**Abstract**

**Aim** The aim of the present study was to evaluate in an adult population the correlation between different methods for the evaluation of halitosis and investigate the influence of prosthetic rehabilitation on halitosis level.

**Materials and methods** A sample of 50 adult patients was selected at the Dentistry Unit of the University of Rome Tor Vergata, Italy, who were divided in Group 1 (absence of prosthesis), Group 2 (fixed prosthesis), and Group 3 (removable prosthesis). The assessment of oral malodor was carried out by organoleptic evaluation, measuring the concentration of H2S, CH3SH, and (CH3)2S with Oral Chroma™ and quantification of salivary β-galactosidases (Sβ-g) activity through the spectrophotometric method. Statistics: Anova, Postdoc LSD test, Pearson’s correlation coefficient (P) and Spearman’s correlation coefficient (Rho) were used; p values less than 0.05 were considered significant.

**Results** A positive and significant correlation between organoleptic evaluation, Sβ-g, levels of volatile sulfur compounds Oral Chroma™ measurements was found (p<0.05). By stratifying patients with and without a dental prosthesis, it was possible to show a significant increase of organoleptic scores (P<0.05), β-galactosidase (p<0.05), food stagnation (p<0.01) and a higher scores of H2S (p<0.05) and CH3SH (p<0.05) in patients wearing a prosthesis. Stratifying results between Groups 1–2–3, it was possible to see that some parameters were higher in Group 3, followed by Group 2.

**Conclusion** The presence of prosthetic rehabilitation negatively affected halitosis in the patients according to both clinical and self-reported evaluations.

**Keywords** β-galactosidases; Dental prosthesis; Oral malodor.

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**Introduction**

Halitosis is characterized by the presence of a malodorous breath, perceived both by the patient himself and by the other individuals with whom he interacts. A fairly high prevalence of halitosis, more precisely 31.8% (95% CI 24.6–39.0%), manifests in the world population. In particular in developed countries, its prevalence is 29% while in low-middle income countries it is 39.8% (1). The percentage of patients suffering from halitosis tends to progressively increase with advancing age (2), and is strongly correlated to various disorders of the oral cavity. Epidemiological studies state that 90% of the Dutch population has regularly dealt with a person having halitosis, 40% at least once a week (3); 53.51% of Italian adult population experienced oral malodor (4); 31.5% of adults in Switzerland has perceptible bad breath (5). In addition, 45% of Indian dental students reported this problem, with >80% of them especially in the morning (6); 42% of Japanese school children and 22.8% of subjects in Saudi Arabia also reported that they had oral malodor (7,8).

Halitosis mostly derives from the microbial activity in the mouth and in particular the glycoprotein deglycosylation is considered the first step in the onset of this condition. The rupture of some amino acids (such as methionine, cysteine, tryptophan, and lysine) leads to the formation of volatile sulfur compounds (VSCs) such as methyl mercaptan, hydrogen sulfide, indole and cadaverine (9,10). Among the various proteins, the most frequent ones that lead to the onset of halitosis, are salivary mucins and components of epithelial cells (11).

Halitosis is classified in physiological, pathological (oral or extraoral), pseudoalitosis and halitophobia (12). Among the patients suffering from halitosis, there may be orthodontic or prosthetic patients with fixed and removable devices (13–16). Removable appliances are composed of both metal components that are poorly retentive and resin, a plastic material that is highly retentive to plaque and significantly changes its qualitative and quantitative formation.
The aim of the study was to evaluate, in an adult population, the correlation between different methods (organoleptic evaluation, gas chromatography, salivary β-galactosidases activity, self-assessment) for the evaluation of halitosis and clinical parameters. The secondary objective was to investigate the influence of prosthetic rehabilitation on halitosis level.

**MATERIALS AND METHODS**

Oral malodor was detected in 50 adult patients (mean age 42.26±3.88 years old), 40% males and 60% females, at the Dentistry Unit of the University of Rome Tor Vergata (Rome, Italy). All patients were interviewed about their medical history. Inclusion criterion was the age comprised between 18 and 80 years old. Exclusion criteria were: edentulous patients, pregnant or lactating women, females using hormonal contraceptive methods, antibiotic treatments within 1 month prior to the study or showing the evidence of diseases of the respiratory or gastrointestinal tract, diabetes, liver or kidney conditions that may influence breath odor. Before starting the clinical examination, all patients expressed their perception of the quality of their oral breath using a scale comprised between 0 (no malodor) and 5 (severe malodor) (self-assessment). The level of agreement between the two operators, calculated by using the organoleptic intensity scale, based on Rosenberg et al. (18), as follows: 0 = absence of odor; 1 = questionable malodor; 2 = slight; 3 = moderate; 4 = strong; and 5 = severe malodor. The level of agreement between the two operators, calculated through the Cohen’s kappa coefficient, was 0.851.

- **Halitosis measurement**

  Each participant followed a protocol that included abstaining from certain food and drugs (as indicated by Petrini et al.) and procedures of oral hygiene during the previous 3 h (17).

  - **Organoleptic evaluation**: oral malodor assessment was carried out by two calibrated judges (dentists) who scored the air exhaled from patients mouth by using the organoleptic intensity scale, based on Rosenberg et al. (18), as follows: 0 = absence of odor; 1 = questionable malodor; 2 = slight; 3 = moderate; 4 = strong; and 5 = severe malodor. The level of agreement between the two operators, calculated through the Cohen's kappa coefficient, was 0.851.

- **Evaluation of β-galactosidases activity**: the quantification of β-galactosidases activity was performed on saliva samples collected using the spitting method and the assay of salivary β-galactosidases was carried out both with the Colorimetric method (Cβ-g) (19) and spectrophotometrically (Sβ-g) (17,20).

- **Oral Chroma™ analysis**: a portable gas chromatograph (Oral Chroma™, Abilit Corporation, Osaka City, Japan) was used to measure the concentration of H2S, CH3SH and (CH3)2S.

Breath sample was collected using a disposable syringe (all-plastic syringes, 1 ml), and the concentration of the three gases was displayed in either ng/10 ml or ppbv (nmol/mol) (21).

Subjects were divided into three groups: Group 1 (absence of prosthesis), Group 2 (fixed prosthesis), Group 3 (removable prosthesis).

**Statistical analysis**

Data were expressed as average values ± standard deviation of the means. Statistical analysis was performed using SPSS for Windows version 21 (IBM SPSS Inc., Chicago, IL, USA), analysis of variance (ANOVA) and the Least Significant Difference (LSD) test were used to compare the parameters analyzed in the study for intra- and inter-group analysis. Data were analyzed using linear regression and descriptive statistics. p values less than 0.05 were considered significant. The Pearson’s correlation coefficient (P) was used to evaluate the linear relationship between continuous variables (e.g. Sβ-g vs. VSCs values) and Spearman’s correlation coefficient (Rho) was calculated for ordinal variables (e.g. organoleptic scores) vs. other clinical parameters.

**RESULTS**

By categorizing patients based on the presence or absence of dental prostheses it was possible to verify that those with prostheses had higher levels of β-galactosidase (Spearman correlation, Rho=0.369, p<0.05) and received higher scores on organoleptic evaluation (Rho=0.308, p<0.05), compared to those with only natural teeth. In addition, those patients exhibited increased food stagnation (Pearson analysis R=0.446, p<0.01) and received higher scores on the Oral Chroma™ quantification of H2S (P=0.265, p<0.05) and CH3SH (P=0.387, p<0.05) compared to those with only natural teeth. Patients with a reduced number of teeth had higher salivary β-galactosidase levels (Spearman negative correlation (p<0.05). Moreover, the patients wearing a prosthesis were characterized by a significantly (p<0.05) older age (44.50±3.22) with respect to those with natural teeth (42.24±3.59).

There were no significant differences concerning age in subjects with fixed prosthesis (44.43±3.86) with respect...
Figure 1 shows the quantification of halitosis performed with different methods in different groups. It is possible to see that all parameters regarding the measurement of halitosis were higher in Group 3, but results were statistically significant (p<0.05) at Anova and LSD analysis only for what concerned salivary \( \beta \)-galactosidase (S\( \beta \)-g) and food stagnation. The Spearman correlation (Rho) of the data resulted from this trial has shown that the spectrophotometric analysis of \( \beta \)-galactosidases was highly correlated with the colorimetric method (Rho=0.821, p<0.01) and the

**FIG. 1** Quantification of halitosis performed with different methods.

None = Group 1 (patients with only natural teeth); Fixed p. = Group 2 (patients with fixed prosthesis); Removable p. = Group 3 (patients with removable prosthesis). Error bars = standard deviation; * = p-value < 0.05.
organoleptic scores issued by the dentists ($Rho=0.742, \ p<0.01$). Moreover, the level of these enzymes was also significantly correlated with the tongue coating score index ($Rho=0.434, \ p<0.01$), oral breathing ($Rho=0.363, \ p<0.01$), food stagnation ($Rho=0.516, \ p<0.01$), presence of dental infections ($Rho=0.404$) and periodontal disease ($Rho=0.603$). On the contrary, there was an inverse correlation with the index of oral
hygiene (Rho=-0.528 S, p<0.01) and BMI (Rho=-0.313 S, p<0.05).
The Oral Chroma™ analysis showed a positive correlation between salivary β-galactosidase and hydrogen sulfide (Pearson P=0.746, p<0.01) and methyl mercaptan (P=0.610, p<0.01); by contrast, no correlation was found with dimethyl sulfide levels.

Self-evaluation of halitosis was positively correlated with both the spectrophotometric analysis of β-galactosidases (Rho=0.501, p<0.01) and the organoleptic analysis carried out by the two dentists (Rho=0.696, p<0.01). In particular, stratifying results on the time of the day when patients reported the occurrence of halitosis, the results were never (32%), only in the morning (30%), only in the afternoon (8%), and always (26%). ANOVA analysis found significant differences concerning the time of the day when patients reported halitosis and the level of β-galactosidases (p=0.010), organoleptic scores (p<0.01), H2S (p=0.03), CH3SH (p=0.022) and (CH3)2S (p=0.084) (Fig. 2). The postdoc LSD analysis showed that for all parameters the intergroup differences were significant between those who always manifested halitosis and those who never complained about this problem or only in the morning (p<0.05).

**DISCUSSION**

In this study we analyzed the halitosis level of adult patients with different methods: organoleptic, instrumental and chemical. All these methods were significantly correlated with each other and also with clinical parameters such as low level of oral hygiene and periodontal inflammation. These results were in accordance with our previous research on pediatric patients: high levels of β-galactosidase are correlated with high levels of periodontal disease, tongue coating score, organoleptic scores, VSCs, dental infections and food stagnation (22).

Stratifying patients with and without dental prosthesis, we recorded a significant increase of age, organoleptic scores, β-galactosidase, food stagnation, higher scores of H2S (P=0.265, p<0.05) and CH3SH (p<0.05) in patients wearing a prosthesis, fixed or removable.

A recent study has shown that the presence of fixed orthodontic devices in children was correlated with increased scores of halitosis, evaluated with the organoleptic methods, Oral Chroma™ and salivary β-galactosidases quantification (13). The Oral Chroma™ analysis was very important because it permitted to discriminate between the three main VSCs implicated in halitosis etiology: it has been shown that an increase of H2S is indicative of gram-negative metabolism mainly from the dorsum of the tongue and CH3SH is predominantly higher in periodontal pockets. On the contrary, (CH3)2S can be of either periodontal or systemic origin (23). The increase of halitosis in the group wearing dental prosthesis could be a consequence of increased food stagnation and poor oral hygiene, which consequently causes an increased gram-positive bacterial β-galactosidases activity that synergically works with gram-negatives VSCs production (24). Indeed, as shown by Tanabe et al., mucin deglycosylation exerted by bacterial β-galactosidase is fundamental for VSCs production and the enzymatic inhibition is associated with a decrease of VSCs production (25).

Our results are in accordance with previous researches, which hypothesized that the increase of halitosis in denture wearers could be related to factors, such as bacterial plaque on the tongue, oral dryness, burning mouth, overnight denture wear, and low educational level (26,27). The significant differences in age between patients with or without a dental prosthesis could be connected to a reduction of manual dexterity in oral hygiene. However, a mean difference of three years of age is not so significant to affect oral hygiene parameters. This hypothesis is confirmed by the fact that stratifying results between patients with natural teeth, those wearing a removable and a fixed prosthesis, it was possible to see that all parameters were higher in subjects wearing removable dentures, followed by fixed prosthesis (Fig. 1), but there were no differences in age between these groups. Only β-galactosidase activity and food stagnation remained statistically significant (p<0.05). So we believe that the increase of halitosis parameters and the worsening of oral hygiene condition could be related to the impact of the type of prosthesis in plaque accumulation and on the ability of the patients to clean these devices.

The impact of different types of prostheses on oral microbiota has been previously studied. The worsening of clinical parameters could be related to the increased area of a removable prosthesis, which mostly in the upper jaw covers a great part of the palate. Indeed removable prostheses that are mainly made of resin represent a large hotbed for bacteria and fungi that could cause the onset of halitosis (23). This study confirmed that self-reported halitosis was highly correlated with the other objective measurements of halitosis. This is a very important concern because the awareness of suffering from this problem can generate anxiety, stress and a deterioration of the quality of life (28,29) for these patients. This problem is so serious that literature suggests managing halitosis not only by treating the biological causes, such as oral hygiene, but also with psychological support (30).

This study could represent an opportunity to identify some predictive factors for bad breath in a group of adults. In fact, β-galactosidase activity quantification is a very sensitive tool that permits to achieve a diagnosis of halitosis also in its early phases of onset and could be used for patient’s hygiene motivation.
CONCLUSION

The presence of prosthetic rehabilitations negatively affects halitosis in patients evaluated both clinically and self-reported. These results seem to be highly influenced by the presence of higher food stagnation and lower level of oral hygiene. However, although halitosis levels resulted to be higher in patients wearing a removable denture, results were significant only for salivary β-galactosidases activity and food stagnation.

Considering the high correlation between the level of salivary β-galactosidases in the adult population with clinical parameters, halitosis scores, and patient's self-assessment, oral hygiene instructions, and motivation could represent a valid tool to solve or reduce this problem, both in patients with natural teeth and in those wearing a dental prosthesis.

Salivary β-galactosidases evaluation, performed with the spectrophotometric or colorimetric method, could represent a valid diagnostic tool for genuine oral halitosis, especially in those cases at risk of halitophobia.

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