The Ph Salivary Biomarker – is There a Difference Between Cleft and Non-Cleft Orthodontic Patients?

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Abstract

Cleft lip and/or palate (CL/P) is one of the most common congenital malformations. In view of the important role of salivary pH and the scarcity of studies on this topic in patients with CL/P, the aim of this study was to analyze salivary pH in patients with and without CL/P undergoing orthodontic treatment with fixed appliances. The sample consisted of 45 patients wearing orthodontic appliances (20 boys and 25 girls; mean [SD] age, 15 [2.70] years), who were divided into 3 groups: control group (patients without CL/P, n = 15); CL/P group (patients with CL/P without oronasal communication, n = 15); and CL/P-O group (patients with CL/P with oronasal communication, n = 15). Saliva samples were collected and evaluated for pH by using indicator strips, with a reading scale ranging from 2.0 to 9.0. Data were tested for normality using the Shapiro-Wilk test, and results were compared by the Kruskal-Wallis test (p ≤ 0.05).

The median pH was 7.5 in the control group, 7.0 in the CL/P group, and 6.5 in the CL/P-O group. Although there was no statistically significant difference among the 3 groups (p ≤ 0.05), the CL/P and CL/P-O groups tended to have lower and more scattered pH values than the control group.

Salivary pH did not differ statistically between patients with and without CL/P wearing fixed orthodontic appliances. However, those with CL/P tended to have more acidic and scattered pH values. Further studies and trials involving a larger number of patients are important to clarify this issue.


Keywords: Saliva, biomarker, cleft lip, cleft palate.

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Introduction

Cleft lip and/or palate (CL/P) is one of the most common congenital malformations. Its global prevalence is estimated at 14.29 per 10,000 live births, with considerable ethnic and geographic variation ¹. The incidence of CL/P varies with race, being higher in Asians than Caucasians but lower in individuals of African descent ². Boys are more commonly affected by CL/P than girls (2:1), with an inverse ratio for isolated cleft palate (male:female, 0.5:1)³. Using the best available evidence, a systematic review showed that in low- and middle-income countries, including Brazil, 1 in every 730 children is born with CL/P ⁴. In Brazil, the prevalence of this malformation was estimated at 0.19 per 1000 live births between 1975 and 1994⁵. It has already been reported that the mutation of C677T and 1298C genes can cause fusion failure during oral and maxillofacial fusion failure during oral and maxillofacial
development. The hereditary pattern is probably related to an interaction between genetic susceptibility and environmental stimulation. CL/P are common birth defects and present an complex etiology, involving genetic factor and environmental exposures. It is suggested that a possible risk of reduced DNA repair ability occurs in CL/P patients. Despite the small sample, in this recent clinical study, healthy individuals and cleft patients had some degree of methylation of the MGMT promoter; however, CL/P patients had significantly higher prevalence of fully methylated status than the control group.

The American Cleft Palate-Craniofacial Association emphasizes the importance of multidisciplinary care for these patients within the first few days of life. A majority of the individuals have marked skeletal discrepancies in the anteroposterior, transverse, and vertical dimensions, such as alterations in the number and shape of teeth and malpositioned teeth. Thus, orthodontic treatment, irrespective of whether it is combined with orthognathic surgery, is necessary for the rehabilitation of patients with such conditions. The use of orthodontic appliances induces changes in the oral environment, rendering plaque control difficult, and consequently alters salivary characteristics (pH, buffering capacity, and salivary flow rate). Saliva is a complex fluid with multiple functions that play an important role in maintaining a balanced microbiota. Furthermore, saliva is responsible for providing lubrication to intraoral surfaces and protecting the teeth and oropharyngeal mucosa. It maintains neutral pH through its buffering capacity, prevents tooth demineralization and dental caries, exerts antimicrobial activity, aids in taste recognition and bolus formation, initiates enzymatic digestion of starch, and is imperative for mastication, swallowing, and speech production.

Individuals with cleft palate are more likely to develop secretory otitis media, due to Eustachian tube dysfunction, and hearing loss. Opening of the Eustachian tube is compromised, and the middle ear cavity is not properly ventilated. This problem leads to a negative pressure, which results in a retracted tympanic membrane and secretion of mucus from the tissues through osmosis into the middle ear cavity. The involvement of structures posteriorly to the incisive foramen and oronasal communication are strong predictors of increased occurrence of otitis media.

Patients with CL/P often show deficient plaque control and are at high risk for caries owing to difficulties in maintaining hygiene in the cleft area and to associated dental anomalies. The low self-esteem that is often experienced by these patients may contribute to their lack of oral health care. In addition, it is hypothesized that the salivary pH of patients with CL/P may be altered because of the presence of nasal mucus in the oral environment, which interferes with important functions of saliva, such as buffering the acids produced in the oral biofilm and its antimicrobial properties. Buffering capacity of saliva represents an important protective factor against caries; therefore, this function must be controlled especially in patients with CL/P, who already are at increased risk due to their condition.

In view of the scarcity of studies on the salivary characteristics of such patients, the aim of this study was to analyze the salivary pH of patients with CL/P (with and without oronasal communication) vs patients without CL/P undergoing orthodontic treatment and to determine whether they differ from each other.

**Materials and methods**

We selected 45 patients undergoing orthodontic treatment (20 boys and 25 girls; mean [SD] age, 15 [2.70] years) to participate in this preliminary study (Table 1, Figure 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
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<tbody>
<tr>
<td>A</td>
<td>15</td>
</tr>
<tr>
<td>B</td>
<td>15</td>
</tr>
<tr>
<td>C</td>
<td>15</td>
</tr>
</tbody>
</table>

**Table 1.** Patients divided into control, CL/P, and CL/P-O groups.

**Figure 1.** Examples of patients in each group: A) Patients without CL/P (control group); B) Patients with CL/P without oronasal communication (CL/P group); and C) Patients with CL/P with oronasal communication (CL/P-O group).
Thirty patients with CL/P with or without oronasal communication were recruited from the Cleft Lip and Palate Rehabilitation Center at the School of Health and Life Sciences of Pontificia Universidade Católica do Rio Grande do Sul (PUCRS), southern Brazil. Fifteen patients without CL/P undergoing treatment were recruited from the Orthodontics Program at PUCRS School of Health and Life Sciences.

After providing written informed consent, patients and their legal representatives agreed to participate in the study, which consisted of saliva collection and salivary pH testing. This randomized clinical trial was approved by PUCRS Research Ethics Committee.

Inclusion criteria for the control and experimental groups were good general and oral health, ongoing orthodontic treatment with fixed appliances, and absence of CL/P for the control group and presence of CL/P for the experimental groups. Patients were excluded if they had active carious lesions or periodontal disease, had systemic alterations or syndromes, reported current use of medications, or had diseases associated with genetic disorders.

Two researchers performed the clinical tests to assess the presence or absence of oronasal communication. For this assessment, the patient was instructed to perform the Valsalva maneuver, which consists in expelling air against closed nostrils, while the clinician checks if air hisses \(^2\). A hissing noise and/or expelling saliva or mucus from air leakage through the maxillary sinus and nose indicates a positive test (presence of oronasal communication).

**Saliva collection**

Collection was performed during orthodontic treatment after complete assembly of the fixed appliances. Patients were instructed not to ingest any food or liquid (except water) within 1 h prior to collection and not to engage in physical activity, chew gum, or brush their teeth on the day of collection. Usual oral hygiene instructions were given to patients (toothbrushing and flossing). In all groups, saliva samples were collected between 9:00 and 11:00 AM. Patients were instructed to accumulate unstimulated saliva in the mouth for 5 min and then spit into a plastic bottle (50 mL Falcon tube) labelled and identified with the patient’s name and group. Only 1 saliva sample was collected from each patient.

**Salivary pH testing**

Test strips were used to read salivary pH (Macherey-Nagel, Düren, Germany / Ref. 921 18), with a reading scale ranging from 2.0 to 9.0, with intervals of 0.5, to measure the acidity of the unstimulated saliva sample. Immediately after saliva collection, a pH indicator strip was placed in the plastic bottle containing the saliva sample for 3 min before measurement (Figure 2). The salivary pH was determined by 2 blinded operators who compared the color on the test strip with the color on the pH chart supplied by the manufacturer. In case of disagreement between the readings, a third operator was consulted.

![Color-fixed pH indicator strips](image_url)

**Figure 2.** Color-fixed pH indicator strips.

**Statistical analysis**

The pH values for all patients were entered in tables. Quantitative data were analyzed descriptively. The data were tested for normality using the Shapiro-Wilk test. Because of skewed distribution, the nonparametric Kruskal-Wallis test was used for data analysis at 5% significance level. Data were analyzed in Jamovi version 1.6.3.0 (Sydney, Australia) for Windows.
Results

The salivary pH values in each group, including medians, minimum–maximum values, and 25th and 75th percentiles, are shown in Table 2. The pH values for each patient in each group are expressed in a scatter plot, and each box plot shows the distribution of pH values in each group (Figure 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>P25</th>
<th>P75</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.5</td>
<td>6.5</td>
<td>7.5</td>
<td>7.0</td>
<td>7.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B</td>
<td>7.0</td>
<td>5.5</td>
<td>8.0</td>
<td>6.5</td>
<td>7.5</td>
<td>0.040</td>
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<tr>
<td>C</td>
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<td>5.5</td>
<td>7.5</td>
<td>6.5</td>
<td>7.0</td>
<td>0.112</td>
</tr>
</tbody>
</table>

Table 2. Salivary pH values (median, minimum, maximum, 25th and 75th percentiles) in each group and p-values.

Of 45 patients, 17 had low pH values (between 5.5 and 6.5). Of these 17 patients, 14 had CL/P (8 with and 6 without oronasal communication). Four patients had a critical pH of 5.5, all of whom had CL/P (both CL/P and CL/P-O groups).

There were no statistically significant differences among the 3 groups (p ≤ 0.05). However, there was a trend toward lower and more scattered pH values in the CL/P and CL/P-O groups (Figure 3). The pH values appeared to vary more in the CL/P and CL/P-O groups, and their pH tended to be more acidic than that of the non-CL/P group.

Discussion

Patients with CL/P are subjected to long-term orthodontic treatment, considering the malocclusion complexity in these cases. Maintenance of good oral hygiene is often difficult for patients wearing orthodontic appliances 11. The use of fixed appliances results in more biofilm accumulation on the maxillary lateral incisors and canines, particularly in the gingival area and areas behind arch wires 23. There is also moderate evidence that the presence of fixed orthodontic appliances influences the quantity and quality of oral microbiota 24. Furthermore, fixed orthodontic treatment causes major changes in salivary properties, leading to significantly increased Candida albicans, Streptococcus mutans, and Lactobacillus acidophilus counts and decreased salivary pH during treatment 25. In patients without CL/P undergoing orthodontic treatment, salivary pH significantly increased after the first 6 weeks of treatment, continued to increase during the next 6 weeks, and then decreased toward baseline within weeks 12 and 18, while Streptococcus mutans and Lactobacillus spp levels also increased significantly, with a major peak at week 12 26.

Few studies have evaluated the salivary characteristics of patients with CL/P. It has been speculated that patients with CL/P with oronasal communication have an altered salivary pH due to the presence of nasal mucus in the oral cavity. Considering an oral environment that enhances the growth of bacteria, these patients may have higher levels of salivary bacteria and a salivary profile that is more conducive to dental caries than those without CL/P 27. Cheng et al. 27 evaluated patients with and without CL/P undergoing orthodontic treatment vs controls with and without CL/P not undergoing orthodontic treatment and found no statistically significant differences among all groups for pH parameters. Patients with CL/P undergoing orthodontic treatment even showed a better buffering capacity, although, overall, the averages were within the normal range. The salivary microbial test showed that patients without CL/P undergoing orthodontic treatment appeared to have the most favorable microbiological environment for the development of dental caries as this group had a high percentage of participants acquiring ≥10⁵ CFU/mL of both mutans streptococci and lactobacilli 27. Patients with CL/P received regular oral health care and advice from dental professionals, which may have encouraged and motivated those in the orthodontic treatment group to maintain an adequate standard of oral hygiene at home 27.

Increased caries risk has been reported in children with CL/P compared with age-matched
controls, and the most influential factors are impaired oral hygiene and elevated salivary counts of lactobacilli. However, despite the significantly higher prevalence of dental caries, only minor differences have been found in the salivary microbial profile between patients with and without CL/P. Moreover, there is often a lack of adequate bacterial plaque control due to dental anomalies and lip and palate defects, which makes good oral practices more difficult. The use of orthodontic appliances has also been associated with changes in dietary habits; patients often switch to a soft diet, which may increase plaque retention at the sites of clefts and fistulas.

Salivary pH usually ranges from 6.2 to 7.4 due to an efficient buffer system, balanced mainly by the carbonic/bicarbonate acid and phosphate systems. Salivary buffering capacity seems to play a crucial role in maintaining salivary pH. Salivary buffering acts by countering decreases in pH and serves as an important protective factor against remineralization and caries. Bicarbonate is the main component responsible for the buffering capacity of saliva, along with the phosphate and protein buffer systems. Low salivary buffering capacity is considered a moderate risk factor for caries prevalence and incidence. In addition to the concentrations of calcium and phosphate, supersaturation of saliva in relation to tooth enamel is regulated by the pH of the environment. Therefore, maintenance of salivary pH at non-critical levels is of primordial importance in preventing the development of carious lesions. Considering the results of the present study, patients with CL/P may have lower pH values, which deserves special attention along with the fact that patients with CL/P already experience difficulties in oral hygiene and a consequent increased risk of developing caries lesions. The pH of nasal secretion shows a sinuous curve, but it is not constant, ranging from approximately 5.5 to 6.5. Interaction between the nasal mucus and oral cavity occurs in patients with CL/P with oronasal communication. A genetic alteration in the salivary transport of some antioxidants occurs in CL/P as a result of early closure of the cleft palate, which may create constant communication between the oral and nasal cavities and an oral biological environment different from that of patients without CL/P. A recent study with salivary analysis showed that total antioxidant capacity and total protein were significantly different between children with and without CL/P, but uric acid concentrations did not differ between them. Salivary flow rate was significantly decreased in children with CL/P, whereas pH was within the normal range for both groups. In the present study, CL/P group tended to have lower pH values than controls and those in the CL/P-O group (Figure 3). However, an analysis of pH values in the 3 groups by the Kruskal-Wallis test confirmed the absence of statistically significant differences among them (Table 2). The median salivary pH values of all groups were within the normal range, i.e., between 6.2 and 7.4.

Cheng et al. evaluated the salivary characteristics and amount of bacteria present in the saliva of adolescents undergoing orthodontic treatment and found no difference in the incidence of oral conditions between patients with CL/P and without CL/P. However, the patients received regular instructions on oral hygiene and diet, and plaque was removed by a dental professional whenever necessary.

The results of the present study, while not indicating a statistically significant difference, did reveal a tendency of patients with CL/P to show wider variations of salivary pH than those shown by patients without CL/P. Of the 17 patients with low pH values (between 5.5 and 6.5), 14 had CL/P, of whom 8 had oronasal communication and 6 did not (Figure 3). Four patients showed critical pH (5.5), all of them with CL/P (both CL/P and CL/P-O groups). Following this research, these patients were contacted again and given additional instructions on oral hygiene. They also received dietary counselling and were advised to rinse their mouth on a daily basis with a 0.05% sodium fluoride solution.

There was a trend toward lower and more widely scattered pH values in the CL/P and CL/P-O groups compared with the control group of patients without CL/P. Lower pH values in patients with CL/P may be explained by poorer oral hygiene practices, more crowded teeth (facilitating plaque accumulation and rendering oral hygiene difficult), lower purchasing power, and lower self-esteem, all of which are characteristic of these patients. Although the median salivary pH was similar in all groups, patients with CL/P showed greater variation in pH measurements, as shown in Figure 3. Salivary buffers play an important role in maintaining...
salivary pH stability by neutralizing the acidity of the nasal mucus in the oral cavity. This occurs because alkaline solutions, such as saliva, contain several ionizable/ionizing substances that are subjected to many variables, such as diet, flow stimulation, and bacterial metabolism (production of acids), which drive the continuous variation of pH values. Salivary buffering capacity corrects pH changes arising from the formation of acid and basic ions and plays an important role in the regulation of pH to 6.9–7.0 in the oral environment, thus preventing lesions produced by the excess of acids and bases. Therefore, both bacterial plaque control and dietary counselling in patients undergoing orthodontic treatment are paramount for the maintenance of pH at non-critical levels, thus helping to prevent carious lesions, periodontal disease, and adverse reactions caused by corrosion of the orthodontic appliance in an oral environment with low pH 27.

The propensity for lower pH levels in individuals with CL/P serves as a warning. Low pH promotes demineralization of tooth structure and favors the growth and metabolism of acidogenic and acid-tolerating bacteria 33. Conditions associated with salivary gland hypofunction, low salivary pH, and altered salivary composition often lead to oral microbiome modification causing dysbiosis with an associated risk of oral disease 34. Patients with CL/P tend to have deficient plaque control and increased risk of caries due to difficulties in maintaining oral hygiene 17, and these data should be associated with the present trend toward lower pH as a warning sign for clinicians. We also emphasize the importance of oral hygiene reinforcement and dental health education during orthodontic treatment, especially for patients with CL/P.

Conclusions

Median salivary pH was within the normal range for all groups, with no statistically significant differences between them. However, patients with CL/P tended to show lower and more scattered pH values than those without CL/P (control group). These preliminary data serve as a warning given the importance of salivary buffering capacity and its influence on the development of dental caries. It is therefore important to conduct more clinical trials and studies with larger sample sizes to clarify these results. Meanwhile, we can highlight this tendency and give special attention to oral hygiene reinforcement and dental health education in patients with CL/P.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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Ethical approval

The study was submitted and approved in the research ethics committee of Pontificia Universidade Católica do Rio Grande do Sul (CEP-PUCRS). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Declaration of Interest

Informed consent was obtained from all individual participants included in the study.

The authors report no conflict of interest.

References


