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ORIGINAL RESEARCH

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# Evaluation of salivary pH in pediatric patients undergoing orthodontic treatment with rapid palatal expander: cross-sectional study

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## Abstract

**Background**: Orthodontic appliances increase the difficulty of maintaining oral hygiene due to their size and complexity. This study aimed to evaluate salivary pH and buffer capacity in pediatric patients aged 6–14 years using the Saliva-Check Buffer test comparing two groups: patients who have completed dental treatments but have not yet started the orthodontic treatment (control group), and those who have been undergoing orthodontic treatment with rapid palatal expander (RPE) for at least six months (trial group). **Methods**: Clinical assessments included the recording of Decayed, Missing, Filled Teeth (DMFT + dmft), Decayed, Missing, Filled Surfaces (DMFS + dmfs), plaque control record (PCR) and Frankl behavioural scale. Salivary pH and buffering capacity were measured using the Saliva-Check Buffer kit which consisted of five different tests. Data were statistically analyzed, assessing data normality of distributions with Kolmogorov-Smirnov test, and subsequently performing Mann-Whitney test and linear regressions analyses (significance threshold: $p < 0.05$). **Results**: Orthodontic patients exhibited significantly higher PCR scores and lower salivary buffering capacity compared to the pre-orthodontic group ($p < 0.05$). No significant differences were observed in DMFT + dmft and DMFS + dmfs scores between the groups ($p > 0.05$), but DMFS + dmfs significantly influenced salivary consistency, pH and buffering capacity ($p < 0.05$). Salivary consistency significantly influenced by age, while salivary pH seemed influenced by PCR and group. Sex and group significantly influenced saliva quantity ($p < 0.05$), and finally PCR significantly influenced salivary buffering capacity ($p < 0.05$). **Conclusions**: The findings suggest that orthodontic treatment may contribute to increased plaque accumulation and reduced salivary buffering capacity, potentially elevating caries risk. Enhanced oral hygiene measures and preventive strategies are recommended for orthodontic patients. **Clinical Trial Registration**: the study was registered on clinicaltrials.gov (NCT06922799).

## Keywords

Dental caries; Pediatric dentistry; Orthodontic appliances; Interceptive orthodontics; Salivary pH

## 1. Introduction

Dental caries is one of the most prevalent chronic diseases globally, significantly impacting oral and systemic health. According to the World Health Organization (WHO), The estimated global average prevalence of caries is 43% for deciduous teeth and 29% for permanent teeth, with differences between country groups [1].

The disease is a multifactorial process involving cariogenic bacteria, high sugar consumption, inadequate oral hygiene, and host factors (*e.g.*, low salivary flow and systemic conditions). In particular, when bacteria such as *Streptococcus mutans* and *Lactobacilli spp.* metabolize fermentable carbohydrates, they produce organic acids that lower the oral pH. If the pH drops below the critical threshold of 5.5, enamel dissolution occurs and it can represent the beginning of carious process. To restore the pH value to a higher level, remineralization of dental surfaces is required, mainly through saliva or fluoride treatment [2].

Saliva plays a crucial role in maintaining oral health by serving as a natural barrier against caries. Its composition, which includes electrolytes, enzymes, proteins, and immunoglobulins, provides mechanical cleansing, buffering capacity, and antimicrobial properties. Saliva's buffering capacity, primar-

ily due to bicarbonates, helps neutralize acids produced by bacterial metabolism, maintaining an optimal pH environment in the mouth. Additionally, it aids in the remineralization of enamel by providing essential minerals such as calcium and phosphate [3, 4]. Enzymes like lysozyme, lactoferrin, and peroxidase contribute to its antimicrobial function by inhibiting bacterial growth [5]. Other important factors that control pH values are maintaining adequate oral hygiene to remove plaque from tooth surfaces, following a low-sugar diet, and avoiding frequent snacking [6].

The present study evaluated caries susceptibility in pediatric patients scheduled for orthodontic treatment (control group) and undergoing orthodontic treatment (trial group) with rapid palatal expander (RPE).

Despite numerous studies on caries prevention and treatment, there is a lack of data on the specific impact of RPE on caries. Orthodontic appliances may increase risk of caries because of various factors: they can promote plaque accumulation in hard-to-reach areas [7] and change salivary flow and composition, making it less effective in neutralizing acids and more difficult to control oral pH [8]. Changes in dietary habits have also been observed: orthodontic patients often avoid hard and fibrous foods, such as raw fruits, vegetables, and whole grains, preferring soft, processed foods high in sugars [9]. These foods provide an ideal substrate for bacterial fermentation, resulting in acid production and a drop in pH [10].

Considering these premises, it is expected that RPE patients will show a lower salivary buffering capacity and higher plaque index compared to non-RPE patients, that can accelerate the caries formation process.

Therefore, the aim of this study was to evaluate the effect of RPE treatment on salivary pH, buffering capacity, and plaque index in children aged 6–14.

The first null hypothesis of this study is that there is no statistically significant difference in salivary pH between the two groups. The second null hypothesis is that there is no difference in salivary parameters recorded. The third null hypothesis is that there is no difference in plaque accumulation between the two groups.

## 2. Materials and methods

### 2.1 Study design

This was a cross-sectional study approved by the Unit Internal Review Board (2024-0605). The protocol was registered on clinicaltrials.gov (NCT: NCT06922799).

### 2.2 Participants

Pediatric patients aged 6–14 years presenting for dental care at the Unit of Orthodontics and Pediatric Dentistry, Section of Dentistry, Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy were asked to participate in the study. Written informed consent was signed by parents/legal guardians of patients for the participation in the study. Patients were allocated to two groups: the control group which included patients who had completed general dental treatments but had not yet started orthodontic treatment, and the trial group, which included patients undergoing orthodontic treatment with Hyrax RPE for at least six months. RPE was bonded on first permanent molar or on second deciduous molars only if these teeth weren't decayed and if the second premolar cusp was apical to HPC (half-pulp-chamber) line [11].

The following inclusion criteria were considered: children aged 6–14 years; no previous orthodontic treatment for allocation in the control group, ongoing orthodontic treatment with RPE for at least 6 months for the allocation in the trial group; parental or legal guardian consent for the participation.

The following exclusion criteria were considered: systemic conditions affecting salivary composition (diabetes, Sjogren's syndrome); recent antibiotic use or other medication that could influence saliva composition in the weeks prior to testing; patients with oral anomalies that may interfere with data collection or affect test results.

### 2.3 Interventions and outcomes

After signing the informed consent, patients underwent a series of clinical and salivary tests to evaluate caries risk, dental plaque, saliva quality, and bacterial composition. Each parameter was monitored before the beginning of the orthodontic treatment (for the control group) or during a routine check-up (for the trial group). All tests were performed by an instructed operator, just as the periodontal and oral indices.

To assess caries experience in mixed dentition, "DMFT + dmft" (Decayed, Missing, Filled Teeth) and "DMFS + dmfs" (Decayed, Missing, Filled Surfaces) were adopted [12]. DMFT + dmft index includes decayed teeth (teeth with a visible cavity that requires treatment), missing teeth (teeth that have been extracted due to advanced caries or other reasons) and filled teeth (teeth that have been treated with a dental filling to repair previous decay). DMFS + dmfs (Decayed, Missing, Filled Surfaces) index was determined after a clinical examination by considering all tooth surfaces damaged by caries or filled (mesial, distal, occlusal, buccal, and lingual).

To assess patients' level of cooperation, Frankl Index was used [13]. The scale consists of four categories. In Frankl 1 the cooperation is definitely negative: the child refuses treatment, cries forcefully, exhibits fearfulness, or is extremely uncooperative. In Frankl 2 the child is reluctant to accept treatment, is uncooperative, and has some negative attitude but is not forcefully resistant. In Frankl 3 the child accepts treatment but may be cautious or reserved. The child follows the dentist's directions cooperatively but may show some hesitation or minor signs of negativity. In Frankl 4 the child displays a positive attitude throughout the treatment. Frankl parameter was included to assess the baseline level of anxiety.

To assess the presence of dental plaque, the plaque control record (PCR) was used for both primary and permanent teeth: GC Tri Plaque ID Gel disclosing agent (400392718, GC International AG, Luzern, Switzerland) was placed on all tooth surfaces, and the presence or absence of plaque was recorded as a percentage of the number of tooth surfaces with plaque relative to the total number of tooth surfaces examined [14].

The Saliva-Check Buffer kit (219983445, GC International AG, Luzern, Switzerland) was used to evaluate the amount

of saliva, salivary pH, viscosity, flow and composition thanks to five different salivary tests: tests 1, 2 and 3 concerned resting saliva, while tests 4 and 5 concerned stimulated saliva. The salivary tests and the periodontal indexes were performed/recorded by a single examiner. The intra-rater reliability was assessed using strips with different pH colours and two assessments were assigned with a time frame of 14 days. An intra-operator reliability of 0.999 was obtained. All tests were conducted on the same day, in the morning and before the RPE review. Before data collection, patients were instructed as follows: to avoid eating before the dental appointment, to refrain from using mouthwashes, and to brush their teeth three times a day with a soft-bristled manual toothbrush and a provided toothpaste (Elmex Junior, Colgate-GABA, Switzerland).

"Salivary hydration test" consisted in a visual inspection of level of hydration, considering the lower lip labial gland secretion. After the eversion of the lower lip, the labial mucosa was gently blotted with a small piece of gauze and the mucosa was observed under good light to assess the time for visible production of saliva droplets at the orifices of the minor glands. The resting flow was considered as "normal" or "low" if the saliva production time was respectively less or greater than 60 seconds [15].

"Salivary consistency test" visually assessed the resting salivary consistency in the oral cavity. The viscosity was considered as "normal" with watery clear saliva, "increased" with frothy bubbly saliva or with sticky frothy saliva residues [15].

For "Salivary pH test", the patient expectorated any pooled saliva into the collection cup. A pH test strip was placed into the sample of resting saliva for 10 seconds, and then the colour of the strip was checked and compared with the testing chart available in the package. Green nuances indicated healthy saliva (pH 6.8–7.8), yellow nuances indicated moderately acidic saliva (pH 6.0–6.6), red nuances indicated highly acidic saliva (pH 5.0–5.8) [16, 17] (Fig. 1, Ref. [18]).

"Salivary quantity test" was performed as follow: the patient was instructed to chew standardized 1 × 1 × 1 cm piece of tasteless paraffin wax to stimulate salivary flow, after 30 seconds the patient expectorated into the spittoon, and then continued chewing for a further 5 minutes, collecting all the saliva into the collection cup at regular intervals. The quantity of saliva was measured by checking the mL markings on the side of the cup. Quantity of saliva at 5 minutes was considered as follow: "very low" <3.5 mL, "low" between 5.0–3.5 mL, "normal" >5.0 mL [18, 19].

To perform "Salivary buffering capacity test", a buffer test strip was removed from the foil package and placed onto an absorbent tissue with the test side up. Using a pipette, saliva was drawn from the collection cup (Fig. 2A, Ref. [18]) and one drop was dispensed onto each of the 3 test pads (Fig. 2B). The strip was immediately turned 90° to soak up excess saliva on the absorbent tissue. This prevented the excess saliva from swelling on the test pad and possibly affecting the accuracy of the test result. The test pads began to change colour immediately and after 2 minutes the final result could be calculated by adding the points according to the final colour of each pad as follow: "green" 4 points, "green/blue" 3 points, "blue" 2 points, "red/blue" 1 point, "red" 0 points (Fig. 2C).

Buffering capability of saliva was defined as "very low" if the score was 0–5, "low" if the score was 6–9, "normal/high" if the score was 10–12 [15, 19].

## 2.4 Sample size

Sample size was calculated on "salivary pH" as the primary outcome. Considering alpha = 0.05 and power = 80%, an expected mean of 6.287 with an expected mean difference of 0.462 and a standard deviation of 0.52 were hypothesized based on data from previous literature [19]. The study required 40 patients with an equal distribution into the two groups.

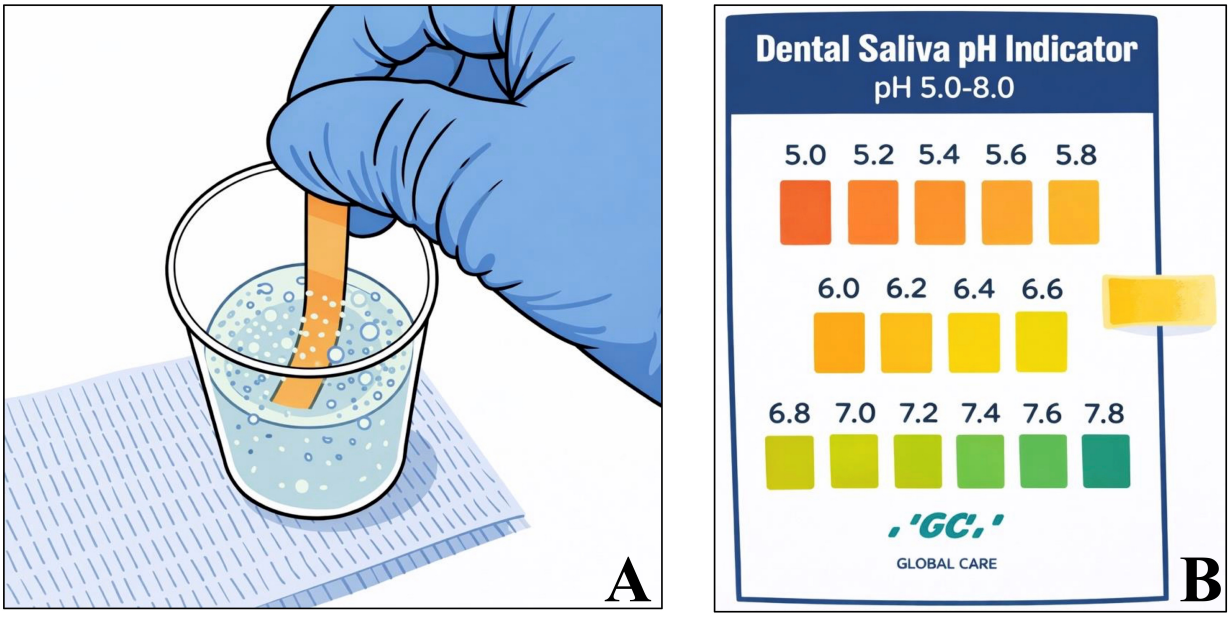


**FIGURE 1. Salivary pH evaluated with test 3.** (A) strip placed in the dispensing cup; (B) pH test legend. Adapted from [18] under Creative Commons Attribution (CC-BY) license.

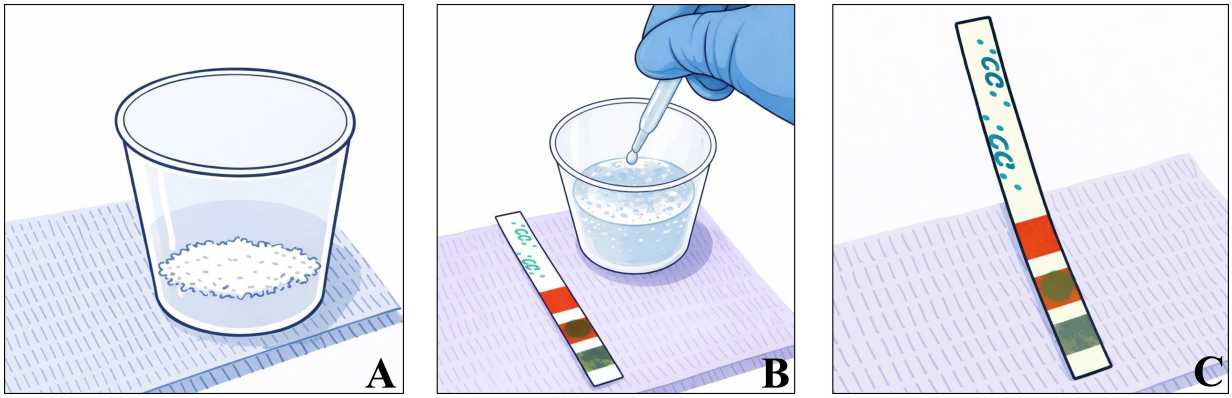


**FIGURE 2. Salivary buffer capacity test.** (A) saliva collected in the dispensing cup; (B) use of a pipette to dispense saliva drops onto each of the 3 test pads; (C) Buffer test value. Adapted from [18] under Creative Commons Attribution (CC-BY) license.

## 2.5 Statistical analysis

Data were statistically analysed with R software (R version 3.1.3, R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics (mean, standard deviation, median, minimum and maximum values) were calculated for each variable. Data normality of distributions was assessed with the Kolmogorov-Smirnov test. Subsequently, the Mann-Whitney test was applied for all the variables of the study.

Linear regressions were performed to evaluate the influence of independent variables (the five salivary tests) on dependent variables (DMFT + dmft, DMFS + dmfs, PCR, Frankl, group, sex, age).

Significance was predetermined for $p < 0.05$.

## 3. Results

The study started in April and ended in May 2025. 40 patients were recruited for the study. They all fulfilled the inclusion criteria and accepted to participate in the study, they all received the allocated interventions, and none was excluded from analysis.

The mean age of the participants at the beginning of the study was 9.75 ± 2.7 years. The baseline characteristics of the study sample are shown in Table 1.

Data about plaque, caries and compliance of patients included are shown in Table 2. According to the findings of the study, there was a significantly lower PCR score in the control group in contrast with the trial group ($p < 0.05$), while no significant differences were found for the other variables ($p > 0.05$). No significant changes for Frankl score imply that neither the Trial Group and the Control group perceived the procedures as invasive and that no change in cooperation was observed over the study period.

Data about the five salivary tests are shown in Table 3. As regards "Salivary hydration" and "Salivary consistency", no statistically significant differences were found between the two groups ($p > 0.05$).

"Salivary pH" and "Salivary buffering capacity" showed a statistically significant difference between the two groups, with lower values for the trial group ($p < 0.05$).

"Salivary quantity" showed a statistically significant difference too, with higher values for the trial group ($p < 0.05$).

Linear regressions were calculated considering DMFT + dmft, DMFS + dmfs, PCR, Frankl tests, group, sex, age as independent variables and are shown in Table 4. Salivary consistency test resulted significantly influenced by DMFS + dmfs and age ($p < 0.05$). Salivary pH test resulted significantly influenced by DMFS + dmfs, PCR and group ($p < 0.05$). Group and sex appeared to significantly influence saliva quantity test ($p < 0.05$). Salivary buffering capacity test resulted significantly influenced by DMFS + dmfs, PCR, Frankl and group ($p < 0.05$).

Table 5 was provided in order to explain the influences of independent variables on dependent variables.

**TABLE 1. Baseline demographic data of the study sample.**

| | | n (mean age ± standard deviation) | |
|---|---|---|---|
| | | Control | Trial |
| Group | | 20 (9.55 ± 2.72) | 20 (9.95 ± 2.72) |
| Sex | | | |
| | Males | 10 (8.70 ± 2.06) | 9 (10.22 ± 2.91) |
| | Females | 10 (10.40 ± 3.13) | 11 (9.73 ± 2.69) |

*n, number.*

**TABLE 2. Assessment of oral indexes: DMFT + dmft, DMFS + dmfs, PCR.**

| Oral index | Groups | Mean | SD | Min | Mdn | Max | *p* Value |
|---|---|---|---|---|---|---|---|
| DMFT + dmft | | | | | | | |
| | Control | 3.45 | 1.85 | 1.00 | 3.00 | 7.00 | 0.892 |
| | Trial | 3.15 | 1.14 | 1.00 | 3.00 | 5.00 | |
| DMFS + dmfs | | | | | | | |
| | Control | 3.95 | 1.26 | 1.00 | 3.00 | 8.00 | 0.305 |
| | Trial | 4.45 | 1.67 | 2.00 | 4.00 | 8.00 | |
| PCR | | | | | | | |
| | Control | 32.98 | 9.86 | 18.00 | 31.50 | 53.00 | <0.0001* |
| | Trial | 59.05 | 13.66 | 37.00 | 58.50 | 81.00 | |
| Frankl | | | | | | | |
| | Control | 3.10 | 0.79 | 1.00 | 3.00 | 4.00 | 0.789 |
| | Trial | 3.20 | 0.70 | 2.00 | 3.00 | 4.00 | |

*SD, standard deviation; Min, minimum; Mdn, median; Max, maximum; DMFT + dmft, Decayed, Missing, Filled Teeth; DMFS + dmfs, Decayed, Missing, Filled Surfaces; PCR, plaque control record. *p < 0.05.*

**TABLE 3. Salivary tests.**

| Salivary Test | Groups | Mean | SD | Min | Mdn | Max | *p* Value |
|---|---|---|---|---|---|---|---|
| Salivary hydration | | | | | | | |
| | Control | 0.45 | 0.51 | 0.00 | 0.00 | 1.00 | 0.341 |
| | Trial | 0.65 | 0.49 | 0.00 | 1.00 | 1.00 | |
| Salivary consistency | | | | | | | |
| | Control | 0.85 | 0.67 | 0.00 | 1.00 | 2.00 | 0.556 |
| | Trial | 0.90 | 0.31 | 0.00 | 1.00 | 1.00 | |
| Salivary pH | | | | | | | |
| | Control | 6.65 | 0.45 | 6.00 | 6.60 | 7.60 | 0.0004* |
| | Trial | 5.88 | 0.60 | 5.00 | 5.80 | 6.60 | |
| Salivary quantity | | | | | | | |
| | Control | 0.90 | 0.72 | 0.00 | 1.00 | 2.00 | 0.003* |
| | Trial | 1.60 | 0.50 | 1.00 | 2.00 | 2.00 | |
| Salivary buffering capacity | | | | | | | |
| | Control | 1.55 | 0.51 | 1.00 | 2.00 | 2.00 | <0.0001* |
| | Trial | 0.60 | 0.50 | 0.00 | 1.00 | 1.00 | |

*SD, standard deviation; Min, minimum; Mdn, median; Max, maximum. *p < 0.05.*

**TABLE 4. *p* values of linear regressions for the variables considered in the study.**

| | Dependent Variables | | | | |
|---|---|---|---|---|---|
| Independent Variables | Salivary hydration test | Salivary consistency test | Salivary pH test | Salivary quantity test | Salivary buffering capacity test |
| DMFT + dmft | 0.247 | 0.051 | 0.102 | 0.239 | 0.988 |
| DMFS + dmfs | 0.295 | 0.026* | 0.047* | 0.574 | 0.038* |
| PCR | 0.306 | 0.985 | 0.002** | 0.062 | <0.001* |
| Frankl | 0.581 | 0.349 | 0.620 | 0.091 | 0.026* |
| Group | 0.214 | 0.764 | <0.001* | <0.001* | <0.001* |
| Sex | 0.734 | 0.147 | 0.666 | 0.032* | 0.797 |
| Age | 0.602 | <0.001* | 0.451 | 0.902 | 0.342 |

**p < 0.05. DMFT + dmft, Decayed, Missing, Filled Teeth; DMFS + dmfs, Decayed, Missing, Filled Surfaces; PCR, plaque control record.*

**TABLE 5. Interpretation of regression analysis, considering only significant influences according to Table 4.**

| Independent variables | Dependent variables | | | | |
|---|---|---|---|---|---|
| | Salivary hydration test | Salivary consistency test | Salivary pH test | Salivary quantity test | Salivary buffering capacity test |
| ↑ DMFT + dmft | | | | | |
| ↑ DMFS + dmfs | | ↓ | ↓ | | ↓ |
| ↑ PCR | | | ↓ | | ↓ |
| ↑ Frankl | | | | | ↓ |
| Group | | | ↑ in trial group | ↑ in trial group | ↑ in trial group |
| Sex | | | | ↑ in females | |
| ↑ Age | | ↓ | | | |

*↓, decrease; ↑, increase. DMFT + dmft, Decayed, Missing, Filled Teeth; DMFS + dmfs, Decayed, Missing, Filled Surfaces; PCR, plaque control record.*

## 4. Discussion

The aim of this study was to evaluate caries susceptibility in RPE and non-RPE patients, by analysing parameters about saliva, plaque and caries before beginning the orthodontic treatment for the control group and after six months of treatment for the trial group. To the best of our knowledge, there are no studies in literature that analyse these parameters in patients with rapid palatal expander, but only in patients with fixed orthodontic appliance. As a consequence, our results were compared with studies on fixed orthodontic treatment. Fixed appliances influence the oral environment, in particular both clinical and salivary properties. Many studies have been conducted to evaluate the microbiological changes in orthodontic patients [20–22]. On the other hand, a few previous reports investigated the changes in saliva pH, saliva buffering capacity and saliva amount. Moreover, results are not consistent [8, 23–30].

The first null hypothesis was rejected, as RPE patients showed a significantly lower pH value than non-RPE patients. Kanaya *et al.* [22] also found a decreased pH associated with an increased number of acidogenic bacteria, comparing 35 orthodontic patients and 36 non-orthodontic patients. These results are consistent with studies of other authors [8, 23]. In contrast with previous observations, some studies found a significant increase in pH values in orthodontic patients [25–28], that indicates higher basicity. These differences could be explained by the fact that RPE remains in the mouth for only a short time. During this time, maintaining oral health is possible through good oral hygiene practices, dietary advice, and topical fluoride application.

The second null hypothesis was partially rejected. As regards saliva flow, a significant increase of saliva quantity was found in RPE patients. This data agrees with current evidence [25–27], and it could be related to a higher stimulation of oral mechanoreceptors that increase salivary secretion. In particular, these studies saw that the salivary flow rate increased significantly with the weight and dimension of the stimulus. Saliva quantity showed to be significantly influenced by group and sex. In females "saliva quantity test" showed higher values. Other authors [27] found that males showed a higher buffer capacity than females, in contrast to the present report. The differences were attributed to two theories: women present smaller salivary glands in comparison with men [31], and the female hormonal pattern may contribute to diminished salivary secretion [32]. It should be highlighted that the experimental conditions are different and variability among studies should be considered.

About saliva buffering capacity, there is not a consistent agreement in literature. In this study, RPE patients had a significantly lower salivary buffer capacity. A relationship could exist between the reduction of pH and buffering capacity that have been found in patients with RPE: a lower buffering capacity translates into a reduced ability to neutralize acids produced by acidogenic bacteria, and, as consequence, pH decreases toward acidic values. On the other hand, findings of other authors [25, 27, 29] showed an opposite attitude, considering this behaviour as a physiologic response to maintain oral homeostasis in adverse situations. Other studies [26] did not find a significant difference between the two groups, suggesting that fixed orthodontic appliances are not the only factors increasing the patient's caries risk during orthodontic treatment. Different buffer capacity between the present study and the study abovementioned [26] might be explained by the lower mean age of the patients enrolled in the present study. Another possible explanation could be related to the use of different orthodontic devices: RPE and fixed orthodontic appliances. Additionally, age might affect this parameter: salivary consistency resulted significantly influenced by age in the present study, but this finding was not confirmed by a recent systematic review [30] on the topic, as these parameters are recorded using tests that are not standardised with each other.

About the correlation between saliva amount and saliva buffer capacity, literature is not consistent: some studies found a significant increase in salivary flow rate without significant changes in buffer capacity, suggesting that salivary flow rate is more sensitive to the placement of orthodontic appliances than buffer capacity [25, 27, 29]. Other authors found a significant increase in salivary flow rate and an increase in buffer capacity [26]. In the present study, a significant increase in salivary flow rate was found together with a significant decrease in

buffer capacity. Maybe other factors interfered with these variables (sex, age, type of orthodontic appliance). It could be interesting to analyse in future studies comparable samples in age and type of orthodontic appliance.

The third null hypothesis was rejected. PCR test in this study showed a significant increase of plaque in RPE patients in respect to non-RPE patients, as seen previously [29, 33], and it significantly influence salivary pH and buffer capacity. The evidence of a correlation between these variables is supported by literature, and it's due to bacteria in oral environment [25–27]. As reported above, buffer capacity depends on sex for hormonal factors [26], salivary consistency is significantly influenced by age.

Despite statistically significantly changes in pH, buffer capacity and plaque amount, no significant changes in DMFS + dmfs and DMFT + dmft index were found in RPE versus non-RPE patients. Varma S *et al.* [33] and Choi YY [34] also obtained similar results. This finding was possibly due to the lack of sensitivity of DMFS + dmfs and DMFT + dmft indices that are used conventionally at the D3 thresholding [35, 36] that is the point at which a lesion has penetrated dentine. This implies that carious lesions are often not detected at D1 (non-cavitated yet clinically detectable enamel lesions with intact surfaces) and D2 (cavitated lesion penetrating the enamel or shadowing) [37]. Another explanation could be attributed to the relatively short duration of RPE treatment in the analyzed patients. It is important to notice that even if there were no significant changes in DMFS + dmfs and DMFT + dmft index between the two groups, caries experience significantly influence many variables (salivary pH, buffer capacity, and consistency). In particular with an increase of DMFS + dmfs index, there is a decrease in salivary consistency, pH and buffering capacity. These data can be explained by considering that the presence of caries causes a change in the oral environment. In particular, pH is more acidic and buffer capacity is reduced [25–27].

About cooperation, it was found that Frankl index significantly influences buffer capacity; even though the Frankl scores were quite high, this finding can be explained with a stress-induced production of bicarbonate, which correlates tightly with flow rate.

The findings of the present report suggest that RPE orthodontic treatment may contribute to increased plaque accumulation and reduced salivary buffering capacity, potentially elevating caries risk. Enhanced oral hygiene measures are recommended for orthodontic patients. Future studies testing the different preventive strategies and the use of various remineralizing agents [38–41] during orthodontic treatment are needed.

This study presents some limits, such as difficulties in qualitative and quantitative saliva assessments, the use of operator dependent tests, and the cross-sectional study model. Since there are no studies in the literature evaluating these parameters in patients with rapid palatal expander, it might be interesting to continue the research in this category of patients. In particular, future studies might evaluate if there are differences in saliva parameters by using different types of rapid palatal expanders (RPE, Leaf Expander, with or without skeletal anchorage).

It could be interesting to evaluate salivary biomarkers to overcome the operator's influence on variables' evaluation. A recent systematic review highlighted the potential role of salivary biomarker concentration levels in detecting and assessing dental caries in both children and adults, although it emphasized the need for methodological standardization before routine clinical application [42]. Other evidence has also demonstrated that salivary protein biomarkers may reflect the biological processes underlying orthodontic tooth movement, suggesting their potential use in monitoring treatment-related tissue remodeling [43]. It could be interesting to analyze the relationship of salivary biomarkers and changes in plaque and saliva in orthodontic patients, taking into consideration also microbiological samples that could be directly implicated in caries development. Additionally, the addition of toothbrushing frequency, dental floss usage and use of other preventive agents like mouthwashes as variables could be of great interest for future studies.

## 5. Conclusions

The result of this study suggest the presence of different risk factors of caries between patients undergoing orthodontic treatment with rapid palatal expander and non-RPE patients. In particular, patients undergoing RPE treatment showed a significantly higher plaque retention on dental surfaces, and a significantly lower salivary pH value and buffering capacity. A significantly higher quantity of saliva was also found in RPE patients than in non-RPE patients. However, despite these differences, DMFS + dmfs and DMFT + dmft index scores between the groups did not show statistically significant variations.

### AVAILABILITY OF DATA AND MATERIALS

Data are available on request from the corresponding author.

### AUTHOR CONTRIBUTIONS

MCV, MFS, MAM and AS—designed the research study. AMS—performed the research. AS—analyzed the data. EM and MP—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Unit Internal Review Board (approval number: 2024-0605) and registered on clinicaltrials.gov (NCT06922799). Written informed consent from parents/legal guardians of the patients involved was previously collected.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest. Andrea Scribante is serving as the Editor in Chief of this journal. Maurizio Pascadopoli is serving as one of the Editorial Board members of this journal. We declare that Andrea Scribante and Maurizio Pascadopoli had no involvement in the peer review of this article and have no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to BN.

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