Original article

Halitosis and volatile sulfur compounds in monastic residents: A cross-sectional study of mediating mechanisms

Nguyen Thi Nhat Vy[1*], Nguyen Thi Thanh Duyen[2], Nguyen Thi Phuong Thao[1], Nguyen Duc Quynh Trang[1]
[1]Faculty of Odonto-Stomatology, University of Medicine and Pharmacy, Hue University, Hue city, Vietnam
[2]Faculty of Odonto-Stomatology, Duy Tan University, Danang, Vietnam

Abstract

Background: Oral malodor is primarily caused by volatile sulfur compounds (VSCs) produced by anaerobic bacteria. Limited research exists on VSCs patterns in monastic populations with homogeneous lifestyles. **Objective:** To investigate relationships between oral health parameters, VSC concentrations, and self-reported malodor among monks and nuns. **Materials and Method:** This cross-sectional study examined 118 monastic residents from four monasteries. Clinical assessments included dental status, periodontal health, oral hygiene indices, and tongue coating. VSCs (hydrogen sulfide, methyl mercaptan, dimethyl sulfide) were measured using OralChroma. Self-reported malodor was assessed using visual analog scale. Statistical analyses included correlations and mediation analysis. **Results:** Most demographic and oral hygiene behavioral factors showed no significant associations with VSC levels. Strong positive correlations were observed between VSCs and clinical parameters including bleeding teeth (r = 0.211 - 0.297), debris index (r = 0.310 - 0.384), and tongue coating (r = 0.233 - 0.349). Participants with self-reported malodor had significantly higher methyl mercaptan and dimethyl sulfide concentrations. Mediation analysis revealed methyl mercaptan as the primary mediator linking oral health parameters to perceived malodor, while hydrogen sulfide showed limited mediation and dimethyl sulfide showed no significant mediation. **Conclusions:** Methyl mercaptan serves as the key mediator between poor oral health and subjective malodor perception in this monastic population, supporting targeted therapeutic approaches for oral malodor management.

Keywords: *halitosis, volatile sulfur compounds, self-report, periodontal health, mediation analysis.*

1. INTRODUCTION

Oral malodor, commonly known as halitosis, is a prevalent condition that can significantly impact social interactions and quality of life [1]. The primary culprits behind oral malodor are volatile sulfur compounds (VSCs), such as hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide ($(CH_3)_2S$), which are produced by anaerobic, proteolytic bacteria residing predominantly on the dorsum of the tongue and within periodontal pockets [2-4]. These bacteria metabolize sulfur-containing amino acids from food debris, desquamated epithelial cells, and inflammatory exudates, leading to the release of malodorous gases [2, 4].

The etiology of oral malodor is multifactorial, with the majority of cases originating from intraoral sources. Poor oral hygiene, periodontal disease, tongue coating, and reduced salivary flow are well-established contributors to increased VSCs production [3-5]. While exogenous factors such as diet, tobacco use, and certain systemic conditions can exacerbate halitosis, the oral cavity remains the principal site of odor generation in most individuals [4, 6]. Epidemiological studies indicate that the prevalence of self-reported oral malodor can reach up to 35% in older adults, with many individuals adopting various behavioral strategies to manage or mask their condition [7].

Self-reported halitosis plays a crucial role in both clinical assessment and patient motivation for treatment and improved oral hygiene practices. Research demonstrates that self-reported halitosis prevalence ranges from 21.4% to 48.5% across different populations, with individuals showing high willingness to seek professional treatment when they perceive oral malodor [8-10]. Studies consistently show that patients with self-reported halitosis are more likely to adopt enhanced oral hygiene behaviors, including increased frequency of tongue cleaning, regular toothbrush replacement, and use of mouth rinses [8]. The psychological impact of perceived halitosis often serves as a powerful motivational factor, driving individuals to seek professional dental care and maintain better oral hygiene practices [8].

Despite extensive research in general and clinical

**Corresponding author: Nguyen Thi Nhat Vy. Email: nguyenthinhatvy@hueuni.edu.vn*
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populations, there is limited data on oral malodor and its determinants in unique lifestyle groups such as monks and nuns, whose daily routines, dietary patterns, and oral hygiene behaviors may differ substantially from the general population. Understanding the interplay between social, behavioral, and clinical factors in such populations can provide valuable insights into the fundamental mechanisms of VSC production and oral malodor. Therefore, this study aims to investigate the relationships between clinical oral health parameters, VSC concentrations, and self-reported oral malodor among monks and nuns residing in monasteries.

2. MATERIALS AND METHODS

Study Design and Population

This cross-sectional study was conducted among monks and nuns from four monasteries who volunteered to participate. The participant recruitment procedures, inclusion/exclusion criteria, and general study methodology have been previously described [11]. The examinations and data collection were performed on-site at the monasteries to facilitate participation and ensure convenience for the subjects. Inclusion criteria included adult monastic residents (≥ 18 years old) who reside in the monastic community for at least six months and provided informed consents. Those who had acute systemic diseases, recently used antibiotics or were undergoing active periodontal treatment were excluded [12].

Data Collection Procedures

Demographic information, general health status, and oral hygiene behaviors—including gender, age, educational level, frequency of dental visits, tooth brushing, flossing, tongue cleaning, and mouth rinsing—were obtained through a self-administrated questionnaire at the monasteries.

Oral Health Examination

One qualified dental professional conducted thorough oral health assessments for all subjects involved in the study. The status of oral hygiene was evaluated utilizing the Greene-Vermillion Oral Hygiene Index (OHI) [13]. Dental caries were measured through the World Health Organization's Decayed, Missing, and Filled Teeth (DMFT) index [14]. Periodontal health was assessed through two parameters: the highest Periodontal Probing Depth (PPD) recorded for each participant and the Bleeding on Probing (BOP) score, which quantifies the aggregate number of teeth that exhibited bleeding during probing. The presence of tongue coating was methodically assessed employing the Winkel Tongue Coating Index (WTCI), which entailed a visual examination of six dorsal segments of the tongue, with each segment assigned a score of 0 (indicating no coating), 1 (indicating a thin coating with visible papillae), or 2 (indicating a thick coating obscuring papillae), yielding an overall score range of 0 to 12 [15]. Additionally, salivary flow rates were measured during a state of rest. Participants expectorated all saliva present in their oral cavities into a paper container over a duration of 5 minutes, after which the flow rate (mL/min) was calculated.

Measurement of Volatile Sulfur Compounds (VSCs)

VSC levels-including hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide (CH_2S)-were measured using the Oral Chroma™ portable gas chromatograph (Abimedical Corporation, Osaka, Japan). Measurements were conducted between 9:00–11:00 AM or 2:00-5:00 PM. Participants were instructed to avoid eating, drinking, or performing oral hygiene activities for at least two hours prior to measurement. Mouth air samples were collected using a standardized procedure: each participant's breath was sampled via a disposable syringe and immediately injected into the OralChroma inlet within 30 seconds to ensure sample integrity. The OralChroma device was calibrated before each measurement session according to the manufacturer's guidelines and previous study [16]. After analyzing, the device showed H_2S, CH_3SH and CH_2S concentrations (in ng/10 mL) on a screen.

Self-Reported Oral Malodor Assessment

Participants self-assessed their oral malodor using a visual analog scale (VAS) from 0 (no odor) to 100 (severe odor). For analysis, participants were grouped into "no/mild odor" (VAS ≤ 10) and "malodor" (VAS > 10).

Statistical Analysis

Data analysis was conducted using StataMP version 14.1 (StataCorp). Descriptive statistics summarized participant characteristics and clinical findings. Independent sample t-tests assessed differences in VSC concentrations by demographic and behavioral variables. Pearson correlation coefficients evaluated relationships between VSCs and oral health parameters. Comparisons of VSC levels by self-reported malodor status were performed using independent sample t-tests. Mediation analyses examined the indirect effect of oral health parameters on self-reported malodor through VSC levels, estimating total, direct, and indirect effects.

Statistical significance was set at $p < 0.05$.

Ethical Considerations

All procedures conducted in the realm of research involving human subjects followed rigorously to the ethical principles delineated by the 1964 Helsinki Declaration. This research received ethical approval from Hue University of Medicine and Pharmacy (H2023/135). A comprehensive informed consent form was given to every participant before they took part in the study.

3. RESULTS

Participant characteristics have been previously reported. Briefly, the study included 118 monastic residents with a mean age of 32.9±11.3 years (range: 18-81 years), comprising 39.8% males and 60.2% females. Over half (50.8%) reported self-perceived breath odor scores exceeding 10/100 on the visual analog scale. No participants reported xerostomia symptoms or histories of gastrointestinal, ear-nose-throat, or systemic medical conditions [11].

Table 1. The Volatile sulfur compounds according social and oral hygiene characterisitics.

Characteristic	N (%)	H_2S		CH_3SH		$(CH_3)_2S$	
		mean ± SD	p-value*	mean ± SD	p-value*	Mean ± SD	p-value*
Gender			0.152		0.263		0.256
Male	47 (39.8%)	0.18 ± 0.28		0.38 ± 0.34		0.40 ± 0.54	
Female	71 (60.2%)	0.51 ± 1.52		0.60 ± 1.30		0.57 ± 0.95	
Age (years)			0.384		0.440		0.494
< 30	53 (44.9%)	0.27 ± 0.6		0.43 ± 0.44		0.45 ± 0.48	
≥ 30	65 (55.1%)	0.46 ± 1.5		0.58 ± 1.34		0.55 ± 1.02	
Educational level			0.178		0.071		0.114
Until highschool	98 (83.1%)	0.31 ± 0.88		0.44 ± 0.51		0.46 ± 0.55	
University/college	20 (16.9%)	0.71 ± 2.18		0.90 ± 2.26		0.75 ± 1.56	
Las dental visit			0.091		0.063		0.214
Within 6 months	68 (57.6%)	0.22 ± 0.36		0.36 ± 0.37		0.42 ± 0.49	
> 6 months or never	50 (42.4%)	0.59 ± 1.78		0.72 ± 1.52		0.61±1.10	
Brushing frequency			0.730		0.530		0.842
< 2 times/day	13 (11.0%)	0.27 ± 0.27		0.69 ± 0.52		0.46 ± 0.76	
≥ 2 times/day	105 (89.0%)	0.39 ± 1.27		0.49 ± 1.08		0.51± 0.82	
Flossing frequency			0.125		0.227		0.558
Yes	39 (33.0%)	0.14 ± 0.17		0.35 ± 0.35		0.44 ± 0.51	
No	79 (67.0%)	0.50 ± 1.45		0.60 ± 1.24		0.54 ± 0.93	
Tongue cleaning frequency			0.222		0.322		0.260
Yes	95 (80.5%)	0.31 ± 1.09		0.47 ± 1.09		0.46 ± 0.81	
No	23 (19.5%)	0.65 ± 1.57		0.71 ± 0.75		0.68 ± 0.81	
Mouth rinsing frequency			0.632		0.428		0.463
Yes	84 (71.2%)	0.34 ± 1.14		0.63 ± 0.74		0.54 ± 0.93	
No	34 (28.8%)	0.46 ± 1.35		0.47 ± 1.14		0.42 ± 0.27	

* Independent sample t-test

Table 1 demonstrates how VSCs concentrations vary with demographic and oral hygiene behaviors. The analysis indicates that most demographic factors—such as gender, age, and educational level—do not show statistically significant differences in VSC levels. Similarly, most oral hygiene practices, including brushing frequency, flossing, tongue cleaning, and mouth rinsing, do not exhibit significant associations with the measured VSCs. Nonetheless, a trend indicates that individuals aged ≥ 30, those who have not seen a dentist in the last six months, and those lacking the practices of flossing, tongue cleaning, and mouth rinsing may exhibit elevated VSC concentrations, although this variation is not statistically significant.

Table 2. The pearson correlation between the volatile sulfur compounds and the oral health parameters (N = 118)

Oral health	H_2S	CH_3SH	$(CH_3)_2S$
Number of decayed teeth	-0.073	-0.101	-0.032
Number of missing teeth	-0.054	-0.056	-0.010
Number of filled teeth	-0.070	-0.067	-0.132
Number of bleeding teeth	0.211*	0.297**	0.270**
Highest pocket depth	-0.009	-0.026	
DI score	0.310***	0.339***	0.311***
CI score	0.384***	0.337***	0.302***
WTCI score	0.239**	0.349***	0.271**
Saliva flow rate	-0.086	-0.0243	0.071

*p<0.05, **p<0.01, ***p<0.001

Table 2 highlights the relationships between VSC concentrations and specific oral health parameters. The results reveal that higher score in debris index, calculus index, tongue coating index, and more bleeding teeth on probing are positively correlated with increased VSCs concentrations, especially for methyl mercaptan and dimethyl sulfide. Notably, the number of decayed, missing, or filled teeth shows no correlation with VSCs. Saliva flow rate also does not demonstrate a significant relationship with VSCs in this sample.

Table 3. Mean volatile sulfur compounds concentration (ng/10 mL) by self-reported oral health

VSCs	Total (N=118)	No/mild odor (VAS ≤ 10) (n=58)	Malodor (VAS>10) (n=60)	p-value*
H_2S	0.38 ± 1.20	0.16 ± 0.21	0.59 ± 1.65	0.053
CH_3SH	0.51 ± 1.04	0.30 ± 0.24	0.72 ± 1.41	0.029
$(CH_3)_2S$	0.50 ± 0.82	0.34 ± 0.43	0.66 ± 1.05	0.032

* Independent sample t-test

Table 3 demonstrates the clinical validity of VSCs measurements as objective indicators of self-perceived oral malodor. The data show that participants who perceive themselves as having oral malodor tend to have higher concentrations of all three VSCs, with statistically significant differences for methyl mercaptan and dimethyl sulfide.

Table 4. Mediation analysis: effect (coefficients) of the oral health parameter on the likelihood of self-reported malodor (VAS score: 0-100), as mediated by changes in volatile sulfur compounds level (allowing exposure-mediator interaction) (n = 118)

	Mediator: H_2S		Mediator: CH_3SH		Mediator: $(CH_3)_2S$	
	Estimate	p-value	Estimate	p-value	Estimate	p-value
Number of bleeding teeth						
Total effect	1.38	0.002	2.29	0.002	0.78	0.041
Natural direct effect	0.86	0.017	1.09	0.002	0.71	0.011
Indirect effect	0.52	**0.005**	1.20	**0.001**	0.07	0.706
Calculus index score						
Total effect	8.79	0.001	11.32	<0.001	9.31	<0.001
Natural direct effect	6.87	0.002	8.73	<0.001	8.20	<0.001
Indirect effect	1.92	0.062	2.59	**0.018**	1.11	0.082

Debris index score						
Total effect	7.45	0.016	11.90	0.005	10.96	<0.001
Natural direct effect	7.01	0.005	9.45	0.001	9.66	<0.001
Indirect effect	0.44	0.756	2.45	0.181	1.30	0.106
Tongue coating (WTCI score)						
Total effect	5.17	0.001	8.57	0.002	3.24	0.002
Natural direct effect	3.85	<0.001	4.70	<0.001	3.35	<0.001
Indirect effect	1.32	0.100	3.87	**0.019**	-0.11	0.830

Table 4 presents mediation analyses examining whether VSCs serve as intermediary pathways between oral health parameters and self-reported malodor. The results reveal that methyl mercaptan plays a central mediating role, with the number of bleeding teeth, calculus accumulation, and tongue coating all exerting significant indirect effects on perceived malodor through elevated methyl mercaptan levels. In contrast, hydrogen sulfide demonstrated a more limited mediating function, significantly affecting only the relationship between bleeding teeth and self-reported malodor. Notably, dimethyl sulfide did not play a significant mediating role in the pathway between any oral health parameter and self-reported malodor, as no significant indirect effects were observed for this compound. Besides, the debris index score maintains a direct effect on malodor independent of VSCs mediation, indicating that additional factors beyond VSCs may influence the pathway from poor oral hygiene to self-perceived bad breath.

4. DISCUSSION

This study demonstrate that volatile sulfur compound (VSCs) concentrations, particularly methyl mercaptan, are significantly associated with poor oral health parameters such as the bleeding teeth, calculus, tongue coating, and debris index, while demographic and oral hygiene behaviors showed no significant associations. Mediation analysis additionally showed that methyl mercaptan serves as a key mediator in the relationship between poor periodontal or oral hygiene conditions and the perception of bad breath.

Our findings reveals that most demographic factors had no significant associations with VSCs levels in this monastic population which both align with and diverge from previous research. Miyazaki et al. (1995) examined 2,672 subjects and similarly noted no significant variances in VSCs between genders across various age brackets [17]. Nonetheless, other research has highlighted gender disparities, as Lima et al. (2012) discovered that women exhibited higher VSC values than men under stress conditions [18], while other studies have indicated that males generally present higher VSC concentrations due to factors such as oral hygiene habits, smoking behavior, and hormonal effects [19]. The age-related trends in this study consist with broader findings. Abdullah et al. found VSC levels increase with age, from 161.79 ppb in 15-29 year-olds to 282.89 ppb in those over 59 [19]. Unlike established literature, our study found no significant link between hygiene practices (brushing, flossing, tongue cleaning) and VSC levels [20]. This may be due to the consistent oral hygiene routines in our monastic environment and the limited sample size with low VSCs levels, which could hinder identifying variations.

Regarding oral health, we observed strong correlations between VSCs and clinical indicators, aligning with previous research. Yaegaki & Sanada (1992) and Yeon-Hee Lee (2023) found significant relationships between VSCs and periodontal health [3, 21]. The correlation between bleeding, plaque indices, and VSCs levels supports the role of gram-negative anaerobic bacteria in VSCs production. Bleeding provides heme proteins, a substrate for VSCs production by proteolytic bacteria. Methyl mercaptan showed the strongest associations with clinical parameters, indicating its closer link to periodontal issues than hydrogen sulfide [21, 22]. The tongue coating index was a significant correlator, emphasizing the tongue's role in VSCs production [3], while the lack of correlations with dental status suggests that active inflammation and bacterial overgrowth are more influential than static dental conditions. This finding suggests that DMFT may not be a sensitive indicator of current oral microbial activity or volatile compound production. Individuals with high DMFT scores may have received restorative

treatment that stabilizes oral health, thereby reducing active bacterial metabolism associated with VSC generation. Consequently, current periodontal and soft tissue conditions, rather than cumulative dental history, appear to play a more decisive role in determining oral malodor levels.

The clinical significance of VSCs measurements was confirmed by showing that participants who reported experiencing malodor had notably higher concentrations of these compounds. Specifically, individuals with self-reported malodor exhibited significantly elevated levels of methyl mercaptan (CH_3SH) and dimethyl sulfide ($(CH_3)_2S$), as well as near-significantly higher levels of hydrogen sulfide (H_2S). This aligns with previous studies that indicate a correlation between objective VSCs measurements and subjective perceptions of malodor, although it is important to note that self-perception can sometimes be unreliable [23]. Research by Lee highlighted that methyl mercaptan has greater permeability through the oral mucosa and is more pathogenic than hydrogen sulfide [21]. Additionally, CH_3SH is often reported to have a lower odor threshold and a more unpleasant smell compared to H_2S, which may account for its stronger association with malodor in this context [24].

Mediation Analysis and Mechanistic Pathways

The mediation analysis aimed to clarify the pathways linking oral health parameters to perceived malodor via VSCs production. The fundamental idea is that oral conditions contribute to malodor through VSCs. Our findings indicate that methyl mercaptan (CH_3SH) significantly mediates the effects of bleeding, calculus, and tongue coating on self-reported malodor, aligning with previous research that highlights these clinical factors' roles in VSCs production [3, 22]. The dominant mediating role of methyl mercaptan aligns with established literature showing that methyl mercaptan is more closely associated with periodontal pathology than other VSCs [25, 26]. CH_3SH, primarily derived from bacterial breakdown of methionine found in blood proteins and desquamated epithelial cells, plays a dominant role in oral environments. This is further supported by bleeding, which supplies heme, and the presence of calculus and tongue coating that create rich conditions for bacterial activity [3, 27]. Moreover, CH_3SH has a notably low odor threshold, often perceived as more unpleasant compared to hydrogen sulfide (H_2S), making even small increases in CH_3SH more likely to be detected as malodor [24].

Hydrogen sulfide (H_2S) demonstrated limited mediating effects, significantly impacting bleeding teeth (coefficient 0.52, p=0.05), while its influence on calculus (p=0.062) and tongue coating (p=0.100) was marginal or non-significant. This contrasts with the expectation that H_2S, being the most abundant VSCs in oral breath, would have broader effects [25]. Research indicates that while H_2S is prevalent in healthy individuals, methyl mercaptan (CH_3SH) becomes the dominant mediator in pathological conditions [21]. This discrepancy may arise because H_2S is primarily produced from cysteine metabolism, which, although present in oral substrates, may be less directly associated with clinical signs of inflammation and retentive factors compared to methionine, the source of CH_3SH. Saliva and gingival crevicular fluid (GCF) are key contributors to these processes.

The complete lack of significant mediation by dimethyl sulfide across all oral health parameters represents an important negative finding. Similar to H_2S, $(CH_3)_2S$ showed significant Natural Direct Effects (NDE) for bleeding, calculus, and debris. This confirms $(CH_3)_2S$ production is not significantly influenced by the oral health conditions studied, which suggests that its link to malodor is not explained by these factors. While $(CH_3)_2S$ can be produced from methionine, it involves different bacterial pathways and is more commonly associated with systemic factors, such as liver dysfunction and certain foods, rather than the intra-oral conditions measured [27]. Thus, dimethyl sulfide is typically linked to systemic rather than intra-oral factors.

The three volatile sulfur compounds play distinct roles in oral malodor. Hydrogen sulfide (H_2S) is usually the most abundant but has lower odor intensity, mainly reflecting general bacterial activity in the oral cavity. Methyl mercaptan (CH_3SH), though less abundant, has a much stronger odor and is closely associated with periodontal disease because of its high tissue permeability and cytotoxicity. Dimethyl sulfide ($(CH_3)_2S$), however, is more often linked to non-oral or systemic sources of malodor and showed no significant mediation effect in this study. Overall, methyl mercaptan emerged as the most clinically important VSC connecting poor oral health with perceived bad breath.

The persistence of direct effects after accounting for VSCs mediation suggests additional pathways beyond VSCs influence malodor perception. This finding is consistent with research indicating that other malodorous compounds (such as indole, skatole, putrescine, and cadaverine), psychological factors, and individual sensitivity variations may influence malodor perception [28].

Limitations and strengths

Several limitations must be acknowledged. The cross-sectional design of the study prevents causal inferences from being drawn regarding the observed relationships. Additionally, the specialized population of monks and nuns may limit the generalizability of the findings to broader groups with more varied lifestyles, dietary habits, and oral hygiene practices. The reliance on self-reported assessments of malodor introduces potential bias, although the correlation with objective VSCs measurements support its validity. The relatively small sample size may impact the statistical power and external validity of the results. Furthermore, while the homogeneous lifestyle of the study population reduces confounding variables, it may also obscure important relationships that could be revealed in more diverse populations.

This research, however, presents several notable methodological strengths that enhance its overall impact. By employing a comprehensive assessment that integrates objective VSC measurements, detailed clinical examinations, and self-reported outcomes, the study offers a thorough evaluation of the etiology of oral malodor. Furthermore, the mediation analysis provides valuable mechanistic insights that are rarely explored in oral malodor research. Additionally, the unique population studied contributes important data on VSC patterns in a lifestyle-controlled environment, thereby offering insights into the fundamental biological relationships between oral health and malodor production when confounding lifestyle factors are minimized.

5. CONCLUSION

The mediation analysis indicates that oral malodor perception involves multiple pathways rather than solely relying on volatile sulfur compounds (VSCs). Methyl mercaptan plays a significant role in periodontal issues, linking periodontal disease to perceived malodor. Effective malodor management should address these various pathways, including mechanical cleaning and targeted antimicrobial treatments, rather than focusing only on VSCs.

Conflict of Interest

The authors declare that there are no conflicts of interest related to this manuscript. No financial, personal, or professional relationships influenced the conduct or reporting of this study.

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