LASER - A New Tool for Management of Oral Malodour

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Abstract: Halitosis is an unpleasant odour which originates from the breath. That is caused by dental infections, periodontal infections, carious teeth, tongue coating, improper oral hygiene and non-oral causes like infections of tonsillary area, throat. LASER - Light Amplification by Stimulated Emission of Radiation is widely used in dentistry and periodontics. Lasers are recognized as an adjunctive or alternative approach in periodontal and peri-implant therapy. Recently, lasers have shown effective results in treatment of oral malodour.

Keywords: LASER-Light Amplification by Stimulated Emission of Radiation, VSCs-Light Amplification by Stimulated Emission of Radiation, PDT-Photodynamic Therapy, CCT-Chronic Caseous Tonsillitis, LCC-Laser cryosynthesis by coagulation

1. Introduction

Halitosis is an offensive or unpleasant odour of the breath the origin of which may be oral or non-oral. Oral Malodour may decrease an individual’s well-being and confidence. A newer approach for awareness and treatment of the same is required. Breath malodour is a common complaint in the general population. One in four persons has bad breath at a given time in his/her life1.

The possible causes can vary from intraoral due to dental infections, periodontal infections, carious teeth, tongue coatings &due to improper oral hygiene etc. Extra-oral causes may be various, underlying systemic diseases such as diabetes, nephropathies, infections of ear, nose, throat, tonsils, etc. Oral malodour is a cumulative result of degradation of proteins by specific oral bacteria leading to the production of volatile sulphur compounds (VSCs). Cumulative action of certain anaerobic Gram-negative bacteria (Bacteroides forsythus and Eubacterium Fusobacterium nucleatum, Treponema denticola, Salomonella, Tannerellaforislythia, Porphyromonasgingivalis, Prevotella intermedia) found in the oral cavity on substrates containing sulfur. These various VSCs are ametabolic by-product of the bacteria. Hydrogen Sulfide (H2S) is found on the dorsum of the tongue, methanethiol (CH3SH) in gingival pockets and dimethyl sulfide (CH3SCH3), which has an extra-oral origin12. Halitosis is an indicator of higher concentration of these compounds. Anaerobic Gram-positive bacterium Solobacteriummooori (also known as Bulleidiamooori) a recent addition has shown an association with halitosis as it produces H2S in the presence of different supplements containing especially cysteine & amino acids.

VSCs are capable of altering permeability of the gingival tissues inducing inflammatory responses and modulating functions of gingival fibroblasts. Volatile Sulphur...
compounds play1) a cosmetic role in social interactions; 2) a role as a facilitating agent which may accentuate the effects of other factors participating in early stages of gingivitis; and 3) a role as a pathologic agent which may contribute to the disease process directly.2

Different Diagnostic Measures

1) Organoletic Measurement:
In an organoleptic evaluation, a trained and preferably calibrated "judge" sniffs the expired air and assesses whether it is unpleasant by using an intensity rating. Scoring is normally done according to the intensity scale of Rosenberg3
0 represents the absence of odour
1 is barely noticeable odour
2 is slight malodour
3 is moderate malodour
4 is strong malodour
5 is severe malodour

2) Gas Chromatography
Gas Chromatography is performed with an apparatus that detects sulphur in mouth air which is equipped with a flame photometric detector. It is considered as a gold standard for measuring oral malodour due to its specificity to volatile sulphur compounds (VSCs).4

<table>
<thead>
<tr>
<th>Question</th>
<th>Mean ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1. Mainly mouth breathing</td>
<td>1.21 ± 1.62 (0-5)</td>
</tr>
<tr>
<td>Q2. Frequent tonsillar infections</td>
<td>1.18 ± 1.65 (0-5)</td>
</tr>
<tr>
<td>Q3. Frequent sinus infections</td>
<td>1.12 ± 1.39 (0-4)</td>
</tr>
<tr>
<td>Q4. Worrying about or self conscious about your mouth breath</td>
<td>4.42 ± 0.90 (1-5)</td>
</tr>
<tr>
<td>Q5. Miserable or tense due to halitosis</td>
<td>4.12 ± 1.17 (1-5)</td>
</tr>
<tr>
<td>Q6. Difficulty chewing or limiting certain food due to halitosis</td>
<td>1.76 ± 1.84 (0-5)</td>
</tr>
<tr>
<td>Q7. Change of taste</td>
<td>1.55 ± 1.66 (0-5)</td>
</tr>
<tr>
<td>Q8. Problems speaking (or mouth covering) due to halitosis</td>
<td>3.48 ± 1.42 (0-5)</td>
</tr>
<tr>
<td>Q9. Appearance affected due to halitosis</td>
<td>2.03 ± 1.90 (0-5)</td>
</tr>
<tr>
<td>Q10. Depressed due to mouth breath</td>
<td>2.85 ± 1.91 (0-5)</td>
</tr>
<tr>
<td>Q11. Problems concentrating due to halitosis</td>
<td>2.36 ± 1.95 (0-5)</td>
</tr>
<tr>
<td>Q12. Embarrassed due to halitosis</td>
<td>3.88 ± 1.52 (0-5)</td>
</tr>
<tr>
<td>Q13. Spending time related to halitosis?</td>
<td>4.06 ± 1.22 (0-5)</td>
</tr>
<tr>
<td>Q14. Talking from afar due to halitosis</td>
<td>3.88 ± 1.32 (1-5)</td>
</tr>
<tr>
<td>Q15. Avoid going out due to halitosis</td>
<td>2.61 ± 1.95 (0-5)</td>
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<tr>
<td>Q16. Communication problems due to halitosis</td>
<td>2.91 ± 1.72 (0-5)</td>
</tr>
<tr>
<td>Q17. Mentioned about halitosis</td>
<td>2.61 ± 1.80 (0-5)</td>
</tr>
<tr>
<td>Q18. Suffer financial loss due to halitosis</td>
<td>1.09 ± 1.42 (0-5)</td>
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<tr>
<td>Q19. Suffer social/personal loss due to halitosis</td>
<td>2.76 ± 1.82 (0-5)</td>
</tr>
<tr>
<td>Q20. Reduced life satisfaction due to halitosis</td>
<td>3.18 ± 1.67 (0-5)</td>
</tr>
</tbody>
</table>

6) Finkelstien's tonsil smelling test: Finkelstein's tonsil smelling test, is based on squeezing the tonsils and smelling the squeezed discharge. The scoring criteria are as follows:
1-Severe when bad breath is felt during conversation with the patient.
2-Moderate bad breath is felt when sniffing the air exhaled from the patient's nares or from his/her mouth, or smelling a strong fetid odor from the glove at a distance of 10 cm.
3-Mild when smelling a fetid odor bringing the glove near to the examiner's nose.

7) Tongue Colour Analysis: Developed By Roy Liezer: The tongue is isolated in each picture and uploaded to the application on an iPad-Pro. It analyses the colour, the composition of the photograph and produces the percent distribution of each colour shade. Colours are divided as shades of pink, shades of white to light tan, & shades of tan to brown.
White to light brown: Thick coating on tongue surface
Pink colour: Healthy
Salmon Pink: Healthy
Brown Colour: Foul

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Tongue Colour Analysis for Medical Application:
The human tongue has various features that can be used to diagnose disease, with colour features being the most important. Presence of coatings on the tongue may lead to colour changes which indicate presence of certain diseases. Given a tongue image the tongue colour analysis system is able to first distinguish Healthy versus Disease with an average accuracy of 91.99%. If the image is from Disease it is further assigned to one of three clusters. From these clusters 11 illnesses can be successfully classified given a classification rate of at least 70%. The proposed method uses a special capture device with image correction and extracts a tongue colour feature vector from each image. This vector consists of 12 colour ratios calculated with the tongue colour gamut to better characterize each foreground tongue pixel.\(^\text{16}\)

![Tongue Colour Analysis for Medical Application](image)

**Figure 1**: Three typical Healthy (a) and (b) Disease (b) samples (Courtesy Bob Zhang et. Al Hindawi Publishing Corporation Volume 12 Article ID 264742)

Treatment Modalities
Treatment modalities for Halitosis may vary depending on the cause. The following general treatment strategies can be applied.

1) Mechanical Reduction: Mechanical cleansing can be emphasised as there is extensive accumulation of bacteria on the dorsum of the tongue. Tongue cleaning can be done with a normal toothbrush, but preferably with a tongue scraper if a coating is present. Tongue cleaning using a tongue scraper reduces halitosis levels\(^\text{1}\)

2) Chemical Reduction of Microbial load: Along with tooth brushing mouth rinsing has become a common oral hygiene practice. Antimicrobial and oxidizing agents, are added to formulations of mouthwashes which impacts the process of oral malodour formation. The active ingredients are antimicrobial agents such as chlorhexidine, cetlypyridinium chloride (CPC), essential oils, chlorine dioxide, triclosan, amine fluoride and stannous fluoride, hydrogen peroxide, and baking soda. Some of these agents have only a temporary effect on the total number of microorganisms in the oral cavity. Chewing gums can be formulated with antibacterial agents, such as fluoride or chlorhexidine, helping to reduce oral malodour through both mechanical and chemical approaches\(^\text{1}\).

3) Masking the Malodour: Treatment with rinses, mouth sprays, or lozenges containing volatiles with a pleasant odour has only a short-term effect. Typical examples are mint-containing lozenges and the aroma of rinses without antibacterial components.\(^\text{1}\)

4) Laser as new tool for management for Malodour:

LASER - Light Amplification by Stimulated Emission of Radiationis widely used in dentistry and periodontics. Lasers are recognized as an adjunctive or alternative approach in periodontal and peri-implant therapy. The various characteristics of lasers, such as ablation, hemostasis, bactericidal and detoxification effect, as well as promotion of tissue regeneration and wound healing make it possible to treat soft and hard tissues in a manner that surpasses the scope of conventional techniques. Use of lasers ensures patient safety without producing irreversible changes and less post- operative pain and fewer complications. There is substantial data on successful laser applications in periodontal and peri-implant diseases. Different lasers have been especially useful for periodontal debridement, ablation, vaporization, hemostasis, and disinfection effects as well as biological effects, such as photobiomodulation.\(^\text{14}\)
Types of Lasers

1) **Hard Tissue Lasers**
   Er:Cr:YSGG(2790nm)

2) **Soft Tissue Lasers**
   - Diode laser (810-1064nm)
   - CO2 laser (10,600nm)
   - Nd:YAG (1064nm)

**Diode Laser** (810-1064nm)

Mechanism of Action

The active medium of the diode laser is a solid-state semiconductor made of aluminum, gallium, arsenide, and occasionally indium, which produces laser wavelengths, ranging from approximately 810 nm to 980 nm. All diode wavelengths are absorbed primarily by tissue pigment (melanin) and hemoglobin.\(^{14}\)

A study conducted by Mükerrem HATIPOĞLU et al in 2017 aimed at the efficiency of diode laser for periodontal pocket irradiation as an adjunct to conventional scaling and root planning (SRP) on periodontal parameters and halitosis. SRP was achieved via hand pieces (Gracey Curettes, Hu-Friedy, Chicago, IL, USA) and ultrasonic equipment (EMS SA CH 1260 Nyon, SWITZERLAND). 940 nm indium-gallium-aluminum-phosphate diode lasers (Epic, Biolase, Irvine, CA, USA) were implemented in the same session under local anesthesia. Total 15 J/cm² power of laser in 1.5 W power with 20 ms frequency during 20 ms shots was implemented to periodontal pocket. Laser irradiation was released with fiber optic ends which are of 300 µm diameter. Fiber was implemented by parallelly locating on root surface level inside the periodontal pocket. Fibertip was directed from apical to coronal during light emission. It was implemented in total 20 seconds as 10 second 0 seconds to each tooth in mesiodistal direction in buccal angle.\(^{4}\)

Periodontal pathogens generate endotoxins, proteinase and VSCs.

Persson and colleagues stated that Bacteroides melaninogenicus, Treponema denticola, Porphyromonas gingivalis and Prevotella intermedia types produce VSC.\(^{15}\) Diode lasers act on pigmented chromophores mainly P.gingivalis causing a decrease in VSC production which in turns shows reduction of halitosis.

While measuring halitosis, it was discovered that both treatment methods decreased bad smell at 1st, 3rd and 6th month’s periods. The halitosis measurements were done by Halimeter (InterscanCorp., Chatsworth, Ca, USA)

Erbium family of Lasers

Er:Cr:YSGG (2790nm)

**Mechanism of Action:**
The erbiun ‘family’ of lasers has two distinct wavelengths, Er,Cr: YSGG (yttrium scandium gallium garnet) lasers and Er: YAG (yttrium aluminium garnet) lasers. More often it is the laser of choice for treatment of dental hard tissues. In addition to hard tissue procedures, erbium lasers can also be used for soft tissue ablation, because the dental soft tissue contains a high percentage of water. The adjunctive use of Er,Cr:YSGG laser to conventional periodontal therapy has reported to be more effective in bacterial reduction when compared to conventional periodontal therapy. In addition the Er,Cr:YSGG lasers are also successful in coagulating the opened blood vessels and de-epithelizing the gingival pocket.

A randomised controlled trial conducted by Krespi et al in the year aimed to investigate the potential role of the Er,Cr:YSGG solid state laser to treathalitosis specifically by targeting the tongue. As tongue colour is potentially related to some diseased states and to bacterial colonization, it aimed to explore the validity of tongue colour analysis as predictor of treatment.

Treatment of the control group was by a tongue scraper. Laser treatment included: a single 10-minute treatment with the Waterlase Express (Biolase, Irvine, CA, USA). The dorso-posterior surface of the tongue was treated with the laser in 10 passes of 6 s each with 10 s of rest in between. Laser settings were 60 µs pulse width, 4 W, 40 Hz, 10% air and 5% water irrigation. An MC12 sapphire laser tip (Biolase, Irvine, CA) held 3 mm away from the tongue in a constant sweeping motion during treatment with overlapping passes in alternate direction, side to side motion and front to back motion with laser fluence on the tongue surface calculated at 3 J/cm². The settings were non-thermal& non-ablative. The Er, Cr: YSGG solid state laser with pulsed water was chosen for targeting oral biofilms specifically as it has been shown to be effective in biofilm reduction. As the laser is absorbed by water the successful removal of biofilm was due to a dual action laser and water in couple action in a continuous flow. The efficacy of Er,Cr:YSGG laser particularly showed changes of certain bacteria of oral biofilm such as *Enterococcus faecalis* and *Candida albicans* than other lasers such as Nd:YAG laser. The Er, Cr: YSGG laser-activated irrigation was shown to be effective in increasing the bactericidal efficiency of 0.5% NaOCl against oral *E. faecalis* biofilm as well.\(^{5}\)
Ömür Dereci et al. conducted a study with Er, Cr: YSGG laser and evaluated the efficacy of Er, Cr: YSGG laser assisted periodontal therapy on the reduction of oral malodor and periodontal disease.

The study results were suggestive that Er, Cr: YSGG laser assisted periodontal therapy was more effective in controlling periodontal disease related oral malodor compared to conventional periodontal therapy.

A Waterlase MD Er, Cr: YSGG laser (Biolase, Irvine, California, USA) with a RFPT 5-14 360° firing tip was used in the study. The firing tip of the laser was used with an angulation of 10° to the root surface and a bottom-up technique in which the laser was applied in a bottom to upward direction with circulation movements in contact with the pocket. Only inside of the pockets were irradiated and each pocket was irradiated once per session. The laser settings were 1.5 W, 30 Hz pulse rate, 11% air, 20% H2O and H-Mode (Pulse Duration: 140 us). The laser irradiation was applied three times over a period of 7 days under local anesthesia (40 mg/ml articaine hydrochloride and 0.006 mg adrenaline hydrochloride).

In the above study, there was a dramatic decrease in the VSC levels of patients in the Er, Cr: YSGG laser group as compared to conventional periodontal therapy.

Also a significant decrease in BOP and halitosis was observed in 3rd and 6th months in patients treated with Er, Cr: YSGG assisted conventional periodontal therapy. The significant shift in these parameters indicates the assumption of the reduction in periodontal disease related halitosis may be associated with the reduction of BOP. Bacterial colonies residing in the periodontal pocket are responsible for production of VSCs. Thus, reduction in halitosis may be explained by the elimination of the VSC producing bacteria in the periodontal pocket. However, a more definite examination and a separate study protocol are needed to expose the true relationship between BOP and halitosis.

Mechanism of Action:
The Nd: YAG wavelength is highly absorbed by the pigmented tissue, making it a very effective surgical laser for cutting and coagulating dental soft tissues, with good hemostasis. In addition to its surgical applications, there has been research on using the Nd: YAG laser for nonsurgical sulcular debridement in periodontal disease control. Depending upon the energy level, efficacy of wavelength absorption by target tissues, surface temperatures and various other parameters, lasers have the ability to either stimulate or inhibit the growth of bacteria or to be bactericidal. Due to this bactericidal potential, manufacturers and researchers suggested that lasers could be used to reduce the microbial population of periodontal pockets and thereby used in an adjunctive capacity during periodontal therapy.

The study investigated that whether treating periodontitis with non-surgical periodontal treatment procedures and laser applications can decrease oral malodour. The aim of this study was to evaluate that Nd: YAG laser irradiation (because of the bactericidal potential) would reduce the rate of VSC levels on the treatment of oral malodour associated with chronic periodontitis. A third aim was to compare the effectiveness of the different therapies for the treatment of oral malodour. (Cankat Kara et al. 2008 International Dental Journal).

An Nd: YAG laser (Smarty A10; DEKA, Firenze, Italy: free-running pulsed wave laser with a wavelength of 1064nm) was selected for laser irradiation. The standardised settings were power output (2W), energy (100mJ), 90s and frequency (20Hz). Treatment was performed under local anaesthesia. The delivery contact optic fibre was continuously moved back and forth to cover all surfaces in each periodontal pocket. Use of black ink as an absorption enhancer is recommended when using Nd: YAG laser irradiation, in order to prevent deep penetration of the Nd: YAG laser beam. For this purpose povidone-iodine was used in the periodontal pockets under Nd: YAG laser irradiation to partly absorb the laser beam.

The results confirmed that there was significant reduction in halitosis after treating chronic periodontitis. The data indicated a possible use of Nd: YAG lasers adjunctive to periodontal therapy and treatment of oral malodour. Volatile

Neodymium-Doped Yttrium Aluminium Garnet (1064nm)
sulphides were measured using a Halimeter (Interscan, Chatsworth, CA, USA).  

Carbon Dioxide Laser (10,600nm)

**Mechanism of Action**

The CO2 laser wavelength has a very high affinity for water, resulting in rapid soft tissue removal and hemostasis with a very shallow depth of penetration. Although it possesses the highest absorbance of any laser, disadvantages of the CO2 laser are its relatively larger size and high cost and hard tissue destructive interactions.

The study evaluated the impact of CO2 laser cryptolysis by coagulation (LCC) treatment in the reduction of volatile sulphur compounds (VSCs) halitometry in patients with chronic caseous tonsillitis (CCT). (ANA.C. et al 2006 Journal of Photomedicine and Laser Surgery). CCT is characterised by caesum retention and halitosis. Tonsillectomy was indicated in case of failure of clinical treatment. Recently, a conservative new treatment, CO2 Laser cryptolysis by coagulation, has been introduced. Since the procedure is painless and opens the crypt ostium, therefore decreases case um retention.

LCC procedure was painless and well tolerated by the patients. On an average complete treatment requires 4-6 appointments, with an interval of 4 weeks between laser sessions. The coagulation is achieved with moderate CO2 laser power density, reaching temperatures in the tonsillar tissue of 50–100°C. As a result, there was dehydration, whitening, and contraction of the tissue due to protein and collagen denaturation. This technique is more conservative than conventional one using the CO2 laser, there is only superficial interaction in the epithelial layer.

**Laser cryptolysis technique**

The CO2 laser used in this study was Sharplan 40C with nominal power of 40 W (CO2 Surgical Lasers System, Sharplan Lasers, Inc., Israel) attached to an articulated arm, hand piece, and a scanner accessory (Swiftlase). A frontal light beam to illuminate the oropharynx along with a smoke aspirator with biological filter, protection glasses for the patient and medicalteam were also used. The coagulation technique with CO2 laser, consisted of applications of 6 W of continuous wave(CW) laser power, in scanned and unfocused mode, over a mean area of 2.2 mm2, initially only around the crypts and following the shape of their openings. The laser fluence applied around the crypt was about 54.5 joules/cm2. At a subsequent time, the laser beam was swept over the entire tonsillar surface with a fluence of about 18 joules/cm2. It is important that the handpiece should not touch the tonsils and be lined up with the labial commissura. This procedure was repeated every 4 weeks, 4 sessions in total for each patient.

The laser was swept over the tonsilar surface at an estimated speed of 15 mm/sec, resulting in a fluence of about 18 joules/cm2. The patients were advised not to eat acidic food, spices, seasonings, and to avoid crisp or hard food during the 2 first days after the procedure.

CCT has no gender predominance. CCT can affect any age group; however, teenagers and young adults with CCT seem to be more affected by halitosis. In a selected group of patients with CCT and halitosis complaint, to avoid tonsillectomy a new conservative method of CO2 LCC was performed. This treatment was well tolerated, and no side effects were noted. All patients experienced an improvement of halitosis and other CCT-related symptoms.

The VSC halitometry technique followed the instruction manual for the Halimeter® (Halimeter RH-17 Series, Interscan Corp., Chatsworth, CA) to measure halitosis. All patients tolerated LCC very well, and showed improvement in halitosis after treatment of LCC. VSC reduction was seen by 30.1%, and caesium retention was significantly decreased in this group.

**Use of Low Level Laser Therapy such as Photodynamic Laser Therapy**

Photodynamic therapy (PDT) was discovered in 1900 by Oskar Raab and Hermann von Tappeiner.

**Mechanism of Action:**

PDT uses a photosensitizer that is non-toxic light-sensitive, combined with visible light at the appropriate wavelength which coincides with the absorption spectrum of the photosensitizer, that reaches a state of excitation after absorbing the photons, and reacts with the oxygen in the medium and forms reactive oxygen species. There is destruction of bacterial cells due to this phototoxic reaction induces. This antimicrobial effect is confined to areas which are covered by light-activated photosensitizer, that acts quickly on the target organisms when the appropriate energy dose and output power are used. According to Wainwright bacterial resistance to PDT is unlikely, as the singlet oxygen and free radicals formed interact with different bacterial cell structures and different metabolic pathways.

Considering the scarcity of studies addressing the effect of PDT on tongue biofilm, the aim of the study was to evaluate the effect of PDT on the dorsal of the tongue in adolescents with halitosis by an analysis of VSCs and microbiological analysis of the tongue.

**Photodynamic Therapy**

The THERAPY XT-ESTM (DMC ABC Medical and Dental Equipment, São Paulo, Brazil) with a red (660 nm) and infrared (810 nm) laser and a fine tip (for regions of difficult access) was be used. A single session of PDT was performed with the Chimiolux™ methylene blue photosensitizer (DMC ABC Medical and Dental Equipment, São Paulo, Brazil) at a concentration of 0.005% (165 μm) applied to the middle and posterior thirds of the dorsal 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). A total of six points were irradiated. The device was calibrated with a wavelength of 660 nm, power output of 100 mW, fluency of 320 J/cm2, irradiance of 3537 mW/cm2 and an energy dose of 9 joules for 90 seconds per point. The
punctual application method was used with the conventional tip in contact with the tongue.\textsuperscript{12} 

The main objective of the proposed study was to evaluate the effect of PDT with and without the use of a tongue scraper for the treatment of halitosis in adolescents. The findings were expected to provide convincing evidence that PDT was more effective for the treatment of halitosis. As the penetration of light and spreading of the photosensitizer did not seem to be affected by the posterior papillae of the tongue, treatment with PDT is promising and may achieve satisfactory results, especially when combined with conventional treatment.

The portable Oral Chroma\textsuperscript{TM} device Abilit Corporation, Chuo-ku, Osaka - Japan) device was used for measurement of halitosis as it is a highly sensitive gas semiconductor sensor.

\textbf{Figure 3:} Points of photodynamic therapy application (Courtesy Lopes et al. Trials 2014, 15:443 BMC Journal)

2. Conclusion

Halitosis is a multifactorial condition therefore the treatment varies individually. Laser tongue debridement along with routine treatment with a regular follow up will help patients to eliminate halitosis and lead a healthy and better life. It is known that non-surgical approach for reduction of microbial load of periodontal pathogens with the help of laser disinfection has achieved successful results. Same mechanism may be applied for treatment of halitosis and better treatment outcomes can be achieved. Studies have shown to prove that Laser Tongue Debridement has been effective in 80% in the initial treatment and 70% had effective results within 1month of treatment and 2-3% patients showed no positive results.

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