Detection and measurement of oral malodor in chronic periodontitis patients and its correlation with levels of select oral anaerobes in subgingival plaque

H. S. Grover, Anshu Blaggana, Yashika Jain, Neha Saini

Abstract

Introduction: Oral malodor is generally ascribable to oral microbial putrefaction generating malodorous volatile sulfur compounds. The aim of the present study is to correlate organoleptic recordings with a small handheld portable volatile sulfide monitor and periodontal clinical parameters and correlate the levels of halitosis causing bacteria in plaque between baseline, 1-week, and 1-month. **Materials and Methods:** A total of 20 systemically healthy subjects with self-reported halitosis were subjected to organoleptic examination and FitScan®. Subgingival plaque samples for anaerobic culturing were harvested followed by an assessment of plaque index (PI), gingival bleeding index (GBI), and pocket probing depth. Data derived were subjected to statistical analysis using Wilcoxon signed rank test and Spearman's rank test ($P < 0.05$). **Results:** No correlation was seen between organoleptic measurements and portable volatile sulfide monitor at any time interval. There was a statistically significant ($P < 0.05$) correlation between the scores of PI, gingival index, GBI, and myeloproliferative disease with organoleptic readings at all-time intervals. Anaerobic culture has shown to identify *Fusobacterium* species, *Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia.* However, no correlation could be established in between total microbial load with organoleptic and FitScan® reading at any time interval ($P < 0.05$). **Conclusion:** Significant correlation could be established between organoleptic readings and periodontal parameters.

Keywords: Anaerobic microflora, malodor, organoleptic, portable sulfide monitor, volatile sulfur compound

Introduction

Halitosis is a concern for millions of people, affecting interpersonal social communication with ensuing personal discomfort and social embarrassment.[1] Since bad breath usually emanates from the mouth itself, the dentist is the first professional whom individuals turn for help. Even though the existence of halitosis has been recorded in the literature for thousands of years, it has been a neglected quandary until recently. The emergence of exclusive cosmetic industries and malodor clinics which are explicitly targeting this problem, the situation seems to be changing.[2] In fact now oral malodor ranks only behind dental caries and periodontal disease as the cause of patient's visit to the dentist.[3]

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Halitosis is the general term used to describe any objectionable odor in exhaled air, regardless of whether the odorous substances derive from oral or nonoral sources.[1] Oral malodor specifically refers to such odor originating from the oral cavity itself.[4] The principal underlying reason for the occurrence of this condition in different individuals is usually related to one specific source.[5] Halitosis can be classified into categories of genuine halitosis, pseudo-halitosis, and halitophobia. Genuine halitosis is additionally sub-classified into physiological halitosis and pathological halitosis.[6] Physiological halitosis is temporary and occurs when volatile odoriferous hematologically borne substances from the foods are released into the lungs such as onion, garlic, and alcohol.[7] Pathological halitosis, on the other hand, stems from regional or systemic pathology such as periodontal disease, esophagitis, uremia, diabetic ketosis, pyloric stenosis, respiratory, and gastrointestinal conditions, hepatic and renal failure, or neoplasm.[8] In pseudo halitosis, although patient stubbornly complains of oral malodor but it is not perceived by others. If patient persist in believing that he/she has halitosis even after treatment though no social or physical evidence exists to suggest that halitosis is present then it is classified as halitophobia.[2]

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In about 85% of patients with persistent genuine halitosis, the odor originates from the mouth, as a consequence to a complex interaction between several oral bacterial species (mainly Gram-negative anaerobic flora) with subsequent release of metabolic degradation by products[9] *viz*., volatile sulfur compounds (VSCs), indole, skatole, methyl mercaptan, dimethyl sulfide, and hydrogen sulfide thus imparting an offensive odor to the expired air.[10] The microbes ferment the peptides, mucins and proteins found in blood, lysed neutrophils, desquamated epithelial cells, saliva, gingival crevicular fluid, and any residual food retained on the oral surface[11] has been implicated to produce oral malodor. Among the various ecological niches identified, namely the tooth surfaces, gingival sulcus, etc., the tongue is by far the most important source of these substrates and hence malodor.

Various qualitative and quantitative methods for the measurement of oral malodor employed routinely includes organoleptic measurement, gas chromatography, halimeter, Benzoyl-DL-arginine test, dark field microscopy, saliva incubation test, electronic nose, quantifying β- galactosidase activity, ammonia monitoring, the ninhydrin method, and polymerase chain reaction, etc. Organoleptic measurement has been suggested as a chair side "gold standard" diagnostic test.[8] Presently, available portable VSC monitors boast of high sensitivity, consistency, accuracy, ease of use and measure the cumulative amount of various VSCs present to provide a diagnostic value.[2] One such example of a compact sulfide monitor is Tanita FitScan® Breath Checker (Tanita Corp, Inc., Japan).

Literature amply documents a positive correlation between oral malodor and presence, and severity of gingivitis and periodontitis.[12] Among the Gram-negative bacteria, *Porphyromonas gingivalis* (Pg), *Prevotella aintermedia* (Pi), *Fusobacterium* species (Fu), *Tannerella forsythia* (Tf), and *Treponema denticola* (Td), the so-called periodontopathogens are major contributors of volatile sulfur compounds. Identifying the specific microbes responsible to provide a targeted therapy is, therefore, imperative to completely eradicate the problem. Bacterial sampling from the subgingival sites for anaerobic culture known to be the "gold standard" technique was used to identify the offending microbes.[3]

Effective treatment of oral malodor consists of reducing the total bacterial load on the tongue and the teeth, thereby necessitating a thorough professional prophylaxis in conjunction with twice daily tooth brushing and daily tongue debridement regimen either alone or in combination with the use of antimicrobial mouth rinses such as chlorhexidine.[13]

The purpose of our study was to analyze any potential correlation between organoleptic and portable volatile sulfur compound monitor recordings and their independent correlation with periodontal clinical parameters and subgingival plaque microorganisms at baseline and 1-week and 1-month follow-up intervals in the chronic periodontitis patients.

Materials and Methods

A total of 20 patients[3] both males and females, complaining of bad breath were selected from outpatient Department of Periodontics, Gurgaon. Chronic periodontitis patients with self-reported halitosis, 5–7 mm pocket probing depth[3] with radiographic evidence of bone loss and willing to follow the advised plaque control regimen were included in the study. Subjects taking antibiotics within last 3 months, patients who had undergone any periodontal therapy in the past 6 months, patients suffering from any systemic disease (e.g., diabetes mellitus, respiratory dysfunction, cirrhosis of liver, chronic renal failure, sinusitis, gastrointestinal disorder, and various carcinomas, etc.), female patients who were pregnant and lactating, patients with history of allergy to oral hygiene products and patients or parents/guardians not willing to give a written informed consent were excluded from the study.

The study was designed in three appointments. The first appointment was at baseline while the second and third appointments were at 1-week and 1-month posttreatment. The selected patients underwent organoleptic, and VSC monitor recordings, harvesting, and microbiological analysis of subgingival plaque samples and assessment of clinical parameters *viz*. plaque index (PI)[14] gingival index (GI)[14] gingival bleeding index (GBI),[15,16] and pocket depth (PD) measurement using an UNC-15 Probe (Hu- Friedy®, Chicago)[3,17] at first and third appointments. Except for GI, GBI, and PD measurement, all the parameters were recorded at the second appointment.

For qualifying patients, organoleptic measurement[18] as the primary method for halitosis analysis was performed. The patients were asked to remain quiet and keep their lips closed for a period of 2 min following which they exhaled through the mouth briefly with moderate force at a distance of approximately 10 cm from the nose of the evaluator. The odor detected this way was from the local factors of the oropharyngeal cavity.[18] Depending upon the intensity of offensiveness the following scores were awarded and were considered as a reference standard for oral malodor.[19]

Organoleptic scores

0 = No odor; 1 = Barely noticeable odor; 2 = Slight but clearly noticeable odor; 3 = Moderate odor; 4 = Strong odor; and 5 = Extremely foul odor.

More objective measurement of halitosis was done using Tanita FitScan® HC-212SF Breath Checker, a small handheld

breath checking device, manufactured by Tanita Corp, Inc., Japan, to detect the VSCs and hydrocarbon gases in expired mouth air.[20] As the mouth air is expired, the device measured the amount of VSCs, regardless of type and provided a diagnostic value ranging from 0 to 5 (6 levels) within 9 s.

Bacterial sampling for anaerobic culture, known to be "gold standard" technique[2] was used to culture, Pg, Pi, Fu, and Tf.[3] Before collecting the subgingival plaque samples, all sites were isolated with cotton rolls and air-dried. Subgingival plaque samples were collected with a sterile curette which was inserted to the base of the pocket or sulcus and as much plaque as possible was removed with one single vertical stroke on the root surface. The samples were then placed in the transporting media (reduced transport fluid) and sent to the anaerobic culturing lab within 24–48 h of collection. Samples were first vortexed, then inoculated in the culture medium and then incubated at 37°C for 3–4 days in an anaerobic jar. After completion of incubation, the plates were removed, and the colony characters of the required organism were noted which was picked with the straight wire loop and mixed with a small drop of normal saline on the slide. It was then spread, heat fixed, and following drying, the slide was stained with Gram's staining. The quantification of the suspected microbial colonies was done with a colony counter.

Oral prophylaxis was performed for each patient following the subgingival plaque sample harvesting and was put on rigorous oral hygiene regimen comprising twice daily tooth brushing, and oral rinses with 10 ml of 0.2% chlorhexidine gluconate mouth rinse for 60 s 2 times/day for a period of 1-month. The patients were also instructed to perform a thorough tongue debridement with a toothbrush soaked in chlorhexidine and the protocol.[13] All the collected data were subjected to statistical analysis using Wilcoxon signed rank test for same variables at different time periods. Different variables at same time period were compared by Spearman's rank test, and $P = 0.05$.

Results

Table 1, Figure 1 shows that organoleptic examination and FitScan readings improve after oral prophylaxis, but no significant correlation was seen. The results confirmed in Table 2, Figure 2 that organoleptic readings correlated well with scores of GI, PI, GBI, and mean probing depth. However, FitScan readings did not correlate with the above mentioned periodontal parameters *viz*. GI, PI, GBI, and mean PD as can be seen in Table 3, Figure 3.

In Table 4, Figure 4 and 5 the microbial examination showed a decrease in microbial load after oral prophylaxis. A correlation was seen between total microbial load and organoleptic examination, but it was nonsignificant. Similarly in Table 5, Figure 5 and 6 a nonsignificant correlation was seen between FitScan® readings and the total bacterial count.

Table 1: Comparison of organoleptic measurements and FitScan® readings at baseline, 1-week, and 1-month time intervals

Parameters	Mean±SD	*P*
OG versus FS baseline		
OG	3±0.65	0.642#
FS	1.1±1.59	
OG versus FS 1-week		
OG	1.2±0.52	0.198#
FS	0.5±0.76	
OG versus FS 1-month		
OG	1.9±0.45	0.603#
FS	0.25±0.55	

#Nonsignificant. SD: Standard deviation; OG: Organoleptic scores; FS: Fitscan scores. Significant at $P<0.01$

Table 2: Comparison of organoleptic measurements with PI, GI, GBI, MPD

Parameters	Baseline	1-week	1-month
OG versus PI	3±0.65**	1.2±0.52**	1.9±0.45*
	2.12±0.33	1.15±0.16	1.35±0.13
OG versus GI	3±0.65**	-	1.9±0.45*
	2.01±0.12	-	1.42±0.25
OG versus GBI	3±0.65**	-	1.9±0.45**
	92.64±0.34	-	39.74±21.24
OG versus MPD	3±0.65**	-	1.9±0.45**
	4.85±0.55	-	4.04±0.65

$P<0.05$. PI: Plaque index; GI: Gingival index; GBI: Gingival bleeding index; MPD: Mean probing depth; OG: Organoleptic scores; *: Significant; **: Highly significant

Table 3: Comparison of FitScan® readings with PI, GI, GBI, MPD

Parameters	Baseline	1-week	1-month
FS versus PI	1.10±1.59#	0.5±0.76#	0.25±0.55#
	2.12±0.33	1.15±0.16	1.35±0.13
FS versus GI	1.10±1.59#	-	0.25±0.55#
	2.01±0.12	-	1.42±0.25
FS versus GBI	1.10±1.59#	-	0.25±0.55#
	92.64±0.34	-	39.74±21.24
FS versus MPD	1.10±1.59#	-	0.25±0.55#
	4.85±0.55	-	4.04±0.65

PI: Plaque index; GI: Gingival index; GBI: Gingival bleeding index; MPD: Mean probing depth; FS: FitScan scores; #: Non significant

Discussion

Periodontal disease is a chronic infection of the periodontium affecting the soft and mineralized tissue surrounding the teeth.[21] The toxic products are released from the pathogenic plaque bacteria and is compounded by the host response elicited against these bacteria and their products.[22] The putrefactive action of these microorganisms on endogenous

Table 4: Comparison of organoleptic measurements and total bacterial count at baseline, 1-week, and 1-month time intervals

Parameters	Mean±SD	*P*
OG versus TBC baseline		
OG	3±0.65	0.931#
TBC	80.2±62.47	
OG versus TBC 1-week		
OG	1.2±0.52	0.719#
TBC	8.6±21.05	
OG versus TBC 1-month		
OG	1.9±0.45	0.436#
TBC	21.0±75.68	

SD: Standard deviation; OG: Organoleptic scores; TBC: Total bacterial count; #: Non significant

Table 5: Comparison of FitScan® readings and total bacterial count at baseline, 1-week, and 1-month time intervals

Parameters	Mean±SD	*P*
FS versus TBC baseline		
FS	1.10±1.59	0.172#
TBC	80.2±62.47	
FS versus TBC 1-week		
FS	0.50±0.76	0.623#
TBC	8.6±21.05	
FS versus TBC 1-month		
FS	0.25±0.55	0.294#
TBC	21.0±75.68	

SD: Standard deviation; FS: FitScan scores; TBC: Total bacterial count; #: Non significant

and exogenous proteinaceous substrates, including saliva, exfoliated cells, blood, food debris, and leukocytes, produces VSC's in the oral cavity attributing to oral malodor.[4,23]

Oral microorganisms play an important role in the production of malodor. In the absence of microorganisms, the odoriferous compounds are not released.[2] Subjects with periodontal disease frequently suffer from oral malodor and a positive correlation has been demonstrated between severity of periodontitis and VSC's levels.[24] McNamara *et al*.,[25] John and Vandana[3] established the importance of oral microorganisms in the production of oral malodor. Patients suffering from periodontitis produce more of methyl mercaptan which is 3 times more odoriferous than hydrogen sulfide.[4] Hence, qualitatively malodor will be perceived more in these patients.

Direct sampling and assessment of oral malodor by human judges (organoleptic measurements) is the most rational approach since it is perceived as an olfactory stimulus.[26] Oral malodor measurement by the organoleptic method has been suggested as the "gold standard" for qualitative bad breath measurement.[27] In the present study, organoleptic measurements showed a statistically significant reduction

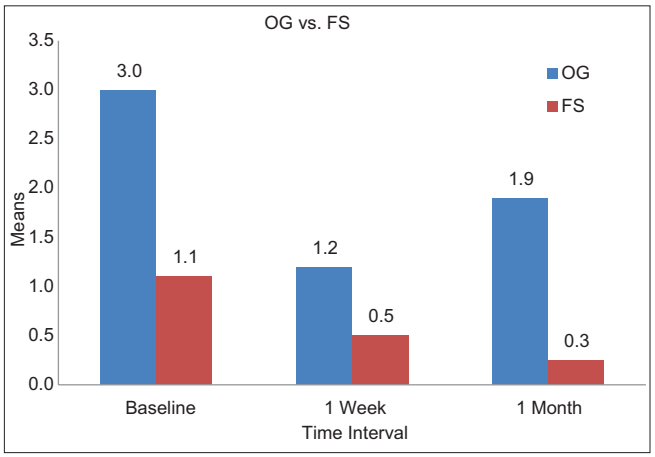

Figure 1: Comparison of organoleptic and FitScan® readings at baseline, 1-week, and 1-month time intervals

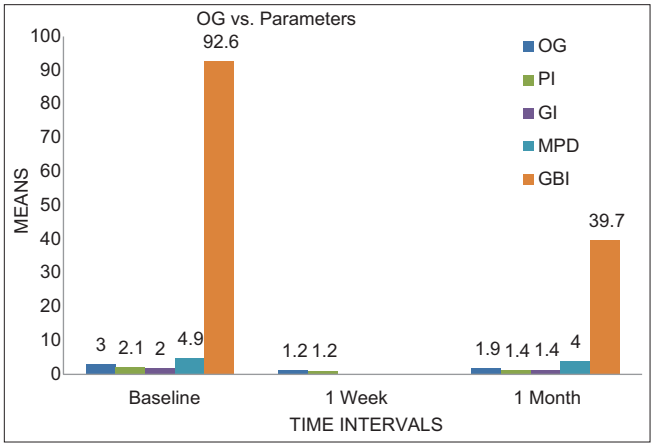

Figure 2: Comparison of organoleptic measurements with plaque index, gingival index, gingival bleeding index, and mean probing depth

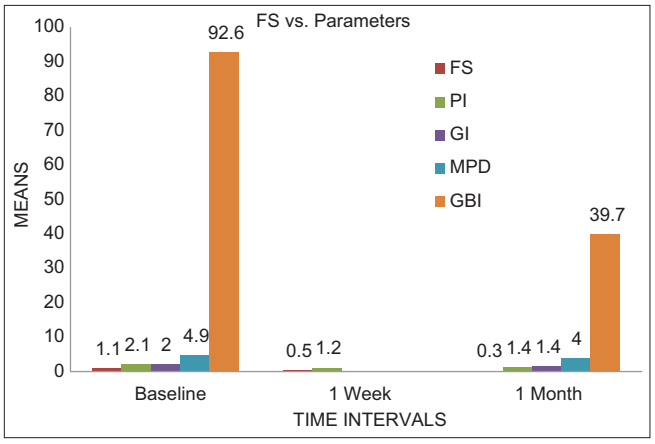

Figure 3: Comparison of FitScan® readings with plaque index, gingival index, gingival bleeding index, and mean probing depth

from baseline to 1-week and baseline to 1-month similar to the observations by Roldán *et al*.,[28] Sulser *et al*.,[29] and Quirynen *et al*.[30] who showed oral malodor reduction following oral prophylaxis. This may be attributable to

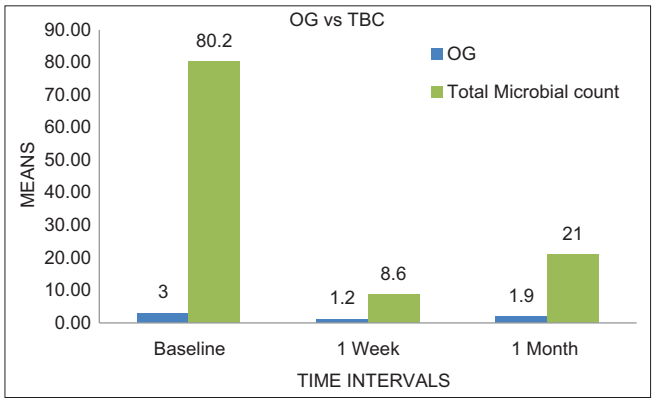

Figure 4: Comparison of organoleptic measurements and total bacterial count at baseline, 1-week, and 1-month time intervals

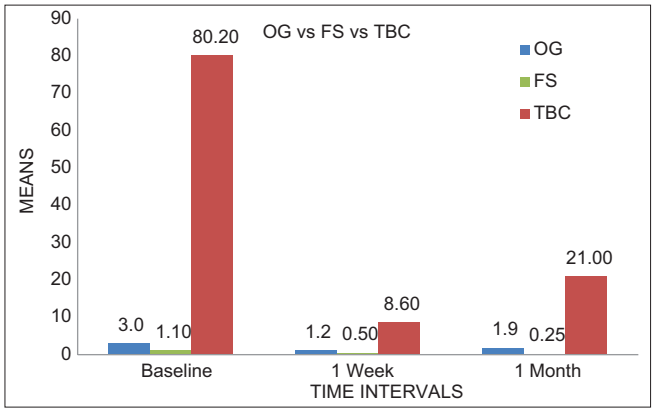

Figure 5: Comparison of organoleptic measurements, FitScan® readings and total bacterial count at baseline, 1-week, and 1-month time intervals

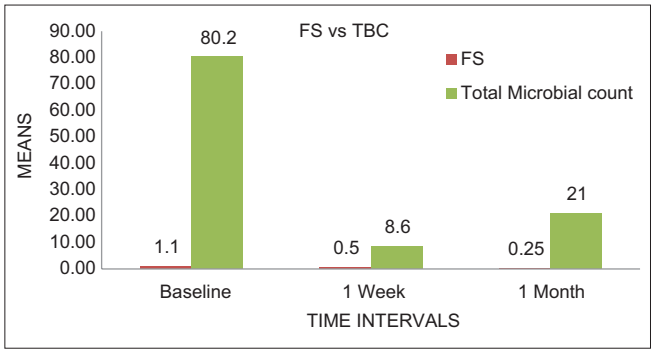

Figure 6: Comparison of FitScan® readings and total bacterial count at baseline, 1-week, and 1-month time intervals

the professional oral prophylaxis performed and the prescribed twice daily tooth brushing with mouth wash and tongue cleaning regimen. Organoleptic measurements, however, showed rebound from 1-week to 1-month posttreatment ($P > 0.05$). The treatment of periodontal disease and tongue cleaning, which can control bacterial plaque growth and progression, are dependent on the patient's daily practice of oral hygiene. However, the treatment is highly reliant on patient's compliance and hence a possibility exists, that subjects might have altered their oral hygiene habits and subsequently elevated levels of oral malodor were observed even though it remained statistically insignificant at the end of 1-month observation period.

The subjectivity of organoleptic ratings may undermine the reliability of this approach; hence an additional quantification method can restrengthen the results obtained. Several methods have been advocated for the measurement of oral malodor. A compact and relatively inexpensive portable volatile sulfide monitor with simple and objective characteristics was adopted. On comparing statistically the Tanita FitScan,® readings have shown a significant reduction from baseline to 1-week and baseline to 1-month consistent with organoleptic ratings but a nonsignificant reduction from 1-week to 1-month. This can be attributable to the possible interference with the detection of VSC's due to the presence of high levels of ethanol in the prescribed mouthwash thus reducing its sensitivity. Additionally, it has been established that Tanita FitScan® is unable to detect any other odoriferous compounds other than VSC. Moreover, it is a quantitative measure showing decreased instrument sensitivity over time that necessitates periodic recalibration.[31] Bosy *et al*.,[18] Brunner *et al*.[32] also showed the consistency of the instruments in contrast to findings of Pedrazzi *et al*.[33]

When an attempt was made to establish a correlation between the qualitative, that is, organoleptic measurements and quantitative, that is, FitScan readings, we found nonsignificant correlation at baseline, 1-week and 1-month. These observations were similar to the one made by Figueiredo *et al*.,[34] Quirynen *et al*.[30] and in contrast to the study done by Bosy *et al*.[18] This could be ascribed to the fact that the Tanita FitScan® analyses the concentration of hydrogen sulfide and methyl mercaptan of the mouth air, without discriminating them. In patients with periodontal disease, methyl mercaptan (CH_3SH) was found to be more abundant[24] and has a threshold of objection ability at least four times lower than that of H_2S therefore, exhibiting significantly higher organoleptic ratings as compared to FitScan® readings.

In an attempt to establish a correlation of various periodontal parameters viz. Plaque score, GI, GBI, and Mean pocket probing depth scores with Organoleptic scores, statistically significant results were obtained at the baseline, 1-week, and 1-month time intervals. It is an established fact that oral malodor is generally ascribable to oral microbial putrefaction generating malodorous volatile sulfur compounds.[35] In oral mucosa proteoglycans and glycoproteins once synthesized intracellularly are held in an aggregated state through disulfide bridges in the extracellular matrix as stated by Hascall.[36] VSC may induce de-aggregation of proteoglycans by cleaving disulfide bonds, thus inducing an increase in permeability of oral mucosa[37] which may promote the ability of antigenic substances such as endotoxin to penetrate the tissue barrier. Whereas the aeration of H_2S-treated mucosa

appeared partially to nullify the effect of H_2S, the process was unable to counteract the effect of CH_3SH hence causing more tissue injury. However, Tonzetich and McBride[38] observed copious production of methyl mercaptan than hydrogen sulfide by periodontally associated pathogenic microorganisms, thus amplifying the inflammation. The complex microbial interactions in the supra-gingival, subgingival plaque and tongue coating are directly causative of gingival inflammation of which the gingival bleeding is the most objective among the various manifestations. Further extension of this inflammatory component in periodontal tissues usually results in an increase in pocket probing depth. Hence, a positive association could be instituted between PI, GI, GBI scores, and mean pocket PD with organoleptic ratings of the patient at the baseline.

On analyzing the association between Organoleptic measurements and total bacterial count (bacterial colonies of Fu, Pg, Pi, and Tf) at baseline, 1-week and 1-month statistically nonsignificant results were obtained similar to the observations by Willis *et al*.,[39] which is in contrast to the study done by De Boever and Loesche[40] who showed correlation between organoleptic measurements and microbial colonies.

Multiple sites in the oral cavity have been implicated in the formation of oral malodor, including the protected proximal surfaces of the teeth, dorsum of the tongue and periodontal pockets[4,41,42] out of which tongue surface serves as the chief source for production of VSC. In our study, however, we have not included the microbiological assessment of the tongue which would be the reason for the noncorroborative results obtained between organoleptic measurements and the subgingival bacterial count.

To the best of our knowledge, the portable volatile sulfide monitor employed in the study has not been evaluated before in relation to various clinical periodontal parameters and subgingival microbial plaque to establish its efficacy as routine self-assessment device for oral malodor. No significant correlation could be established between Tanita FitScan® breath checker and other parameters at all-time intervals. This may be attributable to one or a combination of several reasons. First, Tanita FitScan® may be unable to measure odoriferous gases other than VSCs. The equipment reading may be biased by ambient conditions such as local winds, humid environment, and presence of pollution in the air. Variability in the patient behavior *viz*.-a-*viz*. force of breathing the air on the breath checker may also be an issue since multiple air exhaust on the equipment may be required even though the first exhaust contains most of the odoriferous compounds thereby leaving the subsequent exhaust devoid of VSCs which leads to very low or zero reading on the FitScan.

Till date, there are no published study comparing organoleptic examination, FitScan® reading, and clinical parameters with subgingival microorganisms. Keeping the above facts in mind the present study was conducted to detect and measure oral malodor in chronic periodontitis patients and its correlation with levels of select oral anaerobes in subgingival plaque.

Conclusion

The organoleptic measurement is still a gold standard in the detection of oral malodor in spite of much instrumental advancement. Tanita FitScan® breath checker ratings showed statistically significant reduction at 1-week and 1-month posttreatment but could not show significant changes between 1-week and 1-month. This shows instrument is less sensitive to minor qualitative and quantitative changes in odoriferous gases. After periodontal treatment, periodontal parameters, that is, GI, PI, GBI, and mean PD showed statistically significant reduction at 1-month which is in accordance with organoleptic ratings obtained. There was a significant reduction in subgingival bacterial species count at 1-week and 1-month posttreatment, which is also in accordance with oral malodor readings and periodontal parameters.

This study was a short-term clinical study with a small sample size, and the noninclusion of control groups. Studies, therefore, should be designed with large sample size and longer follow-up periods including control groups to further compare the predictability of the above said procedure.

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Conflicts of interest
There are no conflicts of interest.

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