocket probing [20]. The sum of BOP score was calculated.

To evaluate the TCS, each tongue surface was divided into nine sections. Observers evaluated each section according to TCS criteria (Score 0: Tongue coating not visible; Score 1: Tongue coating thin, papillae of tongue visible; Score 2: Tongue coating very thick, papillae of tongue not visible) and scores from the nine sections of the entire tongue surface were summed to calculate the TCS [21].

Information on the frequency of tongue-brushing was obtained by a four-grade questionnaire: always (code 1), sometimes (code 2), seldom (code 3) and never (code 4). The frequency of tongue-brushing was categorized into participants who always brush their tongue (answered 'always') and participants who do not always (answered 'sometimes', 'seldom' and 'never'). Alcohol consumption and current smoking were also collected from questionnaire: no vs yes.

Statistical analysis

As seen in Figure 1, the role of confounders in the relationship between HBV infection (the independent variable of this study) and VSCs defined halitosis (the dependent variable of this study) can be determined by examining the association of HBV infection with confounders (Figure 1A) and the association of confounders with VSCs defined halitosis (Figure 1B). The adjusted odds ratio of VSCs defined halitosis adjusting for demographic variables (age and gender) represents the un-confounded total impact of HBV infection on VSCs defined halitosis in this study. The relationship of mediators with HBV infection (Figure 1C) and VSCs defined halitosis (Figure 1D) should also be examined to determine the role of mediators. This role of mediators was evaluated with the percentage (%) excess odd explained, which can be calculated as $[(OR_{(\text{adjusted for demographic factors})} - OR_{(\text{adjusted for demographic factors + mediators})})/(OR_{(\text{adjusted for demographic factors})} - 1)]$ in this study [22]. This percentage excess odd explained represents the degree to which a mediator explains the relationship between HBV infection and VSCs defined halitosis.

In this study, the characteristics of study subjects by VSCs defined halitosis were presented with frequency distributions for the categorical variables and means (and standard deviations) for continuous variables (Table I). Chi-square tests for categorical variables and *t*-tests for continuous variables were used to assess the associations of VSCs defined halitosis with HBV infection, confounders and mediators. We then presented characteristics of study subjects according to HBV infection (Table II) and examined the association with chi-square tests and *t*-tests. Series of multiple logistic regression analysis were used to estimate the adjusted odds ratio (AOR) of VSCs defined halitosis according to HBV infection after adjusting for mediator variables (Table III). The model adjusting for confounders were base model in this analysis. Based on these analyses, percentage excess odd explained were calculated. We also presented findings with full adjustment for HBV infection (Table IV).

Results

Significant differences were found in TCS, BOP and tongue brushing habit by H_2S defined halitosis; periodontitis, TCS and tongue brushing habit by CH_3SH defined halitosis; alcohol consumption, tongue brushing habit and HBV infection by $(CH_3)_2S$ defined halitosis (Table I).

Table II shows the relationship of HBV infection with confounder and mediators. There were no significant differences in the distribution of HBV infection with regard to all confounders, mediators and VSCs levels.

Table I. Characteristics of subjects according to the VSCs (n = 182).

	H2S defined halitosis			CH3SH defined halitosis			(CH3)2S defined halitosis		
	No (n = 108)	Yes (n = 74)	p^a	No (n = 97)	Yes (n = 85)	p^a	No (n = 160)	Yes (n = 22)	p^a
Age (years), mean ± SD	48.19 ± 9.68	48.88 ± 10.81	0.656[b]	48.66 ± 10.03	48.26 ± 10.29	0.791[b]	47.88 ± 10.30	52.82 ± 7.61	*0.031*[b]
Gender, n (%)									
Male (n = 112)	66 (58.9)	46 (41.1)	0.886	61 (54.5)	51 (45.5)	0.690	102 (91.1)	10 (8.9)	0.098
Female (n = 70)	42 (60.0)	28 (40.0)		36 (51.4)	34 (48.6)		58 (82.9)	12 (17.1)	
Alcohol consumption, n (%)									
No (n = 75)	42 (56.0)	33 (44.0)	0.442	37 (49.3)	38 (50.7)	0.370	61 (81.3)	14 (18.7)	*0.023*
Yes (n = 107)	66 (61.7)	41 (38.3)		60 (56.1)	47 (43.9)		99 (92.5)	8 (7.5)	
Current smoking, n (%)									
No (n = 142)	85 (59.9)	57 (40.1)	0.788	75 (52.8)	67 (47.2)	0.807	124 (87.3)	18 (12.7)	0.647
Yes (n = 40)	23 (57.5)	17 (42.5)		22 (55.0)	18 (45.0)		36 (90.0)	4 (10.0)	
Periodontitis, n (%)									
No (n = 111)	71 (64.0)	40 (36.0)	0.112	67 (60.4)	44 (39.6)	*0.017*	101 (91.0)	10 (9.0)	0.111
Yes (n = 71)	37 (52.1)	34 (47.9)		30 (42.3)	41 (57.7)		59 (83.1)	12 (16.9)	
Tongue coating score, mean ± SD	7.33 ± 4.74	9.51 ± 5.63	*0.005*[b]	7.32 ± 4.91	9.25 ± 5.40	*0.013*[b]	8.14 ± 5.24	8.77 ± 5.18	0.598[b]
Bleeding on probing, mean ± SD	3.65 ± 3.54	5.14 ± 3.54	*0.006*[b]	4.06 ± 3.86	4.47 ± 3.30	0.447[b]	4.19 ± 3.51	4.73 ± 4.28	0.512[b]
Tongue brushing habit, n (%)									
No (n = 99)	71 (71.7)	28 (28.3)	*< 0.001*	64 (64.6)	35 (35.4)	*0.001*	94 (94.9)	5 (5.1)	*0.001*
Yes (n = 83)	37 (44.6)	46 (55.4)		33 (39.8)	50 (60.2)		66 (79.5)	17 (20.5)	
Viral hepatitis, n (%)									
No (n = 169)	101 (59.8)	68 (40.2)	0.676	92 (54.4)	77 (45.6)	0.266	152 (89.9)	17 (10.1)	*0.002*
Yes (n = 13)	7 (53.8)	6 (46.2)		5 (38.5)	8 (61.5)		8 (61.5)	5 (38.5)	

[a]Obtained by Chi-square test.
[b]Obtained by independent t-test.
H_2S defined halitosis: $H_2S \geq 95$ ppb; CH_3SH defined halitosis: $CH_3SH \geq 12$ ppb; $(CH_3)_2S$ defined halitosis: $(CH_3)_2S \geq 18$ ppb.
SD, standard deviation.
Italics denote statistical significance.

Table II. Association of viral hepatitis and confounders and mediators (n = 182).

	Viral hepatitis		
	No (n = 169)	Yes (n = 13)	p^a
Age (years), mean ± SD	48.41 ± 10.30	49.23 ± 7.77	0.780[b]
Gender, n (%)			
Male (n = 112)	102 (60.4)	10 (76.9)	0.237
Female (n = 70)	67 (39.6)	3 (23.1)	
Alcohol consumption, n (%)			
No (n = 75)	69 (40.8)	6 (46.2)	0.707
Yes (n = 107)	100 (59.2)	7 (53.8)	
Current smoking, n (%)			
No (n = 142)	134 (79.3)	8 (61.5)	0.136
Yes (n = 40)	35 (20.7)	5 (38.5)	
Periodontitis, n (%)			
No (n = 111)	105 (62.1)	6 (46.2)	0.255
Yes (n = 71)	64 (37.9)	7 (53.8)	
Tongue coating score, mean ± SD	8.11 ± 5.21	9.69 ± 5.36	0.292[b]
Bleeding on probing, mean ± SD	4.30 ± 3.61	3.69 ± 3.59	0.562[b]
Tongue brushing habit, n (%)			
No (n = 99)	91 (53.8)	8 (61.5)	0.592
Yes (n = 83)	78 (46.2)	5 (38.5)	
H_2S (ppb), mean ± SD	164.08 ± 270.53	253.54 ± 432.34	0.276[b]
CH_3SH (ppb), mean ± SD	35.09 ± 58.75	94.31 ± 141.79	0.160[b]
$(CH_3)_2S$ (ppb), mean ± SD	7.06 ± 18.81	14.38 ± 17.26	0.175[b]

[a]Obtained by Chi-square test.
[b]Obtained by independent t-test.
SD, standard deviation.

Table III shows a series of AOR of VSCs defined halitosis by HBV infection after adjusting for mediators. The base model was the model adjusted for confounders (age and gender). Analysis results revealed that HBV infection had significantly higher AOR for $(CH_3)_2S$ defined halitosis (AOR = 7.40, 95% CI = 1.99–27.53). The magnitude of AOR of $(CH_3)_2S$ defined halitosis by HBV infection attenuated with adjustment of tongue brushing habit (% excess odd explained = −52.3%).

Table IV shows AOR of VSCs defined halitosis in the fully adjusted model. The magnitude of the

Table III. Roles of mediators in the relationship between viral hepatitis and VSCs defined halitosis: Adjusted odds ratio (95% confidence interval) of VSCs defined halitosis by viral hepatitis and percentage excess odd explained (n = 182).

	H_2S defined halitosis		CH_3SH defined halitosis		$(CH_3)_2S$ defined halitosis	
	OR (95% CI)	% excess odd explained	OR (95% CI)	% excess odd explained	OR (95% CI)	% excess odd explained
Base model (B)	1.26 (0.40–3.92)		1.98 (0.62–6.33)		7.40 (1.99–27.53)	
B+alcohol	1.21 (0.39–3.81)	19.2	1.91 (0.59–6.15)	7.1	7.06 (1.87–26.59)	5.3
B+smoking	1.24 (0.39–3.88)	7.7	2.00 (0.62–6.46)	−2.0	7.13 (1.88–26.98)	4.2
B+periodontitis	1.19 (0.38–3.75)	26.9	1.84 (0.56–6.04)	14.3	7.17 (1.92–26.73)	3.6
B+tongue coating score	1.14 (0.36–3.66)	46.2	1.84 (0.56–6.04)	14.3	7.20 (1.92–26.98)	3.1
B+bleeding on probing	1.37 (0.43–4.39)	−42.3	2.03 (0.63–6.50)	−5.1	7.73 (2.07–28.82)	−5.2
B+tongue brushing habit	1.41 (0.43–4.61)	−57.7	2.25 (0.68–7.48)	−27.6	10.75 (2.53–45.61)	−52.3

Base model was adjusted for age (continuous) and gender.
H_2S defined halitosis: $H_2S \geq 95$ ppb; CH_3SH defined halitosis: $CH_3SH \geq 12$ ppb; $(CH_3)_2S$ defined halitosis: $(CH_3)_2S \geq 18$ ppb.

Table IV. Odds ratio of VSCs defined halitosis by viral hepatitis, confounders and mediators in the fully adjusted model (n = 182).

	H_2S defined halitosis	CH_3SH defined halitosis	$(CH_3)_2S$ defined halitosis
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Viral hepatitis (reference = no)	1.31 (0.39–4.39)	1.92 (0.56–6.61)	*9.22 (2.08–40.95)*
Age (years)	0.99 (0.95–1.02)	0.97 (0.94–1.01)	1.05 (0.99–1.12)
Gender (reference = male)	0.84 (0.37–1.93)	1.25 (0.56–2.82)	2.31 (0.62–8.69)
Alcohol consumption (reference = yes)	1.79 (0.81–3.93)	1.59 (0.74–3.43)	2.49 (0.73–8.51)
Current smoking (reference = yes)	1.15 (0.47–2.78)	1.46 (0.61–3.52)	0.53 (0.10–2.69)
Periodontitis (reference = no)	1.16 (0.55–2.44)	*2.64 (1.24–5.64)*	1.94 (0.64–5.92)
Tongue coating score (continuous)	*1.07 (1.00–1.15)*	*1.08 (1.01–1.15)*	0.98 (0.88–1.09)
Bleeding on probing (continuous)	*1.12 (1.01–1.24)*	0.98 (0.89–1.08)	1.00 (0.86–1.16)
Tongue brushing habit (reference = no)	*0.37 (0.19–0.72)*	*0.39 (0.20–0.76)*	0.15 (0.04–0.52)

Italics denote statistical significance at $p < 0.05$.

relationship of HBV infection with $(CH_3)_2S$ defined halitosis was increased (AOR = 9.22, 95% CI = 2.08–40.95).

Discussion

The results from the present study show the association between HBV infection and $(CH_3)_2S$ defined halitosis. Analysis results showed that HBV infection had 640% greater odds of having $(CH_3)_2S$ defined halitosis than non-HBV infection in an age- and gender-adjusted model. This association was strengthened with further adjustment of tongue brushing habit. To the best of the authors' knowledge, the present study is the first to report a positive relationship between $(CH_3)_2S$ defined halitosis and HBV infection. $(CH_3)_2S$ has always been considered to be a minor component in intra-oral halitosis compared to the two major components, H_2S and CH_3SH [2,23,24]. Although present in intra-oral halitosis, until recently, the source of $(CH_3)_2S$ in halitosis was unknown. Tangerman and Winkel [3] have shown the $(CH_3)_2S$ concentrations in intra-oral halitosis were almost the same in mouth and nose breath, indicating the main origin of $(CH_3)_2S$ lies outside the oral cavity.

Among the extra-oral factors in the present study, HBV infection was significantly associated with $(CH_3)_2S$-related halitosis. Patients with various degrees of hepatocellular failure and portosystemic shunting of blood may acquire a sweet, musty or slightly fecal aroma of the breath, termed fetor hepaticus, which has been mainly attributed to sulfur compounds [12]. If the metabolizing function of the liver fails, the concentration of the metabolites, normally processed in the liver, will increase and enter the systemic circulation. A portion of the metabolites will then be exhaled, as recently confirmed by Van den Velde et al. [10]. *In vitro* experiments have shown that the thiol CH_3SH, containing a free –SH group, immediately reacts with whole blood within seconds, resulting in irreversible binding and oxidation, thereby preventing transportation of CH_3SH from the blood into alveolar air and, thus, into breath [25]; the same is valid for H_2S. This is not true for $(CH_3)_2S$, a neutral molecule which is stable in whole blood and can be transported by blood from the gut into alveolar air and breath [2,3,26]. Other volatiles found in extra-oral halitosis are also stable in whole blood. The neutral nature of most volatiles found in extra-oral halitosis is one of the reasons these compounds are difficult to remove from the breath, which is in contrast with the very reactive thiols CH_3SH and H_2S found in intra-oral halitosis. In contrast to $(CH_3)_2S$, H_2S and CH_3SH were rapidly metabolized by the colonic mucosa and liver [27]. The biological plausibility of the association between HBV infection and $(CH_3)_2S$ defined halitosis in the results could not be clarified; further studies are needed.

It is well established that VSCs including H_2S and CH_3SH were typical for intra-oral halitosis. These VSCs are produced from methionine and cysteine through the breakdown of oral exfoliative epithelial cells or blood components by intra-oral oral bacteria proteinases [23,28]. The significance of tongue coating in halitosis has also been noted in previous studies, where high correlations among tongue coating, periodontitis and odor formation have been found [24,29–32]. However, several studies have demonstrated that oral malodor is not associated with periodontal status. In one study, the mean number of periodontal pockets ≥ 5 mm and ≥ 7 mm did not correlate with VSC level in 127 subjects [33]. Another study also reported that probing pocket depth had no correlation with oral malodor parameters in 71 Israelis [34]. Since periodontitis is a chronic inflammatory disease that results from a complex polymicrobial infection and this bacterially-driven disease may differ from the accepted definition of infection [35], the interpretation of the results from the present study should be performed

with caution. The differences in the subjects may affect the differences observed with previous studies [33,34] in the correlations between periodontal disease and oral malodor. Since the participants in the present study were recruited from hospital patients, selection bias could have affected the results. There were associations between periodontitis and CH_3SH defined halitosis (percentage excess odd explained = 14.3%; AOR in Table IV = 0.38, 95% CI = 0.18–0.81), which is in agreement with the results from previous studies [24,29,30].

Several studies reported a relationship between periodontal bleeding and VSC level [32,36–39]. However, another study showed no statistically significant relationship between VSC level and bleeding [30]. Blood and cellular elements provide essential substrates for odor production. Additionally, blood provides certain factors that accelerate bacterial growth and stimulates proteolysis and odor production of putrescent saliva. Many women report an increase in gingival inflammation and discomfort in association with their menstrual cycle. In particular, around ovulation, the gingival inflammation index was found to increase, although no significant change in plaque index was detected [40]. In a previous study, VSCs and gingival inflammation, as assessed by BOP, increased in the ovulation phase [36]. The present study supported a relationship between periodontal bleeding and H_2S defined halitosis (percentage excess odd explained = −42.3%; AOR in Table IV = 1.12, 95% CI = 1.01–1.24).

The results from the present study showed that TCS and tongue brushing were the most influential factors in VSCs defined halitosis. Recent systematic review showed a positive effect of mechanical tongue cleaning in addition to tooth brushing on various parameters of oral malodor [41]. Studies have demonstrated mechanical approaches, such as tongue brushing or tongue scraping to clean the dorsum of the tongue, have the potential to successfully reduce breath odor and TCS. The results from the present study support the impact of mechanical tongue cleaning on chronic oral malodor. However, the associations were found in all VSCs including $(CH_3)_2S$, which is the main contributor to extra-oral or blood-borne halitosis [3]. In the present study, the frequency of tongue brushing was measured by a questionnaire, thus a recall bias may exist. Randomized clinical trials for assessing the association between tongue brushing and $(CH_3)_2S$ level will be necessary to clarify the results from the present study.

There were several limitations in the present study. First, among the 1851 subjects who visited the Health Promotion Center, only 10% of the subjects completed this examination. Only 13 out of the 182 participants had HBV infection and, among the HBV infection patients only five had dimethyl sulfide associated halitosis. Because the subjects in the study were sampled conveniently, participation bias could have occurred.

Second, the CPI for defining periodontitis may have several shortcomings [42]. A CPI of 3 or 4 is very unlikely to be associated with destructive disease among young subjects and older subjects are likely to have gingival recession and shallow pockets. Therefore, the possibility of an under-estimation in assessing periodontitis using CPI exists.

Third, the cross-sectional design did not allow for causal relationships to be inferred. Further, well-designed prospective investigations are required to determine the causality between HBV infection and VSCs to reduce the above-mentioned limitations.

Fourth, the use of organoleptic measurement is suggested as the gold standard of halitosis measurement. To improve the reliability and objectiveness of the organoleptic method, measurement by a panel of judges and standardization of the sense of smell were suggested [43]. However, we used a portable sulfide monitoring device instead of organoleptic measurement. Although organoleptic measurement was not performed, information about self-reported oral malodor was collected. However, there were no associations between self-reported oral malodor and VSCs (data not shown). The lack of organoleptic measurements is another limitation of this study. Although organoleptic method could not be the sole measurement method in defining patients with halitosis because of its inherent subjectivity, only the measurement of three major VSCs by OralChroma^{TM} should have been reconsidered.

Fifth, there are several non-oral causes of halitosis including respiratory tract, gastrointestinal tract and some systemic diseases [3]. However, we showed only HBV infection. The participants of our study underwent upper gastrointestinal endoscopy due to gastric problems. During endoscopy, antral gastric biopsy sections were taken from the stomach of each patient and the presence of *Helicobacter pylori* (*H. pylori*) in the sections was evaluated by a pathologist. However, the association between *H. pylori* infection and VSCs was not significant in the present study (AOR = 1.79 for $(CH_3)_2S$ defined halitosis, $p = 0.386$, data not shown). *H. pylori* infection was considered a possible cause of halitosis [44–47]. Further study to assess the other non-oral factors of halitosis will be needed. Seventh, although the infection of hepatitis C virus (HCV) would be low compared with that of HBV, HCV carriers have a higher risk to develop into liver cirrhosis and/or cancer [48]. Although it could be related with oral malodor, we didn't check the infection of HCV. Finally, the concentrations found by the OralChroma^{TM} are sometimes incorrect due to an inaccurate assignment of VSCs in the chromatogram [16]. Therefore, the OralChroma^{TM} must be calibrated with standards of known concentrations of VSCs

before use and the values read from the OralChroma^{TM} display must be validated by the gas chromatograms.

However, the strength of the present study consists of several factors. The oral examination and various measurements were performed clinically by dentists and a trained examiner. Moreover, various confounders such as age, gender, alcohol consumption, current smoking, periodontitis, BOP, TCS and tongue brushing habit were included.

Conclusions

Collectively, the results from the present study suggest that HBV infection might be associated with $(CH_3)_2S$ defined halitosis, suggesting that liver function should be assessed in such afflicted patients.

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