

# Photodynamic Therapy as Novel Treatment for Halitosis in Adolescents: A Case Series Study

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## Abstract:

**Introduction:** Halitosis is a common problem that affects a large portion of the population worldwide. The origin of this condition is oral in 90% of cases and systemic in 10% of cases. The foul odor is caused mainly by volatile sulfur compounds produced by Gram-negative bacteria. However, it has recently been found that anaerobic Gram-positive bacteria also produce hydrogen sulfide (H<sub>2</sub>S) in the presence of amino acids, such as cysteine. Light with and without the combination of chemical agents has been used to induce therapeutic and antimicrobial effects. In photodynamic therapy, the antimicrobial effect is confined to areas covered by the photosensitizing dye. The aim of the present case series study was to evaluate the antimicrobial effect of photodynamic therapy on halitosis in adolescents through the analysis of volatile sulfur compounds measured using a sulfide meter (Halimeter®).

**Methods:** Five adolescents aged 14 to 16 years were evaluated using a sulfide meter before and one hour after photodynamic therapy, which involved the use of methylene blue 0.005% on the middle third and posterior thirds of the dorsum of the tongue and nine points of laser irradiation in the red band (660 nm) with an energy dose of 9 J, power output of 100 mW and 90-seconds exposure time.

**Results:** A 31.8% reduction in the concentration of volatile sulfur compounds was found in the comparison of the initial and final readings. The statistically significant reduction ( $p = 0.0091$ ) led to an absence of halitosis following treatment (mean: 58.2 ppb).

**Conclusion:** Photodynamic therapy seems to be effective on reduction the concentration of volatile sulfur compounds. Considering the positive effects of photodynamic therapy in this case series, further studies involving microbiological analyses should be conducted to allow comparisons of the results.

**Keywords:** photodynamic therapy; laser; adolescent.

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## Introduction

Halitosis (bad breath) is a term used to define a foul, unpleasant odor that emanates from the mouth stemming from either a local or systemic origin.<sup>1-3</sup> This

common problem affects a large portion of the population worldwide and causes considerable embarrassment. Halitosis therefore has a negative impact on social communication and quality of life.<sup>4</sup> While the lack of standardization in the protocol for the diagnosis and

treatment of halitosis hinders the comparison of data from epidemiological studies carried out in different countries, it is believed that 25% of the population are affected by this condition.<sup>6</sup>

Studies on the etiology of this condition report that 2% of cases stem from renal, metabolic, hepatic, endocrinologic and gastrointestinal disorders (such as infection by *Helicobacter pylori* and intestinal blockage), 8% due to conditions of the respiratory system and conditions of the ears, nose and throat (ENT), such as acute tonsillitis, postnasal drip, sinusitis and tonsilloliths, and 80 to 90% are directly linked to conditions of the oral cavity, such as periodontal disease, coated tongue, poor oral hygiene, salivary abnormalities (change in pH and hyposialy), stomatitis, intra-oral neoplasm, pulp exposure, extraction wounds and crowding of the teeth.<sup>5-8</sup>

Bad breath is mainly caused by volatile sulfur compounds (VSCs) produced by the action of anaerobic Gram-negative bacteria (*Fusobacterium nucleatum*, *Selenomonas*, *Treponema denticola*, *Prevotella intermedia*, *Tannerella forsythensis*, *Porphyromonas gingivalis*, *Bacteroides forsythus* and *Eubacterium*) found in the oral cavity on substrates containing sulfur.<sup>9-11</sup> The VSCs produced by the metabolism of these bacteria are hydrogen sulfide (H<sub>2</sub>S), found mainly on the dorsum of the tongue, methanethiol (CH<sub>3</sub>SH) in gingival pockets and dimethyl sulfide (CH<sub>3</sub>SCH<sub>3</sub>), which has an extra-oral origin.<sup>12-15</sup> The concentration of these compounds is used as an indicator of halitosis.<sup>3,16</sup> Recently, the anaerobic Gram-positive bacterium *Solobacterium moorei* (also known as *Bulleidia moorei*) has been associated to halitosis due to the production of H<sub>2</sub>S in the presence of different supplements with amino acids, especially cysteine.<sup>17,18</sup> Studies have demonstrated that the presence of these bacteria on the dorsum of the tongue as well as in saliva and periodontal pockets can lead to both halitosis and systemic problems, such as complications during pregnancy, cardiovascular disease and chronic lower respiratory infection,<sup>19</sup> which is considered the third most common cause of death.<sup>2,20-23</sup>

## Detection

Two main methods are used to evaluate oral malodor: a subjective (organoleptic) evaluation and an objective evaluation (quantitative measure of VSC, GC gas chromatography and monitor analysis).<sup>24,25</sup> Studies comparing the efficacy of these methods report gas chromatography (GC) to be the most objective and efficacious<sup>6,15</sup> and this method is currently considered

the gold standard in the literature.<sup>11</sup> However, the majority of researchers have used a combination of both subjective and objective evaluations, whereas others have only used an organoleptic evaluation due to its ease of execution and low cost.<sup>24</sup>

## Organoleptic evaluation

For the organoleptic evaluation, a trained and calibrated rater positioned at a distance of 10 cm distinguishes the breath through the olfactory sense and a score is attributed using the 0 to 5-point Rosenberg scale<sup>3,6</sup> (0 = absence of odor; 1 = nearly undetectable odor; 2 = mild odor; 3 = moderate odor; 4 = strong odor; and 5 = extremely strong odor).

## Portable gas analysis

Mouth air can be analyzed using a sulfide monitor, such as the Halimeter (Interscan Corporation, Chatsworth, CA, USA),<sup>3,4,26,27</sup> which determines the total amount of VSCs in parts per billion (ppb) under normal conditions. According to the manufacturer, this quantity should be less than 80 ppb. However, the equipment is unable to differentiate the origin or type of VSC, is more sensitive to H<sub>2</sub>S than CH<sub>3</sub>SH and is insensitive to CH<sub>3</sub>SCH<sub>3</sub>.<sup>15,28</sup>

## Gas chromatography

GC is the most appropriate method for detecting halitosis. In 2004, a new GC denominated Oral Chroma™ (Abilit Corporation) was developed in Japan for the individual determination of H<sub>2</sub>S, CH<sub>3</sub>SH and CH<sub>3</sub>SCH<sub>3</sub>, allowing the evaluation of both the intensity of bad breath and its origin.<sup>6,11,15</sup>

## Photodynamic therapy

Photodynamic therapy (PDT) was discovered in 1900 by Oskar Raaband Hermann von Tappeiner. In the 1970s, PDT was used for the treatment of cancer. Recently, antimicrobial PDT has been used as a treatment option for localized infections.<sup>29</sup> PDT involves the use of a non-toxic light-sensitive photosensitizer combined with visible light at the appropriate wavelength to coincide with the absorption spectrum of the photosensitizer, which reaches a state of excitation after absorbing the photons, reacting with the oxygen in the medium to form reactive oxygen species (ROS). This phototoxic reaction induces the destruction of bacterial cells, but the antimicrobial

effect is confined to areas covered by the light-activated photosensitizer, quickly acting on the target organisms when the appropriate energy dose and output power are used.<sup>9,29-33</sup> According to Wainwright (1998),<sup>34</sup> bacterial resistance to PDT is unlikely, as the singlet oxygen and free radicals formed interact with different bacterial cell structures and different metabolic pathways.<sup>32,33</sup>

As a condition with a multifactor etiology but related to bacteria, especially Gram-negative bacteria, halitosis exerts a direct impact on social interactions and quality of life.<sup>6</sup> The conventional treatment of halitosis related to oral conditions consists of the chemical reduction of microorganisms with a mouthwash, such as chlorhexidine (CHX) 0.2%, essential oils, triclosan and hydrogen peroxide, the mechanical removal of nutrients with a tongue scraper or brush, the masking of odor with chewing gum, mints and breath spray and the transformation of VSC using zinc plus CHX.<sup>2,6,10,12,35-37</sup> However, the irregular characteristics of the surface of the dorsum of the tongue make the adequate reduction in bacterial load a particular challenge.<sup>2,36,38</sup> Considering the issues regarding the precise treatment of halitosis and the scarcity of studies addressing the effect of PDT on coated tongue, the aim of the present study was to evaluate the effectiveness of PDT on the dorsum of the tongue in adolescents with halitosis through an analysis of VSCs.

## Methods

This study was carried out in compliance with the norms regulating research involving human subjects and was approved by the ethics committee of the University Nove de Julho (Brazil) under process number 037315/2013. After receiving clarifications regarding the objectives and procedures, all legal guardians who agreed to the participation of their adolescent son or daughter signed a statement of informed consent in compliance with Resolution 196/96 of the Brazilian National Health Board.

Male and female adolescents enrolled at the dental clinic of the university were recruited for the study. Those aged 14 to 16 years with a diagnosis of halitosis and Halimeter results above 80 ppb during the cysteine challenge<sup>11,15,39,40</sup> were included. The following were the exclusion criteria:<sup>41</sup> dentofacial anomalies; currently undergoing orthodontic or orthopedic treatment; current use of a removable appliance, implant or dentures; periodontal disease; teeth with carious lesions; currently undergoing cancer treatment; diabetes mellitus; systemic

(gastrointestinal, renal or hepatic disorder); ENT conditions; respiratory condition; antibiotic therapy in the previous month; current pregnancy; and hypersensitivity to the photosensitizer. The recommendations of the Consolidated Standards of Reporting Trials (CONSORT) were used to ensure greater transparency and quality.

### Evaluation of halitosis

The literature describes a number of methods for measuring halitosis, such as an organoleptic evaluation of the air emanated from the oral cavity,<sup>16,26</sup> the use of a sulfide meter<sup>16,24,42</sup> and GC. Although the latter is currently considered the gold standard,<sup>11,42,43</sup> its high cost can be prohibitive. The organoleptic test can be influenced by the olfactory capacity and emotional state of the examiner as well as climatic conditions.<sup>3</sup> Thus, the portable Halimeter™ (Interscan Corporation, Chatsworth, CA, USA) was employed in the present study, which uses a sensor that is highly sensitive to the VSC to be evaluated (H<sub>2</sub>S), is inexpensive and easy to use. The readings were performed following the manufacturer's instructions (Halimeter® Instruction Manual). The participant was instructed to keep his/her mouth closed for three minutes prior to the exam. A disposable plastic tube was inserted into the mouth over the dorsum of the tongue without touching the oral or lingual mucosa. The mouth was maintained slightly open without breathing as the equipment performed the reading. The highest score during the reading was recorded. The same procedure was performed three times at three-minute intervals, resulting in three Halimeter® readings, the mean of which was calculated by the equipment itself.<sup>27</sup> An hour after the treatment the same halimeter measurement was performed. To standardize the halimetric readings, the participants were instructed to avoid the consumption of garlic, onion, strong spices and alcohol as well the use of an antiseptic mouthwash 48 hours prior to the evaluation. On the day of the evaluation, the most recent meal had to be consumed at least two hours prior and the participant was to avoid coffee, cigarettes, breath mints, chewing gum, oral hygiene product and personal products, such as perfume/cologne, aftershave lotion, deodorant, creams and tonics, and was to brush the teeth with water alone.<sup>27,44</sup>

### Photodynamic therapy

The THERAPY XT-EC® device (DMC ABC Equipamentos Médicos e Odontológicos, SP, Brazil) was used for PDT, with laser emission in the red (660 nm) and infrared (810 nm) range and the tip tapered

for dental use (diameter: 0.094 cm). A single session of PDT was held with the Chimiolux<sup>®</sup> methylene blue photosensitizer (DMC ABC Equipamentos Médicos e Odontológicos, SP, Brazil) at a concentration of 0.005% (165 μm) applied immediately after de last halimeter measurement to the middle third and posterior thirds of the dorsum of the tongue. After five minutes of pre-irradiation time for incubation, the excess was removed with an aspirator to maintain the surface moist with the photosensitizer alone (without the use of water). Before the application of the laser, the participant and researchers present put on protective eyewear and the equipment was encased in a plastic protector. Nine points were irradiated with a distance of 1 cm between points, considering the light scattering halo and effectiveness of PDT. Based on previous studies developed for the treatment of periodontal disease with PDT,<sup>45-51</sup> the device was previously calibrated to operate with a wavelength of 660 nm, energy dose of 9 J, power output of 100 mW, 90-seconds exposure time per point, fluency of 320 J/cm<sup>2</sup> and irradiance of 3537 mW/cm<sup>2</sup>. The punctual method was used in direct contact with the tongue.

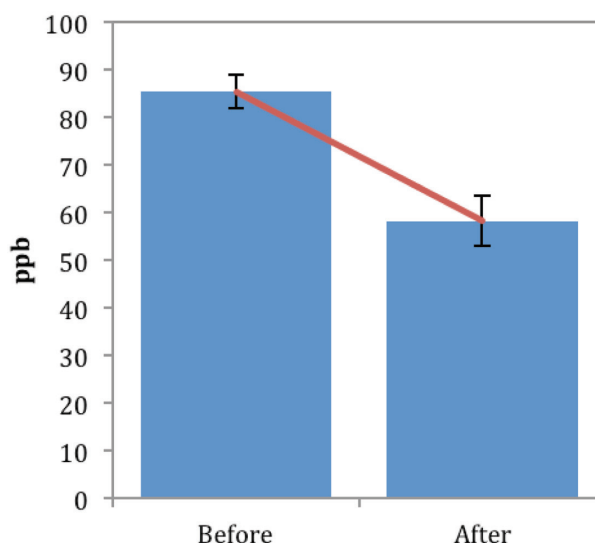
**Statistical analysis**

The data were tabulated and processed using the BioEstat 5.0 program. The Shapiro-Wilk test was used to determine the distribution of the data (normal or non-normal). The paired t-test was used for the comparisons of the evaluation times, with the level of significance set to 5% (p < 0.05).

**Results**

Five individuals were evaluated (2 males and 3 females; mean age: 15 years). Table 1 displays the descriptive statistics of the readings before and after treatment.

Since the data exhibited approximately normal distribution, the differences before and after treatment were determined using the paired t-test. Although only a pilot study with a sample size of n = 5, the test power was greater than 80%. A statistically significant difference was found in halimeter readings (Figure 1), with a mean



**Figure 1.** Mean (± SEM) halimeter measures before and after treatment

of 85.4 ppb prior to treatment and 58.2 ppb after treatment (p = 0.0091).

**Discussion**

In this study, the effectiveness of PDT for the treatment of halitosis in adolescence was evaluated through the analysis of the concentration of VSCs, measured by a sulfide monitor in a single session. PDT applied to the dorsum of the tongue eliminated bad odors by reducing the concentration of VSCs, as demonstrated by the Halimeter<sup>®</sup>, which is highly sensitive to H<sub>2</sub>S.<sup>11,37,44</sup> Despite the lack of a microbiological analysis, the bacteria in the condition of coated tongue were likely affected by PDT, as these bacteria are associated with the production of high concentrations of H<sub>2</sub>S,<sup>9-11</sup> especially in the presence of cysteine, as demonstrated in both *in vivo* and *invitro* models.<sup>11,52</sup>

The effectiveness of PDT on microorganisms has been extensively investigated using different combinations of light and photosensitizers. The degree of photodamage depends on the type and concentration of the photosensitizer, the fluence and fluence-rate of the light as well as the genera of the microorganisms.<sup>53</sup> Most microorganisms tested have proven to be susceptible to PDT and *C. albicans* requires a higher dose.<sup>54</sup> Moreover, Kormerik states that PDT is the best treatment option for localized, superficial oral infections.<sup>55</sup> Based on the present findings, one may hypothesize that PDT caused the direct elimination of pathogens that colonized the dorsum of the tongue, thereby leading to a reduction

**Table 1.** Descriptive statistics of individuals evaluated

	Pre-treatment	Post-treatment
<b>Mean</b>	<b>85.4 ppb</b>	<b>58.2 ppb</b>
Standard deviation	7.9	11.7
Standard error	3.5	5.2
Shapiro-Wilk P-value	0.8625	0.6884

in halitosis. The microorganisms were submitted to high concentrations of ROS due to irradiation of the photosensitizer. Although no evaluation was performed of the microbiological content in the sites treated, microorganisms are considered responsible for the metabolism of substrates and the production of volatile compounds in patients.

The application of punctual PDT on the tongue alone is in line with a previous study involving 2000 patients in whom coated tongue was scored based on a visual inspection: 0 = absence; 1 = 1/3 of the tongue with thin coating; 2 = more than 1/3 with thin coating or 1/3 with thick coating; and 3 = more than 1/3 with thick coating; the findings demonstrated that 43.4% of cases of halitosis stemmed from coated tongue, as demonstrated by the organoleptic test and Halimeter®, whereas 7.4% stemmed from periodontal disease and nearly 2% had an ENT cause.<sup>44</sup> Over the years, studies have demonstrated a small, long-term reduction in the amount of bacteria in coated tongue with the use of a tongue scraper with or without a concomitant mouthwash.<sup>2,56</sup> This limited reduction in bacteria is related to the irregular characteristics of the surface of the tongue,<sup>38</sup> which underscores the need for daily oral hygiene control to maintain a low level of bacterial proliferation. The penetration of light and the flow of the photosensitizing agent were not affected by the posterior papillae. Thus, PDT can achieve promising results in the treatment of halitosis, as suggested by the present study. However, it is possible that the combination of both methods would achieve the best results, as reported in studies involving PDT in conjunction with conventional periodontal treatment methods.<sup>24,49,57,58</sup>

Due to the lack of previous studies involving PDT for the treatment of coated tongue, the parameters employed in the present study were based on papers describing the treatment of periodontal disease with PDT,<sup>45,46,48-50</sup> in which the use of methylene blue and laser at wavelengths ranging from 635 to 670 nm proved successful in reducing the amount of the bacteria analyzed (*Porphyromonas gingivalis*, *Tannerella forsythensis* and *Treponema denticola*),<sup>48,49</sup> which are also found in coated tongue.

Although no microbiological analysis was performed in the present study, the reduction in VSCs was likely associated to the reduction in the amount of bacteria.<sup>11,37</sup> The ease of applicability of PDT is believed to favor the control of oral infection in adolescence, which is a period of intensive hormonal transformations that exert an influence on the gingival inflammation process, facilitating the formation of coated tongue due to the increase in the shedding of the gingival epithelial

tissue.<sup>59,60</sup> This method may also be effective in adolescents who exhibit the mouth-breathing habit, which causes changes in salivary flow and the amount of mucin, thereby favoring the formation of coated tongue and an increase in halitosis.<sup>42,61</sup> Children with postnasal drip may also benefit from this method, as a study involving individuals aged five to 14 years found a significant association between oral mal odor and postnasal drip,<sup>5</sup> which leads to direct contact between the mucus of the nasal sinuses and the dorsum of the tongue.<sup>6</sup>

Considering the easy application of the photosensitizing agent associated with the tapered tip of the laser equipment (THERAPY XT-EC®) in areas of difficult access as the posterior region of the tongue, PDT can be considered a valuable choice for treatment. However, some limitations should be addressed. The irradiation time per point caused patient discomfort and avoidance responses. Thus, the dose should be altered in further studies or a device should be manufactured to allow the single application over a larger surface. Moreover, these measures should be combined to educational counseling regarding the cleaning of the tongue.

## Conclusion

Photodynamic therapy applied to the dorsum of the tongue demonstrated positive results and could be suggested as conservative, noninvasive, fast, effective treatment for halitosis in adolescents. As a preliminary study involving only the analysis of the effect of PDT on the concentration of VSCs, the findings motivate the researchers to develop further studies for the acquisition of more detailed data on this innovating treatment for the treatment of a common problem that affects a large portion of the population.

## Conflict of Interests

The authors declare no conflicts of interest.

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