The correlation between different techniques for the evaluation of oral malodour in children with and without orthodontic treatment

Abstract

Aim  The aim of this work was to evaluate the correlation between different methods (organoleptic evaluation, gas chromatography, salivary β-galactosidases activity) for the evaluation of halitosis in children. The secondary purpose was to investigate the influence of orthodontic treatment on halitosis.

Materials and methods  Study Design: Oral malodour was detected with different methods in 50 children in the Paediatric Dentistry Unit, University of Rome Tor Vergata, Rome, Italy. During the dental visit, level of oral hygiene, tongue coating scores and presence of an orthodontic device, fixed or mobile, were recorded. Two trained and calibrated operators performed the organoleptic evaluation; the Oral Chroma™ device was used for the volatile sulfur compounds (VSCs) quantification and salivary β-galactosidases (β-g) activity was evaluated through the spectrophotometric method. Statistics: The Cohen’s Kappa score was used to evaluate the level of agreement between the operators. The Pearson's correlation coefficient was used to evaluate the linear relationship between continuous variables (e.g., β-g vs. VSCs values) and the Spearman’s correlation coefficient was calculated for ordinal variables (e.g., organoleptic scores) vs. other parameters. The LSD test was used to compare the parameters analysed in the study.

Results  A positive and significant correlation between the organoleptic evaluation, the β-g, the levels of hydrogen sulfide (H2S) and methyl mercaptan (CH3SH) was found. The Spearman’s correlation has shown that organoleptic scores were significantly correlated with β-g (0.664, p<0.001) and the Oral Chroma™ measurements of H2S (0.538, p<0.001) and CH3SH (0.316, p=0.026). The Pearson’s correlation showed that β-g was statistically significantly correlated with the Oral Chroma™ measurements of H2S (0.379 p=0.007) and CH3SH (0.299, p=0.0035). Stratifying results for orthodontic treatment, it was possible to show that children under orthodontic treatment, both fixed or removable, were characterised by higher level of β-g. The organoleptic evaluation and Oral Chroma™ measurements showed that children wearing fixed orthodontic were characterised by higher scores.

Conclusions  There was a significant correlation between the three different techniques for the evaluation of oral malodour in children. Like in the adult population, the increase of β-g activity was associated with oral malodour. The presence of fixed orthodontic appliances was correlated to increased scores of all methods for the evaluation of halitosis.

KEYWORDS  Halitosis; β-galactosidases; VSCs; hydrogen sulfide

Introduction

Halitosis is defined as an unpleasant odor emanating from the mouth, and in 80-90% of subjects the causes lies in the oral cavity [Bollen and Beikler, 2012; van den Broek et al., 2007]. Literature is very variable about the prevalence of halitosis, because of the great heterogeneity of the studies, however, it has been estimated that about the 30% of the world population suffers from this problem [Outhouse et al., 2016; Sanz et al., 2001]. Halitosis affects also the paediatric population with a prevalence comprised between the 23 and 38 % of children [Villa et al., 2014; Lin et al., 2003].

Several causes of oral malodour have been described [Scully and Greenman, 2012]: lingual and/or dental plaque accumulations, periodontal disease [Schorzetti et al., 2013], caries [Li et al., 2015], abscesses, oral ulcers, bone diseases and hyposalivation. Currently, the gold standard technique for the evaluation of oral malodour is still the organoleptic measurement [Naclaci and Sönmeez, 2008]. However, this method is susceptible to such bias, because is subjective and there is the risk to have imprecise scores if operators are not properly trained and calibrated. Moreover, this method cannot detect patients with pseudohalitosis and halitophobia that they are not really affected by oral malodour [Scully and Greenman, 2012]. For this reason, electronic devices and other methodologies have been developed in order to obtain an easy and objective evaluation of oral malodour [Rosenberg et al., 1991].

Oral Chroma™ is a portable gas chromatography device with semiconductor gas sensor that is able to quantify the three main volatile sulfur compounds (VSCs) that more frequently characterise the breath of subjects with oral malodour: hydrogen sulfide (H2S), methyl mercaptan (CH3SH) and dimethyl sulfide (CH2S2) [Krespi et al., 2006; Szabó et al., 2015]. They are the final product of bacterial amino acids decomposition, however, their quantification in breath alone, give us no other information about the real origin of malodour, intra- or extra-oral [Scully and Greenman, 2012]. Currently, the quantification of salivary β-galactosidases is considered only an additional method for the assessment of halitosis, because it does not permit alone to obtain a direct evaluation of halitosis. However, the positivity of this test confirms us that the cause of oral malodour relies on, partially or totally, in the mouth [Scully and Greenman, 2012;]
Petini et al., 2012; Masuo et al., 2012; Sterer and Rosenberg, 2009). In literature the correlation between β-galactosidases activity and VSCs level has been investigated only in the adult population [Masuo et al., 2012; Yoneda et al., 2010]. The aim of this work is to quantify oral malodour and to compare different techniques for evaluation of halitosis in a group of children wearing or not an orthodontic device.

**Materials and methods**

All subjects (50 children) were recruited in Paediatric Dentistry Unit, University of Rome Tor Vergata, Rome, Italy. The inclusion criteria were: children systemically healthy who did not assume any drugs in the 30 days before the consultation. The study protocol was explained and written informed consent was received from the parent/guardian of each child before enrollment in the study. Medical and dental history were obtained for each child and then a trained paedodontist performed a standardised oral visit to all patient, as previously described [Petini et al., 2014]. The level of oral hygiene, the tongue coating scores [Oho et al., 2001] and the presence of an orthodontic device, fixed or mobile, were recorded [Petini et al., 2014]. We considered as fixed, each orthodontic device cemented in the oral cavity (e.g. multibracket or palatal expander), that could not be removed by the patients.

**Halitosis evaluation**

Two different trained operators evaluated and classified the quality of the breath of the patients using the organoleptic intensity scale, based on Rosenberg [Rosenberg et al., 1991], as follows: 0 = absence of odour; 1 = questionable malodour; 2 = slight; 3 = moderate; 4 = strong; and 5 = severe. In order to avoid the risk of bias, all measurements were carried out between 9 and 11 o’clock in the morning. Organoleptic scores were assigned evaluating the intensity of oral malodour at a distance of 10 cm from the mouth of the patient that was invited to count until 10. Each operator was blind about the score expressed by the other. At the end of the organoleptic evaluation, the Cohen’s kappa coefficient was calculated in order to evaluate the level of agreement between the two operators, and then, in cases of different scores, the higher value was reported.

**Measurements of Volatile Sulphur Compounds (VSC)**

The measurement of VSC was carried out through the use of portable gas chromatography (Oral Chroma™, Abilit, Osaka, Japan) that permitted to quantify the three sulfides: hydrogen sulfide, methyl mercaptan and dimethyl sulfide [Tangeman and Winkel, 2008].

**Saliva β-galactosidase activity assay (β-g)**

Saliva was collected by the spitting method. Samples were obtained from the participants between 09:00 a.m. and 11:00 a.m. to minimise the effects of diurnal variability in the salivary composition. β-g activity was evaluated spectrophotometrically, as previously described [Petini et al., 2012, Petri et al., 2014]. Each sample was analysed three times, and the average value was considered.

**Statistical analysis**

Statistical analysis was performed using SPSS for Windows version 21 (IBM SPSS Inc., Chicago, IL, USA). The Cohen’s

<table>
<thead>
<tr>
<th>Organoleptic s.</th>
<th>Spearman’s Correlation</th>
<th>Sig. (2-code)</th>
<th>H₂S</th>
<th>CH₃SHe</th>
<th>CH₃S₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sβ-g</td>
<td>0.664*</td>
<td>&lt;0.001</td>
<td>0.538*</td>
<td>0.316*</td>
<td>0.147</td>
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<tr>
<td>H₂S</td>
<td>0.397*</td>
<td>0.007</td>
<td>0.299*</td>
<td>0.083</td>
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<tr>
<td>CH₃SHe</td>
<td>0.007</td>
<td>0.035</td>
<td>0.568</td>
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<tr>
<td>CH₃S₂</td>
<td>/</td>
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<td>0.268</td>
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**Results**

The correlation between different techniques for the evaluation of oral malodour was calculated in a group of 50 children, 25 males and 25 females (mean age 8.7 ±2.1 years). The level of agreement between the two operators, calculated through the Cohen’s kappa coefficient was 0.85. The 54% of the patients were considered as affected of halitosis, because they received an organoleptic score > 2. The Spearman’s correlation (Table 1) has shown that organoleptic scores were significantly correlated with Sβ-g (0.664, p<0.001) and the Oral Chroma™ measurements of H₂S (0.538, p<0.001) and CH₃SHe (0.316, p=0.026). The Pearson’s correlation (Table 1) has shown that Sβ-g was statistically significantly correlated with the Oral Chroma™ measurements of H₂S (0.379 p=0.007) and CH₃SHe (0.299, p=0.0035). On the contrary, no association was found with the levels of CH₃S₂ (0.083, p=0.568). Also for operator scores, there was not a positive correlation with the level of CH₃S₂ (0.147, p=0.309).

Ten out of 50 participants were in treatment with orthodontic therapy: 6 with fixed and 4 with removable devices. The oral hygiene level and the tongue coating scores, were quite similar in all groups, independently from the presence of orthodontic devices (Fig. 1). However, stratifying results for the presence of an orthodontic treatment, it was possible to observe that the level of Sβ-g (Fig. 2) were significantly correlated with orthodontic appliances, independently if fixed or removable. Spearman’s Correlation= 0.347. However, Fisher LSD found no statistically significant differences between the three groups on the level of this enzyme. On the contrary, higher operators’ scores (Fig. 3) characterised children with fixed orthodontic appliances: results were statistically significantly higher than scores that characterised children with orthodontic removable devices (p=0.024 S).
The Oral Chroma™ measurements (Fig. 4) have confirmed the organoleptic scores: children with fixed orthodontic appliances had higher levels of VSCs; in particular, they had a significantly higher level of H2S than children with removable devices (p=0.045) and without orthodontic treatment (p=0.031).

Discussion

Different techniques were used to detect halitosis in a group of 50 children; a remarkable correlation between the quantification of β-galactosidases activity (Sβ-g), organoleptic scores and VSC concentration in the expired air was found (Table 1). These results are in accordance with Masuo et al. [2012] and Yoneda et al. [2010] who performed a similar research on an adult population without periodontitis. In our previous studies we found a similar correlation between Sβ-g and organoleptic scores in a group of children, however, we did not perform the Oral Chroma™ measurements [Petrini et al., 2014; Petrini et al., 2018]. We did not find any study that correlated VSC measurements with β-galactosidases activity in children. β-galactosidases are essential for the sulfur compounds increment because they deglycosylate salivary glycoproteins, facilitating their further degradation in VSCs by bacterial enzymes like cysteine proteases and methioninase [Chu et al., 1999; Yoshimura et al., 2000; Tanabe and Grenier, 2012; Sterer and Rosenberg, 2006]. It is very interesting to highlight that Sβ-g were statistically correlated with H2S (P=0.379, p=0.007) and CH3SH (R=0.299, p=0.035), that represent the 90% of VSCs in mouth air [Tonzetich, 1971].

Sterer et al. [2009] have shown that β-galactosidases activity and H2S production occur simultaneously in an experimental biofilm but in different bacterial populations: β-galactosidases in the outer layers by gram-positive facultative bacteria and VSCs in the deeper ones by gram-negative anaerobic. It was shown that 0.1–0.2 mm of tongue coating are enough to provide an environment depleted of oxygen, allowing the onset of VSCs production [Kespi et al., 2006]. For this reason, β-galactosidases are considered the first step in VSCs production and the inhibition of their activity, for example as suggested by Morin et al. [2015] with natural compounds like green tea extract, could contribute to reducing the halitosis...
occurrence.

We have also stratified results based on the presence of orthodontic treatment. Considering the very small number of children with orthodontic treatment in our population, our results could only be considered as preliminary. However, the S8-g were significantly correlated with orthodontics, independently if fixed or removable. On the contrary, the organoleptical evaluation found higher scores in children with fixed orthodontic device, and results were statistically significant with respect to children wearing removable devices. Similar results were found with Oral Chrona® measurements that found a statistically significant higher level of H2S in children wearing fixed appliances, with respect to those with removable devices and those without orthodontic treatment.

The increment of H2S is very important because many studies have suggested its contribution in the pathogenesis of connective breakdown and periodontitis, due to its cytotoxicity and ability to stimulate oxidative stress [Rizzo, 1967; Tonzeitch, 1978, Greabu et al., 2016]. The increment of all halitosis indexes are in accordance with literature, which supports the role of fixed orthodontics in the increment of oral malodour. However Babacan et al. have suggested that the direct cause was the increment of plaque accumulation because of the increased difficulties in oral hygiene maneuvers in subjects with not aligned teeth and with fixed orthodontics, that contrast also the natural cleaning of the tongue [Sökücü et al., 2016; Babacan et al., 2011; Delli Mauri et al., 2015]. However, Figure 2 shows that all children included in this study were characterised by a similar level of tongue coating scores and oral hygiene level. The increment of VSCs in children with fixed orthodontics, in our population, could not be a consequence of an increased quantity of plaque accumulation, but of the different quality of bacteria; our theory is confirmed by the increment of VSCs in children with fixed orthodontics, that found a statistically significant higher level of H2S in children wearing fixed appliances, with respect to those with removable devices and those without orthodontic treatment.

Conclusions

The quantification of S8-g activity was significantly correlated with organoleptic scores and Oral Chrona® measurements, especially H2S and CH₄SH concentrations. For this reason, this analysis could permit to understand not only the aetiology of bad breath but also to provide the most effective therapy. The presence of fixed orthodontic appliances was correlated to increased VSCs, organoleptic scores and S8-g activity, with respect to patients with removable devices or without orthodontic treatment.

References