The Relationship between Volatile Sulfur Compounds and the Severity of Chronic Periodontitis: A Cross-sectional Study

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INTRODUCTION

Halitosis is a common condition that draws the attention of both patients and physicians. It is not only an embarrassing issue for many people, but also an indicator of a systemic or dental disease. It is a global medico-social problem but the related epidemiological studies are limited.[1] Porter and Scully[2] reported a prevalence of 30%, whereas Zürcher et al.[3] reported a prevalence of 50%.

It is considered that 80%–90% of etiological factors of halitosis are from the oral cavity.[4] Bacteria present in the dorsum of the tongue have been mostly accused for the halitosis, followed by a periodontal disease, reduced salivary secretion, and prosthetic appliances.[5]

Eid[6] reported that the malodorous breath was directly related to the periodontal pocket depth and was reduced after periodontal therapy. Moreover, a positive correlation was found between chronic periodontitis and oral malodor.[7]

The extraoral causes of halitosis include respiratory and gastrointestinal infections or disease,[8] however, it may also be associated with the consumption of specific types of foods or drinks, such as onions or alcohol. In addition, it can also be related to smoking.[9]

BACKGROUND: Halitosis is a problem that is often ignored or undervalued. It is not considered a major clinical issue; thus, little is carried out to eradicate it. Little is known about the relationship of malodorous breath with the severity of periodontitis. Hence, the study aimed to investigate the relationship between volatile sulfur compounds (VSCs) and chronic periodontitis.

MATERIALS AND METHODS: According to the severity of chronic periodontitis, 80 patients were categorized into four groups (mild, moderate, severe, and control). Probing depth and clinical attachment level were measured. Portable sulfide monitor (Halimeter) is used to assess VSCs. Statistical analyses were conducted using R studio (Northern Ave, Boston).

RESULTS: The differences in the VSC levels among the four groups were found to be statistically significant. The greater the severity of periodontitis, the higher the VSC levels found (P < 0.001). Male had higher levels of VSCs than female (225 and 180.53 parts per billion [ppb], respectively). The lowest mean level of VSCs was found in the subjects aged 15–29 years (161.79 ppb); the mean values increased with the age, reaching 282.89 ppb in subjects aged >59 years.

CONCLUSION: Highly significant differences were found in the VSC levels among different severities of chronic periodontitis. Moreover, an age-related increase in the VSC levels was determined. In addition, male had higher concentration of VSCs than female.

KEYWORDS: Halitosis, periodontitis, volatile organic compounds

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Even though halitosis is a public health concern, its rarely been investigated and the relation between periodontal health and malodor is not well understood in Kuwait.[11,12] Hence, the study aimed to assess the relationship between the volatile sulfur compounds (VSCs) and chronic periodontitis using the probing depth (PD), clinical attachment level (CAL), age, and gender as secondary objectives.

**Materials and Methods**

A cross-sectional, observational study was designed and approved by the Institutional Review Board (IRB; protocol number RC/IRB/2019/109). Patients were selected from four different government dental clinics in Kuwait. The sample population was weighed and calculated by the statistician and found to be 80 patients. The samples were divided into four groups according to the Centre for Disease Control-American Academy of Periodontology (CDC-AAP):

1. Twenty patients with mild chronic periodontitis
2. Twenty patients with moderate chronic periodontitis
3. Twenty patients with severe chronic periodontitis
4. Twenty patients with no clinical attachment loss

**Exclusion criteria**

Participants were excluded if having any of the following conditions:

- Pregnancy
- Age <15 years
- Respiratory tract infections during examination
- Any systemic disease that is associated with malodor
- Dental decay
- Any previous periodontal treatment in the past 3 months
- Complete edentulous (at least 20 teeth were required) or presence of removable or fixed dental prosthesis
- Under any medication for the past 3 months, which could interfere in the VSC production such as antibiotics, antidepressants, and antihistamines

On examination day, patient should refrain from the following:

1. Eating odorous foods for 48 h before the assessment
2. Usual oral hygiene practices, mouth rinses, breath fresheners, and chewing gum for at least 2 h before their assessment
3. Smoking for at least 5 h before assessment

**Clinical measurements**

Each periodontal site was measured for (1) pocket depth and (2) clinical attachment loss. The PD and CAL were assessed at the midbuccal, mesiobuccal, and distobuccal sites as described in previous studies.[12,13]

A partial-mouth assessment was performed as a quick, short-spanned assessment that minimized the subject’s discomfort. All the samples were examined by the main investigator after calibration exercises were performed in four patients twice within a week before starting the actual study. The intra-examiner agreement between the two measurements was 94%.

The PD and CAL measurements were recorded to the nearest millimeter using a calibrated PCP-UNC 15 periodontal probe (Hu-Friedy Mfg Co., LLC, Chicago, Illinois).

According to CDC-AAP, the burden of periodontitis was assessed and based on the measures of PD and CAL at interproximal sites, the degree of severity was considered.[14]

**Volatile sulfur compound measurement**

Intraoral VSC levels were assessed by using portable sulfide monitor (Halimeter, Interscan, Chatsworth, California). All measurements were recorded between 9:30 and 11:30 during the month of Ramadan to ensure that the subjects had refrained from eating or drinking at least 2 h before the assessment. The VSC levels were measured by an electrochemical sensor method. The patients were instructed to keep their mouth close for 120 s prior to the measurement. The samples were collected using a disposable plastic straw attached to the air inlet of the monitor. Subjects were then asked to close the lips allowing a slight gap between the lips and the straw. Mean from three subsequent measurements was recorded in parts per billion (ppb) by taking the highest reached value in each measurement, with an approximately 30-s rest between each measurement.[7]

**Statistical analysis**

Using the RStudio software, multiple linear regressions were performed to predict the VSC levels using the PD, CAL, age, and gender as predictors. The multivariate analysis of variance (ANOVA) was used to compare the means of the VSC levels across different type of chronic periodontitis, gender, and age levels ($P$ value considered significant if <0.05). Frequencies and percentage were used for the categorical variables.

**Results**

**Descriptive statistics of different severities of chronic periodontitis**

Sixteen male subjects (32.7%) had severe chronic periodontitis, whereas only four male subjects (8.2%) had no clinical attachment loss. Conversely, sixteen female subjects (51.6%) had no attachment
loss, whereas only four female subjects (12.9%) had severe chronic periodontitis. Seventeen subjects aged 15–29 years (58.6%) had no clinical attachment loss and four subjects aged >59 years (66.7%) had severe chronic periodontitis [Table 1].

The descriptive statistics of VSC levels among participants with different age and gender revealed that when the levels of VSCs were compared based on the participants age groups, an age-related increase in the VSC values was found. The lowest mean level of VSCs was found in the 15–29-year-old subjects (161.79 ± 32.1 ppb); the mean level was increased with the increasing of age, reaching 282.89 ± 15.89 ppb in subjects aged >59 years. Moreover, male had more severe periodontitis than female (225 ± 40.3 and 180.53 ± 51 ppb, respectively) [Table 2 and Figure 1].

Mean of volatile sulfur compound levels among different severities of chronic periodontitis

The mean VSC level was increased with the increasing severity of periodontitis as diagnosed according to the clinical attachment loss. The highest mean VSC levels were observed in the group of severe chronic periodontitis (263.33 ± 27.44 ppb), and the lowest levels were observed in the group with no clinical attachment loss (147.42 ± 24.14 ppb) [Table 3 and Figure 2].

Result of analysis of variance

A one-way ANOVA was performed to check the differences in VSC levels among the four groups. The results revealed highly significant differences in VSC levels among the different severities of chronic periodontitis ($F$ test = 74.84, $P < 0.001$) [Table 4].

Result of Tukey’s honestly significant difference multiple comparisons test

Multiple comparisons were conducted using the Tukey’s honestly significant difference (HSD) test to investigate the differences between the different stages of periodontitis. Tukey’s HSD showed that all severities differed from each other with a value of $P < 0.001$ for each comparison [Table 5].

Descriptive statistics of clinical attachment level

The descriptive statistics of CAL in relation to the demographic data of age and gender indicated that >59 years old had the highest mean value of CAL (4.69 ± 2.27 mm), whereas 15–29 years old had the lowest mean value of CAL (2.43 ± 0.23 mm). Male had a higher mean reading in CAL (3.38 ± 1.28 mm) than female (2.99 ± 1.13 mm) [Table 6 and Figure 3].

Discussion

The correlation between halitosis and the different severities of chronic periodontitis were assessed by the level of VSCs and the degree of clinical attachment loss, respectively. The results revealed that the mean levels of VSCs were the highest in the participants with severe chronic periodontitis (263.33 ± 27.44 ppb), followed by those with moderate chronic periodontitis (226.03 ± 26.26 ppb), and those with mild chronic periodontitis (194.37 ± 23.57 ppb). The least levels of VSCs were observed in the participants with no CAL (147.42 ± 24.14 ppb). The difference between the groups was statistically significant. This result is supported by that of a study that has shown that periodontitis leads to an increased intensity of the oral malodor.\textsuperscript{15} This study reported that the amount of VSCs in the breath air of

| Table 1: Descriptive statistics of the different severities of chronic periodontitis in relation to the demographic data |
|---------------------------------------------------------------|
| **Severity of periodontitis** | None | Mild | Moderate | Severe | Total |
|--------------------------------|------|------|----------|--------|-------|
| **Gender** |          |            |            |         |       |
| Male | 4 (8.2%) | 15 (30.6%) | 14 (28.6%) | 16 (32.7%) | 49    |
| Female | 16 (51.6%) | 5 (16.1%) | 6 (19.4%) | 4 (12.9%) | 31    |
| **Age** |          |            |            |         |       |
| 15–29 years | 17 (58.6%) | 11 (37.9%) | 1 (3.4%) | 0 (0%) | 29    |
| 30–44 years | 2 (7.4%) | 7 (25.9%) | 11 (40.7%) | 7 (25.9%) | 27    |
| 45–59 years | 1 (5.6%) | 2 (11.1%) | 6 (33.3%) | 9 (50%) | 18    |
| >59 years | 0 | 0 | 2 (33.3%) | 4 (66.7%) | 6     |

| Table 2: Descriptive statistics of volatile sulfur compound levels among demographic data |
|---------------------------------------------------------------|
| **Frequency** | **Percentage** | **Mean of VSC level ± SD (ppb)** |
| **Age** |          |            |                             |
| 15–29 years | 29 | 36.3 | 161.79 ± 32.1 |
| 30–44 years | 27 | 33.8 | 219.32 ± 32.9 |
| 45–59 years | 18 | 22.5 | 239.56 ± 33.14 |
| >59 years | 6 | 7.5 | 282.89 ± 15.89 |
| **Gender** |          |            |                             |
| Female | 31 | 38.7 | 180.53 ± 51 |
| Male | 49 | 61.3 | 225 ± 40.3 |

VSC = volatile sulfur compound, SD = standard deviation
the subjects with periodontal involvements was eight times that of the amount found in the control subjects.

Another study reported that periodontal pockets were positively correlated with VSC concentrations in the mouth.\(^{[60]}\) However, other studies reported no correlation between the periodontal pocket and VSCs.\(^{[17]}\) Moreover, no significant periodontal pathogens were observed in the groups of patients with halitosis.\(^{[18,19]}\)

Male had higher levels of VSCs than female (225 ± 40.3 and 180.53 ± 51 ppb, respectively) in this study. This is similar to the results presented by Morita and Wang.\(^{[20]}\) Similar results were obtained by other groups of researchers, such as Nadanovsky et al.\(^{[21]}\) and Snel et al.\(^{[22]}\) Yet, other studies have reported that halitosis was similarly prevalent in male and female.\(^{[23,24]}\) However, another research that was performed in healthy individuals stated that females had bad breath more often than males in all age groups.\(^{[25]}\) Conversely, a group of researchers found among 35-44 years old a lower level of VSCs in the breathing air of male than in that of female.\(^{[26]}\)

In this study, when the levels of VSCs were compared based on the participants’ age groups, an age-related increase in the VSCs values was noted. The lowest mean level of the VSCs was found in the subjects aged 15–29 years (161.79 ± 32.1 ppb), and the mean level increased with the increasing age until reaching 282.89 ± 15.89 ppb in subjects aged >59 years. Similar results were obtained by Nadim et al.\(^{[27]}\) They found that of the 57% of subjects reporting halitosis, 30% were 50 years old and above. They attributed this to the assumption that older people often have systemic diseases that lead to difficulties in maintaining their oral hygiene.

The age group of 59 years and more in this study also had the highest incidence of severe periodontitis and the greatest concentration of VSCs. According to researchers, periodontitis increases with age.\(^{[28,29]}\) Moreover, same infectious burden in an older adult may have worse consequences than in a younger individual.\(^{[30]}\)

It is difficult to determine the actual influence of age and gender on halitosis, as breath malodor is multifactorial. Halitosis is not directly caused by aging; however, the oral hygiene may be more of a problem in older people who are more likely to have a dry mouth, which can contribute to a bad breath. Researchers have assessed malodor and the factors associated with it among patients of Kuwait.\(^{[10]}\) Their study pointed to the age of 30 and older as a factor that was positively associated with a bad breath.

**CONCLUSION**

The following are the conclusions of this study:

1. Highly significant differences exist in the VSC levels among different severities of chronic periodontitis; the greater the severity of periodontitis, the higher the VSC levels.
2. When the levels of VSCs were compared between the participants’ age groups, an age-related increase in the VSC levels was observed.
3. In addition, males had higher concentration of VSCs than females.

![Mean±SD of VSCs among (Age,Gender)](image1)

**Table 3: Mean and standard deviation of volatile sulfur compound levels among different severities of chronic periodontitis**

| Severity of periodontitis | Frequency | VSC level mean ± std. deviation (ppb) |
|--------------------------|-----------|-------------------------------------|
| Mild periodontitis       | 20        | 194.37 ± 23.57                      |
| Moderate periodontitis   | 20        | 226.03 ± 26.26                      |
| Severe periodontitis     | 20        | 263.33 ± 27.44                      |
| No clinical attachment loss | 20    | 147.42 ± 24.14                      |
| VSC = volatile sulfur compound |         |                                     |
Table 4: Result of analysis of variance (comparing volatile sulfur compound [VSC] levels among different severities of chronic periodontitis and mean and standard deviation of VSC levels among different severities of chronic periodontitis)

| Severity of periodontitis | VSC level mean ± std. deviation (ppb) | F test from ANOVA | P Value |
|--------------------------|----------------------------------------|-------------------|---------|
| Mild periodontitis       | 194.37 ± 23.57                         | 74.84             | <0.001 HS |
| Moderate periodontitis   | 226.03 ± 26.26                         |                   |         |
| Severe periodontitis     | 263.33 ± 27.44                         |                   |         |
| No clinical attachment loss | 147.42 ± 24.14 |                   |         |

VSC = volatile sulfur compound, ANOVA = analysis of variance, HS = high significant P value < 0.05

Table 5: Tukey’s honestly significant difference multiple comparisons test

| Comparisons                        | Mean difference in VSC level (ppb) | P Value | Conclusion                                      |
|------------------------------------|------------------------------------|---------|------------------------------------------------|
| Moderate–mild periodontitis        | 31.67                              | 0.001*  | Moderate has significantly higher VSC level than mild |
| Severe–mild periodontitis          | 68.97                              | 0.001*  | Severe has significantly higher VSC level than mild |
| Severe–moderate periodontitis      | 37.33                              | 0.001*  | Severe has significantly higher VSC level than moderate |
| Mild–no clinical attachment loss   | 46.95                              | 0.001*  | Mild has significantly higher VSC level than no clinical attachment loss |
| Moderate–no clinical attachment loss | 78.6                              | 0.001*  | Moderate has significantly higher VSC level than no clinical attachment loss |
| Severe–no clinical attachment loss | 115.92                             | 0.001*  | Severe has significantly higher VSC level than no clinical attachment loss |

VSC = volatile sulfur compound
* as highly significant (p< 0.05)

Table 6: Descriptive statistics of clinical attachment level among demographic data of age and gender

| Age                        | Mean ± SD (mm) |
|----------------------------|----------------|
| 15–29 years                | 2.43 ± 0.23    |
| 30–44 years                | 3.33 ± 1.06    |
| 45–59 years                | 3.87 ± 1.21    |
| >59 years                  | 4.69 ± 2.27    |
| Gender Female              | 2.99 ± 1.13    |
| Male                       | 3.38 ± 1.28    |

CAL = clinical attachment level, SD = standard deviation

Figure 3: Mean of clinical attachment level (CAL) among demographic data of age and gender

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Conflicts of interest
There are no conflicts of interest.

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