Clinical assessment of oral malodor using autofluorescence of tongue coating

Eun-Song Lee, Hyun-Kyung Yim, Hyung-Suk Lee, Jong-Hoon Choi, Ji Hyun Lee, Baek-II Kim

Aim of this study was to evaluate whether a new method using quantitative light-induced fluorescence-digital (QLF-D) was appropriate for the diagnosis of oral malodor by quantifying the fluorescence of tongue coating.

Methods: This study examined 103 healthy subjects who have an oral malodor as a main complaint. The levels of oral malodor were measured by organoleptic scores (OLS) and volatile sulfur compound (VSC) levels. The fluorescent tongue coating images captured by QLF-D were quantified as the integrated fluorescence score (IF score) by multiplying the intensity and area of fluorescence. The correlations between the fluorescence parameters and OLS as well as VSC levels and the diagnostic accuracy of the IF score were evaluated.

Results: The IF score of tongue coating showed a significant positive correlation with the OLS ($r=0.54, p<0.01$) and the VSC levels ($r=0.49, p<0.01$). This score was significantly differed with the level of oral malodor ($p<0.001$), and its AUC was 0.72 in identifying the patient with definite oral malodor ($\geq$OLS 2).

Conclusions: A new method quantifying tongue coating fluorescence detected by QLF-D can be used to diagnose oral malodor and assess its severity in the clinical practice.

© 2015 Elsevier B.V. All rights reserved.
ment tool for controlling dental plaque by quantifying the degree of maturity and pathogenicity [15,16]. This device has been used for aiding diagnosis and clinical decision making of various dental diseases because it is a rapid, non-invasive, quantitative and convenient tool to assist clinicians [17]. However, no previous study has investigated the use of QLF-D to detect tongue biofilm. If this device could detect tongue coating and assess its status as red fluorescence it would enable the diagnosis of oral malodor by quantifying the fluorescence properties of tongue coating. This could facilitate a more objective and unbiased assessment of oral malodor in clinical fields. The aim of this study was to evaluate whether a new method based on the tongue coating fluorescence detected by QLF-D is appropriate and potentially useful for the clinical diagnosis of oral malodor by quantifying the fluorescence properties.

2. Materials and methods

2.1. Subjects

The cross-sectional study was performed with 103 subjects (46 males and 57 females) between the ages of 19 and 66 years (mean 39.8, standard deviation 15.1) who complained of oral malodor. All subjects were asked to sign an informed consent form and acknowledged their willingness to participate in the study. The study was performed according to the protocols and procedures approved by the Yonsei University Institutional Review Board (2-2012-0007) and followed STROBE guidelines. The exclusion criteria included subjects with systemic diseases, those who had taken antibiotics or other antimicrobial therapy within 3 months prior to the examination, pregnant women and current smokers. Subjects with an extra-oral cause of malodor such as nasal and pharyngeal infection, respiratory conditions, gastro-intestinal conditions and metabolic conditions were also excluded. All patients received instructions before the examinations. Twelve hours prior to examination, the subjects were instructed to avoid drinking coffee and alcohol and to avoid the intake of any foods that might generate oral malodor, such as garlic, onions and spicy foods. The subjects had to refrain from having breakfast and performing any oral hygiene practices such as brushing, use of oral rinses and chewing gum less than 4 h before measurements. All measurements were recorded before lunch between 10:00 am and 12:00 pm.

2.2. Organoleptic measurement

The organoleptic score was determined by two calibrated examiners who tested their ability to distinguish the severity of the oral malodor by a preliminary test to standardize their judging criteria. A specially constructed wall (90 cm × 120 cm) equipped with a Teflon tube (internal diameter, 2.9 cm; length, 10 cm) in the centre hole was placed between the subject and the examiner. For the measurement of only whole-mouth malodor, subjects were instructed to blow out from the mouth through the tube after closing their mouth and holding air for 1 min. The examiners rated the exhaled air on a 0–5 score as described before by Rogenberg et al. [18], where 0 represented absence of odour, 1 was for barely noticeable, 2 was for slight, 3 was for moderate, 4 was for strong and 5 for severe malodor. In cases of measurement disagreement, a representative score was determined by consensus. The organoleptic score was measured before all other tests to avoid any bias.

2.3. VSCs level

Volatile sulfur compounds (VSCs) concentrations in mouth air were measured using a portable gas chromatograph analyser (OralChroma™; Abilit Corporation, Kanagawa, Japan) according to the manufacturer’s instructions. The device responds to the concentrations of each 3 VSCs: hydrogen sulfide [H2S], methyl mercaptan [CH3SH], dimethyl sulfide [(CH3)2S] and provides a reading for the total VSC concentration. After each subject closed the mouth and breathed through the nose for 3 min, a disposable 1 ml plastic syringe was deeply inserted two-thirds into the nearly closed mouth. During the sampling the subjects were instructed to refrain from inhaling or exhaling from the mouth to prevent the tongue from contacting the syringe. The sample was taken, and 0.5 ml was injected into OralChroma™. All procedures were performed by a single trained examiner. Before starting the present study, this system was calibrated with standards of known concentrations of the VSCs. In addition, the resulting chromatogram was reviewed to discard the erroneous data.

2.4. Tongue assessment using QLF-D

2.4.1. QLF-D image capturing

The fluorescence images of the tongue were taken using the QLF-D system (QLF-D Biluminator™, Inspektor Research Systems BV, Amsterdam, The Netherlands) and proprietary software (C2 v1.0.0.7, Inspektor Research Systems BV) to control the camera setting conditions. Normal white-light images and sequential fluorescence images of the tongue were captured with a ‘Live-View’ enabled full-frame sensor digital SLR camera (model 550D, Canon, Tokyo, Japan) using the following settings: shutter speed of 1/45 s, aperture value of 3.2 and ISO speed of 1600 (Fig. 1). All images were taken in a darkened room maintained with the same lighting conditions to maximize the quality of the QLF-D image captured. A subsidiary cylindrical ring was equipped with the illumination tube of the QLF-D camera. This allowed the distance between light sources and the tongue to remain constant while capturing the whole tongue area. The subject was first instructed to protrude the tongue. The assistant used fingers and a sterilized gauze to pull the subject’s tongue as much as possible to expose a maximum area of the tongue and to minimize its movements before QLF-D images were captured.

2.4.2. Image analysis

The tongue fluorescence in the QLF-D images was analyzed using a computer program (Image-Pro PLUS 6.0, Media Cybernetics, Washington, USA). An area of interest (AOI) of the tongue was drawn around the boundary of the tongue from the normal white light image because the boundary was difficult to clearly identify on the fluorescence image. The AOI that had previously been saved then was imported into the fluorescence image. The red fluorescence (RF) intensity value was derived from calculating the average red/green ratio (R/G value) of every pixel within the AOI for each image. The value of the fluorescence area was obtained as a percentage (%) by calculating the ratio of the number of red fluorescent pixels to total pixels within the AOI. This approach accounted for differences in tongue size between individuals. To represent comprehensive fluorescence properties of individual tongue coating, integrated fluorescence (IF) score was calculated by multiplying the calculated value of intensity and the area of the tongue fluorescence [IF score = RF intensity (R/G value) × RF area (%)].

2.5. Statistical analysis

All variables were confirmed for normality using a Kolmogorov–Smirnov test. The differences in median values of the test results among the groups were classified according to the level of the oral malodor based on the organoleptic measurement and were examined using a Mann–Whitney U-test with the Bonferroni’s adjustment. The associations between fluorescence variables and other measurement data were analysed using the
Spearman correlation coefficients. To investigate the overall diagnostic performance of QLF-D for oral malodor, a receiver operating characteristic (ROC) analysis was employed. In this study, the organoleptic score was used as a gold standard indication for assessing the severity of oral malodor. The sensitivity and specificity values were calculated and displayed with 95% confidence intervals, and statistical significance was set at 0.05. The area under the ROC curve (AUC) for the QLF-D score was calculated to assess and compare the accuracy for detecting each diagnostic threshold. The optimal cut-off values were determined by the highest sum of sensitivity and specificity at each threshold. The data were analysed using PASW Statistics 18.0 (SPSS, IBM Corporation, Somers, NY 10589, USA) and MedCalc® (MedCalc® Software, Mariakerke, Belgium) statistical software.

3. Results

From the 103 patients enrolled, subjects with definitive oral malodor based on organoleptic evaluation (OLS 2–5) accounted for 84% of the total subjects. The percentage of subjects classified into the moderate (OLS 3) and strong (OLS 4) level of oral malodor represented 31% of the subjects and was the highest percentage among total subjects. The mean total VSC level measured with the OralChoma™ was 410 ppb (SD: 592.1). The median of VSCs level of each malodor group increased with the organoleptic scores (Fig. 2).

From the fluorescence tongue images of the participants, the fluorescence intensity and the area varied by individual with the different level of oral malodor (Fig. 1). Also, it was observed that the red intensity of the tongue varied in each part of the same tongue surface. These fluorescence properties was quantified into 3 variables (area, intensity and IF score) individually. All of the calculated variables increased with the malodor levels. Especially, the IF scores of each 5 OLS group present the same tendency (Fig. 3).

The participants were sub-divided into 3 groups based on the organoleptic score. Table 1 shows the fluorescence variables and IF scores of 3 groups, which were significantly different between the groups. Among the groups, there was a difference between the highest level group and the other groups ($p=0.003$, $p<0.001$.

Fig. 1. Examples of tongue images captured by QLF-D with white light (A–C) and blue light (a–c) according to the severity level of oral malodor determined by organoleptic score: (A)(a) OLS 1, (B)(b) OLS 3, (C)(c) OLS 5.
Table 1. Table 2 lists the correlation coefficients between the results of malodor assessments and all fluorescence variables from the tongue. All fluorescence variables were also significantly associated with organoleptic scores and total VSC levels. In all three types of VSC, the significant correlations were observed between the IF scores and H2S (r = 0.48, p < 0.001) and CH3SH (r = 0.29, p < 0.001) concentrations, while there was no significant correlation between the fluorescence scores and (CH3)2S concentrations (r = 0.08, p = 0.419) in mouth air. Both the correlations with the organoleptic score and the VSC level were higher for the fluorescence intensity than for the fluorescence area, which had the highest correlations for the IF score (Table 2).

The sensitivity, specificity and AUC for the IF scores at all diagnostic thresholds based on organoleptic assessment as a reference are presented in Table 3. Fig. 4 shows the ROC curves of IF scores for detecting patients with different levels of oral malodor to assess the ability of the IF score to discriminate between those with different levels of oral malodor. With an AUC of 0.72 (CI: 0.61–0.79, p < 0.001), the discrimination performance of the IF scores between OLS 0–1 and OLS 2–5 indicates moderate accuracy for identifying
Table 2 Correlation coefficients between fluorescence variables and oral malodor.

<table>
<thead>
<tr>
<th>OLS</th>
<th>VSC level (ppb)</th>
<th>Fluorescence variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intensity</td>
</tr>
<tr>
<td>OLS</td>
<td>0.71</td>
<td>0.47</td>
</tr>
<tr>
<td>Total VSCs (ppb)</td>
<td>0.71</td>
<td>0.39</td>
</tr>
<tr>
<td>H2S</td>
<td>0.60</td>
<td>0.79</td>
</tr>
<tr>
<td>CH3SH</td>
<td>0.38</td>
<td>0.53</td>
</tr>
<tr>
<td>(CH3)2S</td>
<td>0.32</td>
<td>0.45</td>
</tr>
</tbody>
</table>

OLS: organoleptic score; IF score: integrated fluorescence score calculated by multiplying the intensity and area of the fluorescence. Total VSCs level was measured by OralChromaTM.

Table 3 Area under the ROC curve, optimum sensitivity, specificity and cutoff for the IF score at each diagnostic threshold measured by organoleptic scores.

<table>
<thead>
<tr>
<th>Oral malodor criteria</th>
<th>AUC</th>
<th>95% CI</th>
<th>p-value</th>
<th>ROC</th>
<th>SE (%)</th>
<th>SP (%)</th>
<th>Cutoff score</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLS 0.1/2–5</td>
<td>0.72</td>
<td>0.61–0.79</td>
<td>&lt;0.0001</td>
<td>72.1</td>
<td>64.7</td>
<td>162.7</td>
<td></td>
</tr>
<tr>
<td>OLS 0–2/3–5</td>
<td>0.76</td>
<td>0.65–0.83</td>
<td>&lt;0.0001</td>
<td>64.8</td>
<td>78.1</td>
<td>188.3</td>
<td></td>
</tr>
<tr>
<td>OLS 0–3/4–5</td>
<td>0.74</td>
<td>0.65–0.82</td>
<td>&lt;0.0001</td>
<td>70.3</td>
<td>74.2</td>
<td>200.7</td>
<td></td>
</tr>
<tr>
<td>OLS 0–4/5</td>
<td>0.84</td>
<td>0.75–0.90</td>
<td>&lt;0.0001</td>
<td>85.7</td>
<td>72.9</td>
<td>217.9</td>
<td></td>
</tr>
</tbody>
</table>

AUC: area under the curve; CI: confidence interval; SE: sensitivity; SP: specificity.

the patients with definite oral malodor. The AUC scores for detecting the patients with above-moderate levels (≥ OLS 3) were higher and ranged from 0.74 to 0.84 (Table 3).

4. Discussion

The present study evaluated whether a QLF-D fluorescence detection device could diagnose oral malodor by evaluating the red autofluorescence emitted from tongue coating. We confirmed the potential of the QLF-D system to analyse the properties of tongue coating objectively and to aid diagnosis of the level of oral malodor. The novel diagnostic test using QLF-D could be used as a valid method for oral malodor by providing the cutoff score to diagnose its severity.

This study suggested an individual integrated fluorescence score for comprehensive evaluations of tongue coating status. In the previous studies, the various properties of tongue coating such as distribution area, discoloration and thickness, have been evaluated to quantify the degree of tongue coating more accurately [1,9,19,20]. According to the results of the study, we observed that the differences in the patterns of tongue fluorescence detected by QLF-D varied in different subjects and in different regions of the tongue (Fig. 1). The fluorescence intensity varied according to the different parts of the same tongue, and the fluorescence area varied between the subjects. Thus, we quantified the status of tongue coating comprehensively by calculating the intensity and the area of fluorescence emission from individual QLF-D images separately as two parameters and then calculated a combined score.

Based on the results, the IF score showed highest correlations with oral malodor among either individual fluorescence parameter (p < 0.001). This finding is in agreement with previous studies that investigated a tongue coating index based on the adhesion and thickness of the tongue plaque. These factors were highly correlated with the oral malodor (r = 0.85) [21]. A comparable correlation of 0.67 was found between the tongue coating score based on the level of thickness and coverage area and the organoleptic score. The correlation with OralChroma™ was 0.53 [22]. The current study results highlight the necessity to score both quantitative and qualitative properties of the tongue coating for an accurate diagnosis of oral malodor.

The present findings suggested that the red fluorescence emitted from the tongue coating increased in proportion to the severity of oral malodor, which corroborates the findings that the severity of oral malodor increased with increases in the degree of adhesion of the tongue coating. It can be presumed that the pathogenicity of oral malodor from the tongue coating is correlated with the production of porphyrin compounds within the tongue coating. However, the detection of red fluorescence from the tongue does not indicate the presence of oral malodor. Our results confirmed that the patients without definite oral malodor, who were determined to have an OLS 0–1 score, had fluorescent tongues. This would support the fact that the tongue coating is mainly composed of the Gram-negative species and proteolytic obligate anaerobes [23,24], which are well-known for emitting the strong red fluorescence [12,14,25]. However, we could not identify the exact mechanisms of porphyrin compounds associated with malodorous pathogenicity and production of malodorous compounds. Thus, further studies need to be conducted to identify the metabolic compounds of tongue bacteria that affect the tongue fluorescence.

With respect to the malodorous compounds, the IF scores showed significant correlations with the VSCs level detected by OralChroma™ in this study. This result would support the previous finding that tongue coating is closely associated with VSC production [8,26]. According to the previous study, the role of VSCs-producing bacteria colonizing the tongue has been implicated as a main cause for oral malodor and negative bacterial species which colonizes the tongue mainly produce both H2S and CH3SH [27–29]. Especially, the H2S concentrations had the highest correlation with the fluorescence variables and IF score among other VSCs in the present study. These results are in agreement with those of previous studies. It has been reported that Veillonella, Actinomyces and Prevotella species are the predominant H2S-producing bacteria in tongue coating and these species are responsible for the degree of oral malodor [28]. In addition, these anaerobic species shows red fluorescence during excitation with violet–blue light [12,30]. Based on these results, it is presumed that the H2S as well as total VSCs mainly produced by the metabolic activity of anaerobic bacteria is closely related with the fluorescence of tongue biofilm and the fluorescent metabolites like porphyrin. Therefore, the red fluorescence of tongue biofilm could be used as an indicator for detecting VSCs-producing bacteria.

It can be assumed that fluorescence parameters calculated in this study indicate the properties of tongue coating, which implies the fluorescent area was used as an indicator of coverage, and that the fluorescence intensity was used as an indicator of maturity of tongue coating. This finding was inferred from the previous studies that showed that the fluorescence intensity of dental biofilm increased with maturation time and its pathogenicity [15,16] and that fluorescence could be induced by its intrinsic characteristics [12]. Therefore, it is possible to assess the quantitative and qualitative properties of the tongue coating non-invasively by analysing the intensity and the area of the tongue fluorescence.

The new IF score proposed in this study shows a comparable or higher level of correlation than conventional diagnostic methods for oral malodor with the organoleptic score. According to previous findings, the correlations of organoleptic score with gas chromatography ranged from 0.28 to 0.78, and sulfide monitoring was 0.41–0.64 [31]. The correlation for salivary tests based on the detection of specific bacteria associated with their metabolites ranged from 0.27 to 0.4 [32]. Based on these findings, the IF score might be a more valid and reliable method for the clinical assessment of oral malodor compared to other diagnostic methods.

This study confirmed that the QLF-D could be used as a meaningful assessment tool for oral malodor due to its ability to focus...
on tongue biofilms as a whole rather than on a single species associated with oral malodor. Oral malodor arises from the production of metabolites by the action of total bacteria on the tongue dorsum and not from the characteristics of a specific single species [2,24]. This finding suggests that tongue coating per se, not just the specific bacteria, might be responsible for oral malodor [1,33]. In addition, it has been reported that each organism can produce different types and amounts of malodorous compounds via different metabolic mechanisms [27,34]. Also, there are many malodorous pathogens that have not been identified yet [27]. Therefore, evaluating the metabolic activity of tongue bacteria is more reliable and valid in diagnosing oral malodor than simply detecting specific causative bacteria or counting their numbers. However, there is difficulty in detecting and evaluating the dorsal 1/3 which is less accessible and partially visible to this fluorescence method, which can be a consideration for the evaluation from the digital tongue imaging systems. Because the patient’s ability to open their mouth and the access to the back of the tongue is limited, it is difficult to detect the more posterior regions of the dorsal third of the tongue within the image. To minimize this limitation, the examiner helped the tongue to be extruded as far as possible to when taking the image of the tongue. In addition, the subject with a limited mouth opening did not included in this study, and the tongue images which did not be exposed more than 2/3 the size of tongue excluded in the final analysis.

Our new diagnostic test has an acceptable level of diagnostic accuracy for determining the presence or absence of oral malodor. The discrimination ability of the suggested score to identify patients with a definite oral malodor was 72%, which is higher than its specificity (65%). When considering the features of oral malodor, these values were reasonable for diagnosing oral malodor. Because oral malodor is very prevalent in the general population, and it can be treated and prevented without painful interventions, the diagnostic test having a higher value of sensitivity than specificity might be preferred in diagnosing oral malodor. Furthermore, the level of diagnostic accuracy of the new method for classifying the severity of oral malodor was above-acceptable level. It appears that the clinicians could diagnose the level of oral malodor more easily in clinical practice by applying the cut-off scores obtained from each threshold.

Interestingly, when the higher thresholds (OLS 3–5) were used, there was an improvement in the AUC value (≥0.74). The new diagnostic method using the QLF-D system could accurately diagnose the patient with an above-moderate level of oral malodor. A possible explanation is that tongue red fluorescence detected by the QLF-D indicates mature tongue coating that has a high possibility to produce oral malodor and does not detect early stage biofilm. We presumed that fluorescent coating indicates a more mature status that is related to severe oral malodor. This finding might be supported by previous study findings demonstrating that the red fluorescence observed in mature biofilm using QLF-D had increased pathogenicity [15].

It has been shown that this system may enable the prevention and management of oral malodor. This system has an ability to visualize and score the tongue coating status, which could help patients understand their condition easily and manage the tongue hygiene. If we could monitor the fluorescence emitted from the tongue by capturing images at different visits using QLF-D in clinical practice, we might be able to assess the effect of mechanical or chemical treatments for tongue cleaning on oral malodor.

Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2013R1A1A2062505) and the Yonsei University Future-leading Research Initiative of 2014(2014-22-0171).

References


Please cite this article in press as: E.-S. Lee et al., Clinical assessment of oral malodor using autofluorescence of tongue coating, Photodiagnosis and Photodynamic Therapy (2015), http://dx.doi.org/10.1016/j.photod.2015.09.001