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Association between the organoleptic scores, oral condition and salivary β-galactosidases in children affected by halitosis

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Abstract: *Objectives:* The goal of this work is to evaluate the association between organoleptic scores, oral condition and salivary β-galactosidases, to facilitate the differential diagnosis of halitosis in children. *Methods:* Fifty systemically healthy children with a primary complaint of oral malodour were included in this cross-sectional study. The organoleptic evaluation was carried out by two judges, evaluating the intensity of malodour of the air exhaled 5 s through the mouth of the patients, at a distance of approximately 10 cm from their noses; the level of salivary β-galactosidases was quantified spectrophotometrically after a chromatic reaction between a salivary sample of each patient and a specific chromatic substrate of the enzyme. Clinical conditions, such as visible plaque and gingival bleeding index, tongue coating score, localized food stagnation and other oral parameters, were evaluated by qualified dentists through an oral check-up. *Results:* The β-galactosidase level was significantly related to the organoleptic scores and clinical parameters, such as the tongue coating score and the visible plaque index. Stratifying results with respect to the different phase of the day at which parents complained halitosis in their children, statistical analysis showed that the organoleptic scores and the level of β-galactosidases were significantly higher in children who suffered of halitosis during the whole day, $A = 40\%$, with respect to those without this problem, $N = 20\%$ ($P = 0.001$ and $P = 0.006$, respectively). *Conclusions:* Certain oral parameters such as halitosis during the whole day, high tongue coating score and high visible plaque index were particularly associated with an increase in the salivary β-galactosidase level.

Key words: β-galactosidases; halitosis; saliva; tongue coating

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Introduction

Halitosis (bad breath) is defined as a foul odour arising from a person's oral cavity or nasal passages (1). It has become great concern to many people in the past few decades because it is a very common condition which may affect up to 30% of the population (2). The aetiology of oral malodour is multifactorial, but its main cause is the decomposition of organic compounds caused by proteolytic anaerobic bacteria in the oral cavity (3, 4). The products of this putrefaction are volatile sulphur compounds (VSCs) which are significantly associated with the intensity of bad breath (5, 6). Many studies have shown that salivary β-galactosidases

are influential on the production of VSCs and consequently are associated with halitosis (7–9).

The main producer of β-galactosidase is the *Streptococcus Salivarius*; however, also some periodontopathogenic bacteria such as *Porphyromonas gingivalis*, *Veillonellas sp.* and *Actinomyces sp.* can be important sources (8–10). This is confirmed by an evident association between periodontal pathology and halitosis (2, 3, 6).

Beta-galactosidase is likely to remove the carbohydrate side chains of salivary glycoprotein, thus allowing proteolysis and the consequent VCSs increment. The capacity of β-galactosidase to increase VSCs production from serum, saliva and mucin is dependent on the presence of an exogenous source of proteases such as pancreatic trypsin or *P. Gingivalis* gingipains (11). In 2002, Sterer described that *P. Gingivalis* alone produces substantial levels of VSCs from mucin, but its pre-incubation in the presence of β-galactosidases results in a significant increase in VSCs (9).

The fundamental importance of salivary β-galactosidase in the aetiology of halitosis has been confirmed by the use of the β-galactosidase inhibitor, the p-aminophenyl-b-D-thiogalactopyranoside, that reduced dose-dependently the VSC production by *Solobacterium Moorei* and *P. Gingivalis* (9).

Consequently, the β-galactosidase activity in saliva has been associated with halitosis, and currently, the method that measures the activity of this enzyme is considered an additional or alternative measurement method for the evaluation of oral malodour (7–9, 12).

The assessment and investigation of halitosis in children is essential because it has the potential to cause social restriction, a reduced quality of life and symptoms of depression and can be a manifestation of an extra-oral pathology (13, 14). Despite the introduction of instrumental analysis which involves sulphide monitors and gas chromatography, organoleptic evaluation (OLT) is still the gold standard. Sulphide monitors and gas chromatography are only useful for identifying VSCs, while OLT detects and recognizes all compounds, discriminates complex mixtures and assesses the degree of social offensiveness of breath odour (15).

A deep understanding of the causes of the oral malodour is fundamental, because it permits to choose the best treatment and reduces the development of other complications. For example, the increment of VSCs, which is associated with an increased β-galactosidase activity, can induce the secretion of IL-8 by gingival epithelial cells, promotes osteoclast differentiation and inhibits osteoblast proliferation, contributing to gingival inflammation and periodontal disease (16). Many studies have investigated halitosis in young subjects; however, no one has analysed the relationship between β-galactosidase activity, halitosis and oral health status (17). A more complete understanding of the association between the onset of halitosis, oral condition and the increase in salivary β-galactosidases would allow the use of targeted therapeutic approaches that could consist, for example, in the administration of probiotic bacteria that compete with the β-galactosidase producers (18).

The objective of this study is to evaluate the association between organoleptic scores, oral condition and salivary β-galactosidases, to facilitate the differential diagnosis of halitosis in children.

Study population and methodology

Study subjects

The study included 50 healthy children (26 boys and 24 girls) who attended the University of Rome Tor Vergata, Paediatric Dentistry Unit, complaining about their bad breath. The age ranged from 5 to 13 years (mean age, 9 ± 2 years). Their parents have been fully informed of the nature of the study, and their written consent for participation of the children in the study has been obtained. The study, in accordance with the Declaration of Helsinki, has been approved by the local ethics committee. Exclusion criteria were as follows: antibiotic treatments within 1 month prior to the study or the evidence of diseases concerning respiratory or gastrointestinal tract, diabetes and liver or kidney problems that may influence the organoleptic evaluation. Children and their parents were instructed to abstain from eating strong-smelling foods at least for 48 h and from using scented cosmetics for 24 h. Moreover, they were advised not to ingest any food or drink and not to make their usual oral hygiene practice 2 h before the assessment day (19). The parents were also interviewed about the intensity and the manifestation during the day of oral malodour in their children: 'Do you think your child is suffering of bad breath? When during the day?' What is the intensity of bad breath in a scale from 0 to 5 (20).

Oral malodour assessment

All measurements have been taken between 9 and 11 o'clock in the morning. The oral malodour assessment has been carried out by two dentists (MC and RD). Before the start of the study, these judges have completed a training protocol that consisted in the introduction to sensory scales, exercises assessing quality, intensity, ranking and matching of different odour (21). Oral malodour was scored using the organoleptic intensity scale, based on Rosenberg *et al.* (20), as follows: 0 = absence of odour; 1 = questionable malodour; 2 = slight; 3 = moderate; 4 = strong; and 5 = severe. For the outcome assessment of whole-mouth malodour, subjects were instructed to exhale 5 s through the mouth, at a distance of approximately 10 cm from the nose of the judges. Average values of organoleptic scores have been used for statistical analysis.

Salivary β-galactosidase activity assay (S β-g)

Saliva has been collected using the spitting method. Samples have been obtained from the participants between 09:00 a.m. and 11:00 a.m. to minimize the effects of diurnal variability in salivary composition. Each saliva sample (20 μl) has been immediately applied to a paper disc, containing

X-gal (5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside), according to the procedure previously described (9). After 10 min of incubation at room temperature (20°C), paper discs have been put (15 min) in test tubes containing 400 μl of N,N-dimethylformamide (DMF, Sigma Aldrich, St. Louis, MO, USA). Thereafter, the discs were removed, and the remaining mixture was frozen at −18°C and then transported to the University of Chieti to be analysed. The assay of salivary β-galactosidases was carried out spectrophotometrically as previously described (22). The level of β-galactosidases was obtained calculating the difference in maximum adsorbance between wavelengths at 627 nm and 560 nm. Each sample was analysed three times, and the average value has been considered.

Oral health status

The clinical oral examination included the assessment of the number of teeth, caries, restoration overhangs, the visible plaque index (0 = absence of visible plaque and 1 = presence of visible plaque in more than six sites), gingival bleeding index (0 = no bleeding and 1 = bleeding), localized food stagnation (0 = absence of food stagnation and 1 = food stagnation in more than three sites), dento-alveolar infections (0 = no infections and 1 = presence of one or more abscesses or other infections) and fissured tongue. Standardized clinical criteria were based on the W.H.O. format and Ainamo and Bay (23, 24). The total area and thickness of the tongue coating were scored according to Oho *et al.* (25): 0 = no tongue coating; 1 = thin, with less than one-third covered; 2 = thin, with one-third to two-thirds covered or thick, with less than one-third covered; and 3 = thin with more than two-thirds covered or thick with more than one-third covered.

Statistical analysis

We used Wessa (26) free statistic software for the statistical analysis. Correlations between salivary β-galactosidase, oral malodour levels and clinical parameters were made using Spearman's correlation coefficient (R). Cohen's κ statistic analysis was used to determine the level of agreement between the outcome assessors (27). Stat View 4.0 software (Abacus Concepts, Berkeley, CA, USA) was used to calculate the Bonferroni/Dunn analysis to detect statistically significant differences between the salivary β-galactosidase activity and the organoleptic scores during the different phases of the day and to analyse the other clinical parameters evaluated in this work.

Results

All children enrolled in the study presented with a primary complaint of oral malodour. The Cohen's kappa value between the two odour judges was 0.680, and the Spearman's correlation was 0.750.

There was an high accordance between the organoleptic scores expressed by the parents and the judges (R = 0.810). However the average parents' values were slightly higher. Among 50 children with a halitosis complaint, 20 children were referred to have oral malodour always during the day (A = 40%), 0 in the afternoon (F = 0%), 1 only in the evening (E = 2%) and 19 only in the morning (M = 38%). The parents of 10 children (N = 20%) did not refer to halitosis in their sons and daughters. The upper portion of Fig. 1 shows mean β-galactosidase scores stratifying results with respect to the different phase of the day at which parents complained halitosis in their children. The Bonferroni/Dunn analysis showed a statistically significant difference for what concerning β-galactosidase activity in children who suffered from halitosis in the whole day and those who suffered of this problem only in the morning (P-value = 0.001) and those who did not complain bad breath (P = 0.006; Fig. 1).

The bottom of Fig. 1 shows mean organoleptic scores stratifying results with respect to the different phase of the day at which parents complained halitosis in their children. There were statistical significant differences between the group of children who did not suffer from halitosis and those who had this problem only in the morning ($P < 0.001$), in the evening (P = 0.168) and for the whole day ($P < 0.001$).

Clinical parameters describing baseline for all participants and the statistical analysis are summarized in Table 1. The Spearman's coefficient, R = 0.643 ($P < 0.001$), between the salivary β-galactosidase activity and organoleptic scores of all subjects showed that the two parameters were correlated (Table 1). Beta-galactosidase was also remarkably positively correlated with the tongue parameters. Its correlation coefficients between the tongue coating scores and the fissured tongue were 0.517 ($P < 0.001$) and 0.204 (P = 0.001), respectively. A poor oral hygiene generalized in the whole mouth (visible plaque index) was also associated with β-galactosidase activity (R = 0.496; $P < 0.001$). On the contrary, the presence of localized food stagnation had only a mild positive correlation (R = 0.144; P = 0.002) with β-galactosidase activity. Similarly, a mild correlation between dental alveolar infections (R = 0.220; $P < 0.001$) and gingival bleeding index (R = 0.146; P = 0.006) was found. Other clinical parameters such as the number of deciduous or permanent teeth, the presence of cavities, incongruous restorations, mouth breathing and orthodontic appliances were not significantly associated with salivary β-galactosidase.

Discussion

In this clinical study, 50 children visiting a halitosis consultation were examined by two clinicians, who scored halitosis and the parameters of the whole oral cavity. Moreover, the level of salivary β-galactosidases was measured. All data were stratified with respect to the phase of the day during which the oral malodour manifested, basing on parents' complaints.

Based on average outcome assessors' scores, 40 subjects (80%) suffered from oral malodour (at a cut-off ≥ 2).

The Spearman's correlation coefficient showed a positive association between β-galactosidase activity and organoleptic

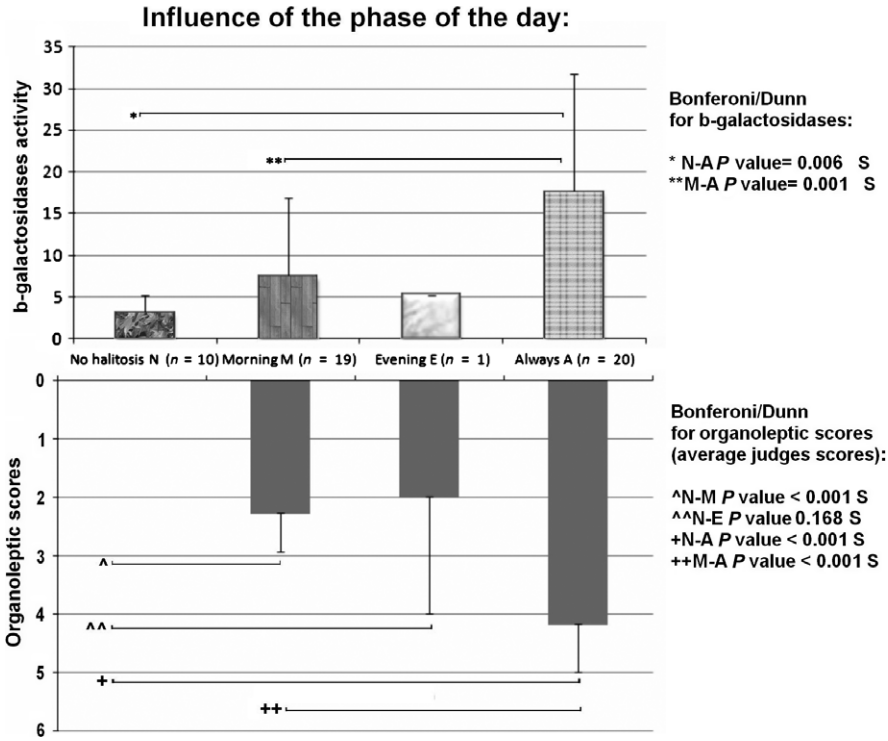

Fig. 1. The data stratified with respect to the phase of the day during which the oral malodour manifested, basing on parents' complaints. 'Do you think your child is suffering of bad breath? When during the day?' The answers have been recorded as follows: *N*, no halitosis (n = 10, 20%); *M*, only in the morning (n = 19, 38%); *F*, only in the afternoon *A* (n = 0, 0%); only in the evening, E (n = 1, 2%); and always, *A* (n = 20, 40%). Bonferroni/Dunn showed statistically significant differences for what concerning the activity of salivary β-galactosidases and average organoleptic scores rated by the two judges.

Table 1. **Clinical parameters describing baseline for all participants and the Spearman statistical analysis**

	Baseline		Spearman with β-gal	
Parameter	Mean value	SD	*R*	*P*-value
β-galactosidase scores	0.010	0.012		
Organoleptic scores (0–5)	2.900	1.741	0.643	<0.001
Tongue coating scores (0–3)	1.320	0.891	0.517	<0.001
Number of deciduous teeth	8.820	4.762	−0.214	0.174
Number of permanent teeth	15.260	5.851	0.188	0.153
Decayed deciduous teeth	0.760	1.598	−0.158	0.548
Decayed permanent teeth	0.600	0.948	−0.126	0.818
Dento-alveolar infections (0–1)	0.040	0.198	0.220	<0.001
Gingival bleeding index (0–1)	0.160	0.370	0.146	0.006
Incongruous restorations (0–1)	0.020	0.141	−0.089	0.001
Mouth breathing (0–1)	0.200	0.404	0.063	0.031
Fissured tongue (0–1)	0.100	0.303	0.204	0.001
Localized food stagnation (0–1)	0.100	0.303	0.144	0.002
Orthodontic appliances (0–1)	0.220	0.418	−0.225	0.542
Visible plaque index (0–1)	0.640	0.485	0.496	<0.001

scores (R = 0.643; Table 1). This result is in agreement with Masuo *et al.* (7), who found that R ranged from 0.400 to 0.700, in an adult population. These data have been confirmed, always for adult population, by other studies and with similar results (8, 9, 22, 28). Moreover, the statistical analysis based on the parents' questionnaire showed statistical significant differences (P-value = 0.006) between the activity of these enzymes in children who suffered the whole day of halitosis (A = 40%) and those without this problem (N = 20%; Fig. 1).

Many studies have shown that oral malodour in children is related to periodontal parameters and in particular to tongue coating (17, 29, 30). Here, we showed that these factors apply in children as well, but also showed the correlation with β-galactosidase activity.

The additional importance of β-galactosidase assay for the differential diagnosis between genuine halitosis and morning breath was confirmed by the Bonferroni/Dunn method; there were indeed statistically significant differences in the β-galactosidase activity between children considered by their parents 'suffering the whole day of halitosis' and those characterized by this problem only in the morning. This result agrees with the fact that morning bad breath is only a transient consequence of the reduction in salivary flow during the night rather an increase in β-galactosidase activity (31).

Many studies have found that oral malodour reaches the critical level during the fixed orthodontic treatment, due to the increase in the plaque accumulation (32). However, the number of volunteers included in this study using orthodontic appliances was not sufficient to verify such hypothesis.

Our data showed no correlations between β-galactosidases and caries. The decrement of the pH caused by the acids produced by mutans streptococci and lactobacilli leads to cavities (33). These bacteria may contribute to the decrease in other

species, such as β-galactosidase producers, through the release of antimicrobial substances, such as mutacins (34).

Although β-galactosidase assay has been associated with organoleptic scores, there was only a mild correlation with gingival bleeding index. These results are in agreement with Masuo *et al*. (7) whose have demonstrated that salivary β-galactosidase affects physiological mouths with halitosis.

Considering that the level of β-galactosidases contributes to the increase in VSCs and that these substances are toxic for periodontal tissues, it is advisable to monitor children who are characterized by high level of this enzyme, in terms of reducing the prevalence and severity of chronic periodontitis in adulthood (35).

Conclusions

Results have shown a high correlation between organoleptic scores expressed by the two blinded outcome assessors and parents' reported complaints.

The level of β-galactosidases and organoleptic scores was particularly high in children who suffered the whole day of halitosis, and these data were correlated with the tongue coating scores and the visible plaque index. We conclude that the β-galactosidase assay is particularly indicated in children suffering of oral malodour and with a poor oral and tongue hygiene. Monitoring of this enzyme in addition to a clinical examination can help to identify the oral origin of halitosis, to accelerate the differential diagnosis with other systemic conditions that may cause this problem.

Clinical relevance

Scientific rationale for the study

Early diagnosis of halitosis is fundamental in children, but currently, in the objective evaluation of breath, instruments are used that measure volatile sulphur compounds only, without the ability to discriminate complex mixtures and to assess the degree of social offensiveness of breath odour.

Principal findings

β-galactosidase activity increased particularly in children with high organoleptic scores in the whole day, a poor oral hygiene and a bad breath during the whole day.

Practical implications

β-galactosidase assay combined with organoleptic rating and objective evaluation of oral cavity is particularly indicated in children suffering of oral malodour, with a poor oral and tongue hygiene.

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