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Original Article

Comparison of Clinical Indices With Halitosis Grading in Chronic Periodontitis: A Randomized Control Trial

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Abstract

Oral malodor is a major periodontal complaint, but the best method for assessing the halitosis grade is still undefined. The primary objective of the study was to detect the halitosis grade in the exhaled breath using the three distinct techniques and to compare the readings with different clinical indices to find out the best method of halitosis grading. A total of 90 patients with chronic periodontitis having oral malodor were included in the study. The subjective assessment of the exhaled breath (halitosis grading) was done by three different methods; using a handheld portable Tanita FitScan sulfide monitor, by Halitox toxin assay, and by organoleptic (Sniff test) method. The findings were then compared with the clinical parameters of poor oral hygiene like plaque index (PI), gingival index (GI), gingival bleeding index (BI), and pocket depth (PD) to detect the best method of halitosis grading. The mean age of the patients included was 38.23 ± 8.83 (mean ± standard deviation) years. The median value of halitosis grading as obtained by Tanita FitScan was 3.0 (95% confidence interval as 2 and 4) which was then compared with clinical indices (PI, GI, BI, and PD) and the results were statistically significant ($P < 0.05$), whereas the other two techniques of halitosis grading gave insignificant results. The results confirmed that the halitosis grading done using Tanita FitScan sulfide monitor is more appropriate with respect to clinical indices when compared with the other two techniques.

Key words: Anaerobic microflora, Chronic periodontitis, Halitosis, Handheld monitor

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Introduction

Oral malodor or bad breath is a commonly experienced problem among the general population. Apart from periodontal diseases and dental caries, oral malodor is the third most common reason for patients to visit a dentist [1, 2]. The common causes of halitosis are periodontal diseases, diminished salivary flow, overhanging restorations, dentures, and colonization of the tongue by microbes [3, 4]. The density of Gram-negative anaerobes present in the subgingival plaque is directly proportional to the amount of sulfur-containing volatile compounds produced resulting in halitosis [5]. The association of halitosis with periodontitis can be confirmed by microbiological analysis of the organisms responsible for the production of volatile sulfur compounds (VSC) and toxins. Oral malodor originates when the breath emerging from the mouth is mixed

with the malodorous compounds present in the oral cavity. These sulfur-containing volatile compounds are produced either from the fermentation of peptides or mucins by the microorganisms present in the saliva. The most common sulfur compounds which are responsible for halitosis are dimethyl sulfide, methyl mercaptan, and hydrogen sulfide which are toxic to the tissues. The thiol-containing agents have the potential to interact with both cellular and ground matrix components of the soft tissue causing increased permeability of ions and bacterial endotoxins [6]. A Halimeter is a type of breath analyzer for halitosis by detecting the level of sulfide gases in the exhaled breath, it was first introduced in the year 1991. The colorimetric analysis can be made by measuring the amount of toxins produced by these organisms, using the reagents like Halitox (Altcorp. Ltd., USA) which serves as a rapid home care tool. Apart from the requirement of elaborate and sophisticated laboratory facilities to detect halitosis, the culture of the plaque and the exudate of the periodontal pocket is the "gold standard" for the detection of microorganisms responsible for halitosis in patients with periodontitis [7, 8]. To the best of our knowledge, the comparison of halitosis grading obtained by halitosis-linked toxins, organoleptically or by Tanita breath analyzer with the clinical parameters of oral malodor have not been linked together to date. Hence, an open-labeled randomized control trial was conducted to evaluate the halitosis grading by different techniques and to compare it with the clinical indices (plaque index [PI], gingival index [GI], bleeding index [BI], and pocket depth [PD]) and with the microbial cultures of the subgingival plaque to find out the best method of halitosis grading in chronic periodontitis patients.

Materials and Methods

After receiving the ethical approval from the Institutional Review Board (VDCH/IEC/15/2018 dated: November 23, 2018) and taking informed consent from the patients, an open-label randomized control trial was planned. The patients were randomized by using a sequentially numbered opaque sealed envelope technique. The study involved a total of 96 patients of either sex suffering from halitosis due to chronic periodontitis having bone loss as seen radiographically with a probing depth of 5–7 mm. The pregnant or lactating females, patients having a systemic illness or on antibiotics or having undergone any periodontal therapy in the past 6 months were excluded from the study.

The evaluation of halitosis grading was done by the organoleptic method (Sniff test), in which the patients were asked to close the mouth and nose simultaneously with their hand, then to exhale out gently by opening the mouth and the malodor was assessed by the clinician [9, 10].

The Halitox toxin assay was done by taking the tongue sample using a sterile cotton-tipped applicator which was immediately dipped in the reaction tubes [11]. The screw cap was then sealed and the reaction was allowed to proceed. After 2 min, the sample tube was held against a color chart printed on the back of the Halitox package. The color chart contains three color scales as follows: Clear-nontoxic, mild yellow–moderate toxin, and bright yellow-high toxin.

The VSC and hydrocarbons in the exhaled breath were detected using the Tanita FitScan breath checker (Tanita Corp., Japan). Patients were asked to keep their mouths closed for 3 min before testing while breathing through the nose. Then, the patients were asked to exhale from the mouth keeping the Tanita breath analyzer close to the mouth for 30 s. The procedure was repeated in three trials for each subject and the mean value was calculated. The readings on the monitor were recorded on a four-point scale of halitosis grading: no odor as 1, slight odor as 2, moderate odor as 3, and strong odor as 4. If no number appeared on the monitor, then it was considered a reading error and the procedure was repeated. After examining every patient, the air opening was cleaned with a dry cloth and the unit was waved gently 4–5 times in the air to remove any odors or moisture left in the unit.

Samples for anaerobic culture (gold standard technique for halitosis) were taken from the infected pockets by means of a sterile curette which was sealed tightly into an Eppendorf containing thioglycolate broth (4 ml) and a transport medium [12, 13]. The samples were sent to the department of microbiology for anaerobic culture to identify the microbial colonies present in the periodontal pockets using culture media such as blood agar, Brewer Anaerobic Agar, and Bacteroides Bile Esculin Agar. The sample was incubated for 72 h at 37°C, and the samples from each colony were taken. The colonies were identified using Gram's stain.

The probing PD was measured with a UNC-15 periodontal probe [14]. The probe was inserted parallel to the long axis of the tooth gently, till resistance was noted and readings were recorded to the nearest millimeters.

The PI was calculated using Silness and Loe scale [15]. The score of zero was given when no plaque was seen. A score of one was given when a film of plaque adhered to the gingival margin or to adjacent areas of the tooth. A score of two on the moderate accumulation of soft deposits within the gingival pocket which can be seen by the naked eyes. When there was an abundance of soft matter seen within the gingival pockets, a score of three was given.
The GI was calculated using Loe and Silness scoring method [15]. A periodontal probe was used to assess the bleeding potential of the tissues. The normal gingiva was rated as a score zero. The gingiva with mild inflammation but with no bleeding on probing (BOP) was scored as one. The gingiva with moderate inflammation with redness, edema, and BOP was scored as two and the gingiva with severe inflammation, marked redness, edema, and ulceration with a tendency for spontaneous bleeding was graded as three.
The gingival BI was measured using Ainamo and Bay scoring system [15]. The presence or absence of gingival bleeding was determined by gentle probing of the gingival crevice with a periodontal probe. The appearance of the bleeding within 10 s indicated a positive score, which was expressed as a percentage of the total number of gingival margins examined.

Statistical analysis
The descriptive data that included mean, standard deviation (SD), and percentage frequency were estimated for each category of halitosis grading. For all tests, a $P < 0.05$ was considered to be statistically significant.

Results and Discussion

A total of 96 patients of either sex suffering from halitosis due to chronic periodontitis were screened for eligibility for 1 year, out of which six patients refused to give consent for the study (CONSORT statement), **(Figure 1)**. Out of 90 patients included in the study 55 were male and 42 were female with a male-to-female ratio of 1.3:1. The mean age of the patients' was 38.23 ± 8.83 (mean ± SD) years.

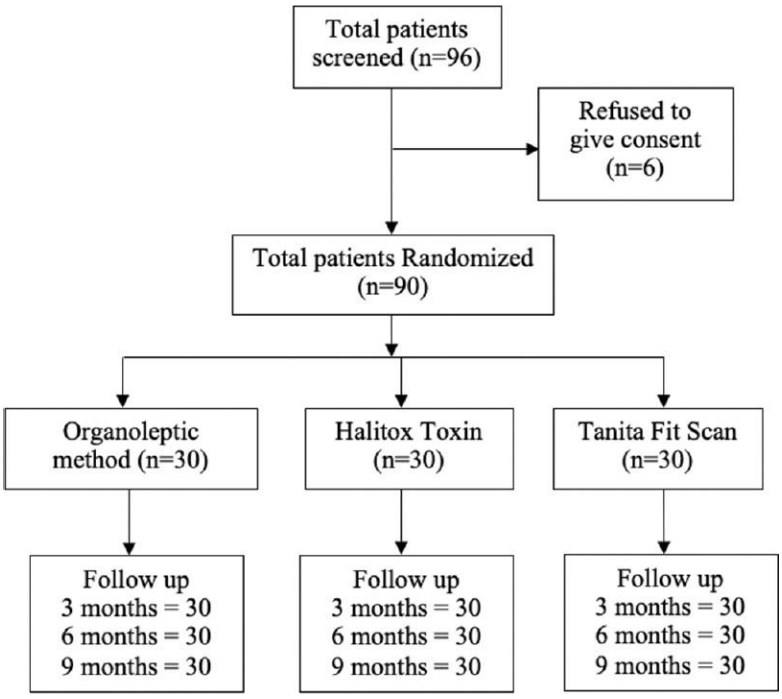

Figure 1. Consolidated Standards of Reporting Trials.*n* – number of patients

The organoleptic assessment of halitosis grading showed that out of 90 patients in the study 36 (40%) had mild malodor (grade 2), 42 (46.7%) had moderate grade malodor (grade 3), and 12 (13.3%) patients had severe oral malodor (grade 4). The

clinical parameters such as PI, GI, and gingival BI, i.e. BOP and PD were obtained from all the quadrants of the mouth and it was correlated with halitosis grading obtained by organoleptic method. The mean values were compared using the ANOVA test **(Table 1)**, and the results were not statistically significant ($P > 0.05$), which showed less reliability of organoleptic method for halitosis grading.

Table 1. Comparison of organoleptic halitosis grading with plaque index, gingival bleeding index %, bleeding on probing, and pocket depth

Organoleptic halitosis grading	Value of the clinical indices (mean±SD)	95% CI for mean		*P*
		Lower bound	Upper bound	
PI				
2.0	1.39±0.23	1.2468	1.5482	0.581
3.0	1.49±0.24	1.3490	1.6281	
4.0	1.50±0.25	1.1009	1.9041	
GI (%)				
2.0	1.91±0.29	1.7285	2.0931	0.244
3.0	1.81±0.21	1.6943	1.9343	
4.0	2.06±0.36	1.4912	2.6338	
Bleeding on probing (%)				
2.0	73.92±14.4	64.747	83.086	0.269
3.0	76.50±10.18	70.622	82.378	
4.0	85.75±12.66	65.607	105.893	
PD (mm)				
2.0	5.73±0.34	5.508	5.942	0.763
3.0	5.77±0.28	5.611	5.932	
4.0	5.85±0.19	5.545	6.155	

P value is nonsignificant as it is more than 0.05. PD – Pocket depth; GI – Gingival bleeding index; PI – Plaque index; SD – Standard deviation; CI – Confidence interval

The Halitox toxin assessment was done in patients with different grades of halitosis. All the patients having halitosis showed medium to high scores of Halitox toxin, with 36.6% of patients with grade 3 halitosis showing medium scores of Halitox toxin but the results were statistically not significant ($P = 0.518$); **(Table 2 and Figure 2)**.

Table 2. Halitosis grading compared with Halitox toxin scores (low scores, medium scores, and high scores; P<0.05 considered as statistically significant)

Halitosis grading	Halitox Toxin-based low score, n (%)	Halitox Toxin-based medium score, n (%)	Halitox Toxin-based high score, n (%)	*P*
2	0	7 (23.3)	5 (16.6)	0.518
3	0	11 (36.6)	3 (10)	
4	0	3 (10)	1 (3.33)	

$P = 0.58$ ($P>0.05$, non significant), *n* – score in each group

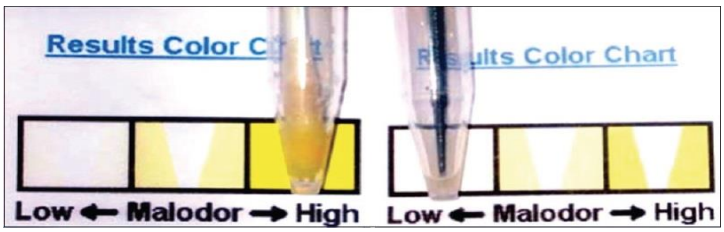

Figure 2. The Halitox toxin assessment with different grades of halitosis

The values of Tanita breath analyzer readings were then compared with PI, GI, PD, and the percentage of bleeding of probing. The results were represented as median and it was statistically significant ($P < 0.05$), **(Table 3 and Figures 3a and 3b)**, indicating the Tanita FitScan a reliable method for halitosis grading in patients with chronic periodontitis.

Table 3. Comparison of halitosis grading by Tanita FitScan breath analyzer with plaque index, gingival index, pocket depth, and the percentage of bleeding

Clinical indices	Mean±SD	Halitosis grading by Tanita FitScan breath analyzer (median)	*P*
PI	1.45±0.23	3.0 (95% CI 2 and 4)	0.004
GI	1.88±0.26		0.0001
PD (mm)	5.76±0.29		0.0001
Percentage of bleeding	76.70±2.51		0.0001

P< 0.05 considered as statistically significant (*P* – probability). SD – Standard deviation; CI – Confidence interval; PD – Pocket depth; GI – Gingival bleeding index; PI – Plaque index

a) b)

Figure 3. a) Patient exhaling out keeping the Tanita breath analyzer close to mouth; b) The Tanita FitScan breath analyzer readings

The cultures were sent from the periodontal pockets **(Figure 4)**. The culture report showed the growth of streptococcal colonies and fusobacterium in a few cases but the growth of *Porphyromonas gingivalis* was seen in 26.6% cases of grade 4 halitosis and *Prevotella* was seen in 33.3% of cases of grade 4 halitosis **(Table 4)**, indicating strong association of bacterial overgrowth with halitosis.

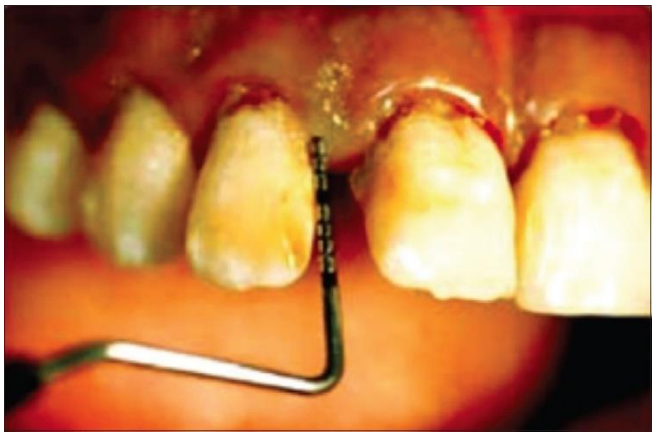

Figure 4. The periodontal pocket from which the cultures were sent

Table 4. Growth of different microorganisms in culture as per different halitosis grading

Halitosis grading	Streptococcus, n (%)	Fusobacterium, n (%)	Porphyromonas gingivalis, n (%)	Prevotella, n (%)	Bacteroides, n (%)
2	4 (13.3)	4 (13.3)	6 (20)	2 (6.66)	5 (16.6)
3	8 (26.6)	12 (40)	4 (13.3)	3 (10)	2 (23.3)
4	18 (60)	14 (46.6)	8 (26.6)	10 (33.3)	1 (3.33)

n – N is the number of colonies grown

Detection of oral malodor is a widespread problem which lacks scientific investigation into its cause and treatment. Early scientific research estimated the effect of oral microbial flora and the hygiene status of the mouth, nose, and sinuses on the production of oral malodor. The detection and quantification of oral malodor can be assessed by different ways such as organoleptic method with single and multiple judges, gas chromatography, portable industrial sulfide monitors, and by small handheld portable monitors [16, 17].

Kapoor *et al*. studied the current concept for diagnosis and management of halitosis, in which nasal sniffing is a commonly used approach to directly sample the expelled mouth air [5]. They defined the organoleptic assessment as the "gold standard" to diagnose halitosis in clinical settings as it was inexpensive, no equipment needed, and a wide range of odors can be detected by a clinician [18, 19]. However, such organoleptic measurement raised the problems such as considerable variation between clinicians on the ranking of the same sample [20, 21]. One major difficulty with this method is that once a subject has expelled the breath from his mouth for estimation by one clinician, the halitus emitted subsequently for the other clinicians may differ in intensity and composition. Our findings differ from Kapoor *et al*., as the newly designed equipment like the Tanita FitScan breath analyzer gives comparable readings every time. Thus, organoleptic method is no longer considered a Gold standard method of halitosis grading.

Alasqah *et al*. did a cross-sectional observational study to compare the different two diagnostic techniques for halitosis [22]. They compared the organoleptic method with Halimeter to detect halitosis. The VSC detected in the exhaled breath using Halimeter were correlated with the organoleptic score but the results were not significant ($P = 0.2170$) and they concluded that the Halimeter was not found to have a good correlation with the organoleptic method due to the diverse influencing factors [23, 24]. In contrary to these observations, our study results showed that the newer devices like the Tanita FitScan breath analyzer showed statistically significant results of halitosis grading when compared with clinical indices such as PI, GI, BI, and PD.

Zürcher *et al*. compared the halitosis grading by organoleptic method with two different instrumental assessments, using Halimeter® and OralChroma™. They concluded that the organoleptic method was superior to the instrumental assessment as both the devices (Halimeter® and the OralChroma™) can detect the sulfur gases only and not any other volatile components (indoles, amines, and acids) which can also contribute to halitosis [25-27]. The observations made in our study differ from Zürcher *et al*., as the Tanita FitScan breath analyzer showed better results of halitosis grading and it correlated with the clinical indices (PI, GI, BI, and PD).

Morita *et al*. conducted a study to compare the relationship between oral malodor and sulfide levels in the periodontal pockets [28]. They concluded that the clinical indices like the volume of tongue coating, extent of periodontal disease, periodontal pockets, and BOP were significantly associated with oral malodor. The volume of tongue coating and percent of sites BOP were significantly associated with oral malodor [29]. Our study also showed similar findings. The clinical indices (PI, GI, BI, and PD) were directly related to halitosis grading as detected by Tanita breath analyzer.

Many bacteria produce H_2S but the production of methyl mercaptans is primarily restricted to periodontal pathogens such as *P. gingivalis* (26.6% of cases) and *Prevotella intermedia*. Direct exposure to either of these metabolites adversely affects protein synthesis by human gingival fibroblasts in culture. Studies have demonstrated that exposure of oral mucosa to either hydrogen sulfide or methyl mercaptan causes a marked increase in its permeability to ions and bacterial endotoxins [30, 31].

Kundu *et al*. reported that the hygiene status of the tongue may play an important role in malodor production as oral malodor was significantly associated with both the percentage of tongue coating and the presence of deep fissures on the dorsum of the tongue [32]. In our study, an attempt was made to isolate *Streptococcus*, *Bacteroides*, *Porphyromonas*, *Prevotella*, and

Fusobacterium colonies from the subgingival plaque. Gram-positive bacteria contribute little to oral malodor production, whereas Gram-negative bacteria produce large amounts of VSCs [33]. In our study, we found that the Gram-negative bacteria, *P.s gingivalis* (26.6% of cases) and *P. intermedia* (33.3% of cases) were the major contributors of bacterial colonies in patients with halitosis.

Falcão *et al*. [34] compared organoleptic tests with Halimeter to detect halitosis in a set of 21 patients. The area under the receiver operating characteristic curve was only 0.67 (95% confidence interval 0.48–0.85). The accuracy of Halimeter was 59% and that of breath analyzer was only 47%, suggesting that the handheld portable devices are not a reliable tool to assess halitosis and may even misdiagnose a considerable number of patients in day-to-day clinical practice.

Conclusion

The halitosis grading done using Tanita FitScan breath analyzer is more reliable than the organoleptic or Halitox method and the findings also correlated with the presence of bacterial colonies in the gingival PDs of the patients with high scores of halitosis grading.

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Ethics statement: None

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