Halimeter ppb Levels as the Predictor of Erosive Gastroesophageal Reflux Disease

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Background/Aims: In a previous issue published in Gut and Liver, we found that erosive changes in the esophagogastrroduodenal mucosa were strongly correlated with increased levels of volatile sulfur-containing compounds (VSC), suggesting that halitosis could be a symptom reflecting the erosive status of the upper gut mucosa. Together with other studies showing a possible association between halitosis and gastroesophageal reflux disease (GERD), under the premise that halitosis could be one of extraesophageal manifestations of erosive GERD (ERD), we investigated the significance of Halimeter ppb levels on ERD compared to non-erosive gastroesophageal reflux disease (NERD).

Methods: Subjects were assigned to the NERD group if there was no evidence of esophageal erosive changes on endoscopy, despite reflux symptoms, and to the ERD group if they had GERD A, B, C, or D (according to the Los Angeles classification). The VSC levels were measured in all patients with either a Halimeter (before endoscopy) or by gas chromatography of the gastric juices aspirated during endoscopy. Results: The VSC level differed significantly between the NERD and ERD groups (p < 0.0001), suggesting that this can be used to discriminate between NERD and ERD. However, the VSC level did not differ significantly with the severity of GERD. Even though hiatal hernia and a body mass index of >24 kg/m² was significantly associated with ERD, there was no correlation with Halimeter ppb levels. Minimal-change lesions exhibited the highest VSC levels, signifying that minimal change lesions can be classified as ERD based on our finding that halimeter ppb levels were descriptive of erosive change. Conclusions: Erosive changes in the esophageal mucosa were strongly associated with VSC levels, supporting the hypothesis that halitosis can be a potential biomarker for the discrimination between ERD and NERD, reflecting the presence of erosive change in the lower esophagogastric junction. (Gut Liver 2010;4:320-325)

Key Words: Volatile sulfur compound; H2S; Halitosis; Gastroesophageal reflux disease; Nonerosive gastroesophageal reflux disease; Hiatal hernia; Body mass index

INTRODUCTION

Halitosis, bad breath or oral malodor, is rather common, but very troublesome symptom, affecting an estimated 10 to 30 percent of the US population on a regular basis. However, patients are very reluctant to consult halitosis to doctors. Because the patients generally regard halitosis as just the result of unsanitary oral health in spite of troublesome discomfort and causing social problem. Moreover, quite many cases with halitosis do not experience improvement with oral gargling or scaling. Halitosis has several origins that are usually categorized as oral, extraoral, or delusional. Halitosis of oral origin is quite well understood and it is thought that in the vast majority of cases (80-90%). Extraoral halitosis originated from respiratory tract, gastrointestinal tract, or pharyngo-tonsilar problem and general systemic condition.
such as liver cirrhosis, chronic renal failure and malignancy.\textsuperscript{2,7} Of the extraoral origin, especially gastrointestinal problems are thought to be major contributor.\textsuperscript{2,7} Some studies including ours\textsuperscript{8-10} have already shown the close relationships between gastrointestinal disease and halitosis.

Volatile sulphur compounds (VSCs) composed of hydrogen sulfide, methyl mercaptan, and dimethyl sulfide, have been known as the main factor of halitosis.\textsuperscript{11,12} In a previous study, we measured VSCs among the patients who had injured gastric mucosa, checking VSC levels by Halimeter of oral air or gas chromatography of gastric juice, and reached to the conclusion that halitosis might have significant correlation with mucosal damage after \textit{Helicobacter pylori} (\textit{H. pylori}) infection.\textsuperscript{13} The core points we have found from this previous study was that the injured esophagogastroduodenal mucosa have potential of generating VSCs supported with the fact that there was statistical difference in VSC levels between eroded esophageal mucosa and noneroded mucosa. Additional report was followed that Korea red ginseng could relieve halitosis through their anti-inflammatory and cytoprotective actions in patients with \textit{H. pylori}-infected gastritis.\textsuperscript{14}

Since halitosis is malodor coming from mouth and stomach, there is high possibility that halitosis is associated with gastroesophageal reflux disease (GERD). In the current study, we hypothesized that halitosis can be an extraesophageal symptom of GERD. The aim of this study was to define the relationship between GERD and halitosis by investigating VSC levels in patients with \textit{H. pylori}-infected gastritis.\textsuperscript{15}

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**MATERIALS AND METHODS**

1. Patients

Among the patients who underwent esophagogastroduodenoscopy due to dyspepsia, heartburn, and acid regurgitation symptoms, 169 patients were enrolled for current study after receiving written consent. In all patients, VSCs concentrations were checked by Halimeter endoscopy in fasting status and by gas chromatography from 5 mL of the gastric juices aspirated from gastric corpus during endoscopy procedure after receiving informed consent about the current study. The aspirated gastric juices were kept frozen in \textdegreeC 70 until analysis and the Halimeter measurements were repeated three times to get the mean value in the early morning before endoscopy. The patients with nonsteroidal anti-inflammatory drugs (NSAIDs) medications, proton pump inhibitor or H\textsubscript{2} receptor antagonists, and other medications including antibiotics were excluded and the patients with systemic illness including diabetes mellitus, liver diseases, renal disease, and malignancy were also excluded.

The total patients (n=169) were grouped into two groups based on their endoscopic findings. Non-erosive gastroesophageal reflux disease (NERD) Group (n=89), who have symptom such as: heart burn, regurgitation, dysphagia, chest pain, belching, and sour taste, but no evidence of definite reflux related erosion or ulcer on endoscopy. We included the patients who can be classified as minimal change lesion in NERD group (n=20). The reason why we included minimal change lesion as NERD group in this study was that many investigators\textsuperscript{15-17} defined minimal change lesion as nonerosive minimal lesion, even though the clear definition is still under debate. Erosive gastroesophageal reflux disease (ERD) group consists of erosive gastro-esophageal reflux disease group (n=80), classified based on LA classification.\textsuperscript{18} Other parameters such as age, gender, presence of erosive gastritis and hiatal hernia, body mass index (BMI), \textit{H. pylori} infection and smoking were also recorded. The presence of \textit{H. pylori} was diagnosed with rapid urease test (CLO test), urea breath test (UBT), and Giemsa staining from biopsied tissue. If two of three tests were positive, the case was diagnosed as \textit{H. pylori} positive and when all of these diagnoses were negative, we defined the case as \textit{H. pylori} negative.

2. Halimeter for halitosis measurement from breath

The portable sulfide monitor (Halimeter\textsuperscript{®}, Model RH-17K; Interscan Co., Chatsworth, CA, USA) has high sensitivity for VSCs. Patients were asked to breathe through the nose, with the mouth closed, for 1 minute. A straw attached to the Halimeter was then inserted into the mouth and air was withdrawn from the mouth for analysis. The results of VSC levels in breath air were recorded as parts per billion (ppb) of sulfide equivalents. Concentrations were determined in triplicate and a mean value was calculated. Measurement of VSCs was usually performed in the morning in fasting condition to avoid the influence from ingested foods.

3. Gas chromatography for gastric juice VSC concentrations

For separation and calibration of VSCs, a gas chromatography (Agilent 6890N; Agilent Technologies, Willington, DE, USA) with a flame photometric detector (FPD) was used. Since we obtained gastric juices for analysis, liquid should be converted into gas, for which we boiled the gastric juice at 60\textdegreeC for evaporating gas, among which 500 \textmu L were entrapped into a syringe in order to perform gas chromatography/FPD analysis. Five milliliters
gastric juices were aspirated from gastric corpus during the endoscope procedure using aspiration tube inserted onto biopsy channel (Olympus, Tokyo, Japan) and were stored at −70°C deep freezer until the assay. After several times of trials and errors at different temperature for the evaporating of gastric juices, we could success in final decision that 60°C were quite ideal for evaporating gastric juices for gas chromatography. Headspace air injection, peak areas and retention times were recorded by ChemStation software (3365 ChemStation revision A09; Agilent Technologies). Our group might be the first investigator in achieving the measurement of VSCs levels using aspirated gastric juices.13

4. Statistics

VSCs concentrations from mouth breath and gastric juice were analyzed using statistical software (MINITAB Inc., State College, Pennsylvania, PA, USA). These data were compared with t-test, all p values of <0.05 were considered statistically significant.

RESULTS

A total of 169 patients presenting with dyspepsia and heartburn were recruited for the study and referred for endoscopy, after which the patients were grouped into two according to the presence or absence of erosive or ulcerative changes in esophago-gastric junctions; NERD (n=89) and ERD (n=80). The 80 ERD patients were further sub-grouped according to LA classification of GERD; LA grade A, n=51; LA grade B, n=22; LA grade C, n= 6; LA grade D, n=1. The demographic characteristics of the study population are shown in Table 1. Male gender, the presence of hiatal hernia, increased BMI > 24, and the absence of H. pylori infection were significantly related parameters in ERD group compared to NERD group (p < 0.05). On the other hand, the mean age, the status of gastric erosions, and smoking were not different between groups.

There was significant correlation between VSC levels measured with Halimeter ppb and the levels of hydrogen sulfide (H2S) measured in gastric juices with gas chromatography with a flame photometric detector (r=0.78, p < 0.001). More than half of the NERD group patients showed “no detectable levels” of VSCs with the measurement of gas chromatography, signifying the levels of VSCs existing in gastric juices less than 0.01 ppm. Since VSC counted as “no detectable levels lesser than 0.01 ppm” should be interpreted as 0, we analyzed this as missing value. Since we confirmed that Halimeter ppb levels reflected the halitosis better than gas chromatography, this is why we used the values of VSCs ppb levels measured by Halimeter in the following analysis to correlate between VSCs levels and parameters of ERD or NERD patients.

The mean levels of VSC levels reflected as Halimeter ppb were significantly different between NERD and ERD (p < 0.042) as shown in Table 2. In subsequent questionnaire analysis, 81.3% of patients (65/80) in ERD group complained of halitosis higher than point 5 from visual analogue scale of 0-10 points, whereas only 33/89 (37.1%) in NERD group complained of halitosis higher than point 5. Taken together of these subjective and objective measurement, halitosis could be an extraesophageal manifestation of ERD. We further analyzed the
association of halitosis according to endoscopic finding as follows; NERD group (n=89) as further subdivision into completely normal gastroesophageal (GE) junction (n=69) and minimal change lesions (n=20) and ERD group (n=80) into LA grade A (n=51) and LA grade >B (n=29) (Table 2). The mean Halimeter ppb levels of minimal change lesions were significantly higher than either NERD or ERD>LA classification grade A (p=0.021), whereas there was no significant difference in Halimeter ppb according to the degree of LA classification from A and higher than B. All of these data suggests that ERD was significantly associated with increased Halimeter ppb levels, but ppb levels did not reflect the severity of mucosal damages. As result, we speculated that halitosis could be the extraesophageal manifestation of ERD, but it might be the results of the presence of esophageal mucosal damages, similar result with our previous publication. Therefore, we assert that minimal change lesion could be a part of ERD rather than NERD in its nature determined by the nature of Halimeter ppb reflecting the presence of mucosal injury.

The mean ppb levels in NERD patients with hiatal hernia were 191.11±32.55 and the levels of ppb in NERD without hiatal hernia were 121.59±13.69, of which statistical difference was significant (p=0.029). The significant difference in Halimeter ppb were also noted in ERD (p=0.016), suggesting that the presence of hiatal hernia contributed to increased levels of Halimeter ppb irrespective of NERD or ERD group (Table 3), that is, simply the presence of hiatal hernia might influence either the development of ERD (Table 1) or halitosis (Table 3). However, since there was no difference in Halimeter ppb levels between NERD and ERD in cases with hiatal hernia, we speculated that hiatal hernia could affect halitosis through its structural trait, not through pathogenic contribution.

The influence of BMI status on ERD was evaluated based on their Halimeter levels (Table 4). Contrary to the analysis of the influence of hiatal hernia on halitosis, BMI was not associated with halitosis as shown in Table 4 (p=0.097 in NERD and p=0.077 in ERD, respectively). However, in patients with BMI <2.4, the mean levels of Halimeter ppb were significantly increased in ERD compared to NERD (p=0.016), suggesting that BMI could contribute to the ERD, but halitosis was not evident related to BMI status. In patients with BMI <2.4, halitosis could be overt extraesophageal symptom of ERD Therefore, we could summarize that neither the presence of hiatal hernia nor high BMI did affect Halimeter ppb according to the degree of LA classification from A and higher than B.

### Table 4. Influence of BMI >24 kg/m² on Halimeter ppb Levels according to Group

<table>
<thead>
<tr>
<th>BMI &gt;24</th>
<th>BMI ≥24</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NERD</td>
<td>98.42±10.52</td>
<td>138.50±21.24</td>
</tr>
<tr>
<td>ERD</td>
<td>249.79±57.37</td>
<td>140.02±16.02</td>
</tr>
</tbody>
</table>

BMI, body mass index; NERD, non-erosive gastroesophageal reflux disease; ERD, erosive gastroesophageal reflux disease. *p between BMI <24 vs BMI ≥24; †p between NERD vs ERD.

### Table 5. Influence of the Presence of Erosive Gastritis on Halimeter ppb Levels according to Group

<table>
<thead>
<tr>
<th>NERD</th>
<th>ERD</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erosive gastritis (−)</td>
<td>118.94±14.16</td>
<td>214.87±32.42</td>
</tr>
<tr>
<td>Erosive gastritis (+)</td>
<td>205.44±27.74</td>
<td>144.04±25.59</td>
</tr>
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NERD, non-erosive gastroesophageal reflux disease; ERD, erosive gastroesophageal reflux disease. *p between NERD vs ERD; †p between erosive gastritis (−) vs erosive gastritis (+).
ported our hypothesis that halitosis could be another extraesophageal manifestation of ERD, discriminating ERD with NERD.

**DISCUSSION**

Only a few studies\(^ {19,20}\) about the relationship of halitosis and GERD have been reported and these a scant studies were all based on either subjective analyses of halitosis symptom or self reported questionnaire, a weak proof of previous reports to ascertain the connection between halitosis and GERD. In order to document that halitosis might be a frequent associated symptom of GERD and could be one of extraesophageal manifestations of GERD, detailed objective measurement of VSCs should be presented. In this background, our study might be the first report documenting the association between halitosis and GERD based on objective measurement of halitosis. As results, the present study convinced a strong association between the level of VSCs and ERD.

As speculation of the underlying mechanism of halitosis related to GERD, injured esophageal mucosa could produce much more \(H_2S\) than intact mucosa\(^ {21-24}\) and halitosis in GERD patients might reflect the presence of erosive changes in the affected esophagus, VSCs levels as discriminating point between NERD and ERD. The fact that no significant difference observed in VSC levels according to severity of esophageal mucosal injury defined by LA classification and minimal lesion showed highest VSC concentration leaves possible insights of \(H_2S\) role in pathogenesis of ERD like intervening of esophageal inflammation before the development of erosive lesions, sloughing of esophageal mucosa, and increased synthesis of VSCs in injured esophageal mucosa.\(^ {22-24}\)

Currently, minimal change lesion is classified as NERD because of no definite presence of erosive or ulcerative changes in esophagogastric junction,\(^ {14-16}\) but there is high possibility that minimal change lesion could be one of early ERD supported with our objective finding that minimal change lesion showed highest levels of VSCs than either NERD or ERD.

Hiatal hernia is closely associated with GERD and ERD was seen more frequently in patients with hiatal hernia than in patients without hiatal hernia.\(^ {19,20}\) Based on the fact that ERD was highly associated with halitosis, we attempted to define the relationship between hiatal hernia associated with ERD and halitosis and found that VSC concentration of ERD with hiatal hernia was higher than that of ERD without hiatal hernia (\(p=0.016\)). However, in NERD group, the influence of hiatal hernia on halitosis was not so significant compared to ERD, we inferred that hiatal hernia could induce halitosis independently regardless of erosive esophagitis. The presence of hiatal hernia is a risk factor for GERD and halitosis, but not essential contributor in causing halitosis.

Nearly all epidemiologic studies have provided an association between increasing BMI and symptoms of GERD.\(^ {25}\) A meta-analysis of 20 studies showed a positive association between increasing BMI and the presence of GERD.\(^ {26}\) Our data also revealed that BMI >24 was significantly higher in patients of ERD group than NERD. However, as far as halitosis is concerned, BMI might affect the NERD cases, never in ERD cases, suggesting that higher BMI could increase the risk of ERD development, but not halitosis symptoms. These findings drive us to search for pathogenic implication of high Halimeter ppb levels, for instances, perpetuation of esophagitis, increased vulnerability to damages, and preponderance to erosive changes, etc.

Conclusively, halitosis could be a one of several extraesophageal symptoms or manifestations of GERD including headache, chronic cough, dizziness, laryngitis, asthma, noncardiac chest pain, recurrent pneumonia, otalgia, etc.\(^ {27}\) In addition to these associations of halitosis with GERD, VSCs could impose pathogenic role in ERD, for which further detailed underlying mechanisms should be investigated. Since erosive changes in esophageal mucosa were highly associated with the levels of VSCs, halitosis might be the result of esophageal erosive lesions, as biomarker for ERD discriminating NERD and minimal change lesion might be reevaluated as early lesion of ERD rather than NERD. Moreover, patients with troublesome halitosis might get better through medicine for GERD including proton pump inhibitor or other cytoprotective medications including mucosal defense enhancing agents. However, further study will be required to draw more concrete conclusion whether improvement of halitosis will happen with the fruitful treatment of ERD.

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