Salivary Streptococcus Mutans and Lactobacillus spp. levels in patients during rapid palatal expansion

**ABSTRACT**

**Aim** To assess the microbial level of Streptococcus Mutans and Lactobacillus spp. during rapid palatal expansion, and compare the data with untreated control patients.

**Materials and methods** Study design: Thirty patients aged between 6-9 years were enrolled in this study (15 males and 15 females). The patients were divided into three groups: 10 patients were treated with rapid palatal expander (RPE) (Test Group 1), 10 patients were treated with McNamara expander, and 10 patients were enrolled in the control untreated group. Whole stimulated saliva was collected from each patient at three time points: before initiation of expansion therapy (baseline at T0), after 3 months (T1), after initiation of treatment, and after 6 months from T0 (T2). The protocol of rapid palatal expansion for the two groups was as follows: at placement of the expander 4 activations were performed by the orthodontist (1 mm expansion), followed by 4 activations per day by the parents (two in the morning and two in the evening, 1 mm per day total) to be repeated for 7 days.

**Results** Statistics: In this study a different trend in the microbial colonisation for the two treated groups was observed. In the Test Group 1, in which patients were treated with the RPE, there was a significant difference between Strp T0 T1 and between Strp T0 and T2 (p<0.05). There was also a significant difference between LAC T1 T0 and LAC T2 and T0 (p<0.05). In the Test Group 2, treated with McNamara expanders, it was found was a significant difference between LAC T2 T0 and LAC T1 T0. In the same group it was also found a significant difference between Strp T2 T0; T1 T0; T1 T2 (p<0.05).

**Conclusion** The level of the various species of bacteria changes during rapid palatal expansion, and this seems to depend on the type of orthodontic expander. During rapid palatal expansion treatment it is also advisable a periodical microbial monitoring using in-office bacteria tests.

**Keywords** Lactobacillus spp; Rapid palatal expansion; Salivary microbial levels; Streptococcus mutans.

**Introduction**

Dental caries is a public health problem in both developed and developing countries; in the latter it is gradually increasing due to the growing consumption of sugary food, alcohol, substance abuse, cigarette smoking, poor oral care practices and inadequate health service [Ser, 1984; Marsh, 2003; Ayele et al., 2013]. In the last 30 years, caries risk assessment has centred on the analysis of bacteriological, salivary, and clinical markers that can be used as risk predictors [Powell, 1998; Sanchez-Perez et al., 2009]. As caries is a multi-factorial disease, a comprehensive risk assessment should evaluate the major factors involved with it, like in the salivary test.

Dental caries is caused by colonisation of oral microorganisms and accumulation of extracellular polysaccharides synthesised by Streptococcus Mutans with the synergistic influence of Lactobacillus spp. bacteria [Dziedzic et al., 2013].

Fixed orthodontic appliances hinder cleaning of teeth and favour the retention of dental plaque. Placement of fixed orthodontic appliances results in a change in the oral environment, leading to increased bacterial density in dental plaque. The number of S. Mutans can increase by up to 5-fold during orthodontic treatment. High numbers of colony-forming units of Lactobacillus spp. have been associated with the use of orthodontic appliances and known to play a role in the increased levels of plaque seen in many orthodontic patients [Huser et al., 1990; Rosenbloom and Tinanoff, 1991; Kupietzky et al., 2005]. In this study the levels of S. Mutans and Lactobacillus spp. were evaluated in the oral saliva of orthodontic patients treated with RPE and McNamara expander and then compared with those of an untreated control group, at baseline and after three and six months. In fact, transversal discrepancies present an unbalance between the transversal diameters of the upper maxilla and those of the mandible which can be due to a reduction in the transverse diameter and to an increase in the mandibular transverse diameter, or both conditions combined: unilateral crossbite and bilateral crossbite (Giua et al., 2009).
Materials and methods

This study was conducted in accordance with the Declaration of Helsinki. The Committee on Ethics in Science of the University of L’Aquila, L’Aquila, Italy approved the study. The subjects participating in the study group were selected from the children attending Amiteurnum’s schools (the schools in the Amiteurnum group have entered into an agreement with our university for a project of oral prevention). Only students whose parents had previously signed the written informed consent were enrolled. Thirty patients were enrolled in this study (15 males and 15 females) aged between 6-9 years and based on the following criteria for inclusion/exclusion.

Inclusion criteria:
• subjects attending Amiteurnum’s schools;
• informed consent signed by parents;
• children treated for rapid palatal expansion.

Exclusion criteria
• refusal to sign the informed consent;
• periodontal diseases;
• History of systemic or metabolic diseases;
• growth disorders;
• cognitive handicaps;
• congenital diseases;
• current antibiotics treatment or use of antiseptic or antibacterial mouth rinse;
• presence of acute oral infections;
• decreased saliva flow (dry mouth syndrome).

The 30 patients were divided into three groups: 10 patients were treated with RPE and 10 patients were treated with the McNamara expander. Ten patients were enrolled in the control untreated group.

Before the beginning of the study, standard oral hygiene instructions were provided to all participants (modified Bass technique), with special attention to the orthodontic appliances. A session of professional oral hygiene was also administered before the beginning of the study. During the study, all patients were highly motivated to careful home oral hygiene (all groups were advised to use the same toothpaste containing fluorinated amine at least twice a day, and a mouthwash containing fluoride once a day).

The authors were unblinded at the time of the first saliva collection. Whole stimulated saliva was collected from each patient at three time points: before initiation of expansion therapy (baseline at T0), 3 months after the initiation of treatment (T1), and after 6 months from T0 (T2).

The subjects had to refrain from eating or drinking beverages, or brushing their teeth for at least 1 hour before saliva collection, as indicated by the manufacturer of the in-office kit, as these actions can affect the mean salivary flow. The protocol of rapid palatal expansion for the two groups was as follows: at placement of the expander 4 activations were carried out in-office by the orthodontist (1 mm expansion), followed later by 4 activations per day by the parents (2 in the morning and 2 in the evening, totaling 1 mm per day) to be repeated for 7 days. The expansion and retention regimens were decided by 1 operator (A.M.), based on clinician’s preference and the patients’ malocclusion.

Salivary tests: assessment of S. mutans and Lactobacillus spp. colonisation

One investigator collected stimulated saliva from each individual, according to the manufacturer’s specification. Saliva samples were stimulated with paraffin-based sticks (1 minute) and collected into sterile flasks (2 ml on the average). The tablets of NaHCO3 were placed at the bottom of the vials. All samples were collected by the same investigator, using the same technique and procedure. The test vials were placed upright in the incubator and incubated at 37°C/99°F for 48 hours. After removal of the vial from the incubator, the density of the MS and LB colonies was compared with the corresponding evaluation pictures in the model chart contained in the kit. The values of <105 and >105 were recorded for the low and high CFU ranking, based on the scale provided in the CRT kit (Ivoclar-Vivadent, Liechtenstein) (Fig. 1). Each sample was evaluated by the same examiner. Bacteria strains, Mutans Streptococci and Lactobacillus spp. isolated from clinical specimens, underwent further incubation.

S. mutans was detected as small blue colonies with a diameter of < 1 mm on the blue agar, while Lactobacillus spp. was detected as white colonies on the clear agar. Comparison with the corresponding pictures in the model chart permitted the assessment of the caries risk [Mummolo et al., 2013]. Findings of 105 CFU or more of Lactobacilli and Mutans Streptococci per ml saliva indicate a high caries risk [Krass, 1988].

Assessment of salivary flow

Salivation was stimulated by asking the subject to chew one 1 g capsule of paraffin for 5 min. Saliva was collected at each 1-min interval in a 15 ml screw-top tube up to 5 min; the minimum volume of stimulated saliva required for processing was 2.0 ml for both tests.

Assessment of the buffering capacity of saliva

The CRT buffer test establishes the protective action
of saliva quickly and effectively. The buffer systems contained in saliva are able to neutralise acids that can damage the teeth. Determination of the buffering power of saliva permits to assess the buffer system in each case. The CRT test is a quick and effective method to evaluate the buffering capacity of saliva, which can be classified as low, medium or high.

Plaque Index

The status of dental hygiene was determined using the O’Leary Plaque Control Record, which is obtained by the examination of four dental surfaces of all teeth. The Plaque Control Record index includes the percentage of dental surfaces with dental plaque (Lee et al., 1986).

Results

Sample size calculation

The sample size calculation determined that 7 subjects per treatment arm would provide 90% power to detect a true difference of 0.7 between test and control group using CFU 105 as the primary outcome variable, assuming that the common standard deviation is 0.4 mm. Accordingly, a sample of 10 subjects per arm were to be recruited to compensate for possible drop-out during the study period. The significance of the differences was evaluated using a mixed ANOVA method. Since significant differences were found, in order to evaluate the differences within each group the Wilcoxon signed rank test was used, while for assessing the differences between the groups at each follow-up was used the Mann-Whitney U test. The level of significance was assumed to be p ≤ 0.05 for all tests. Values are reported as mean ± standard deviation (Table 1). No significant difference between the groups with the exception of Lac T2 was statistically significant between Test 2 and Control Group. In the Control Group there were no significant differences between T0, T1 and T2. In the Group Test 1 there was no statistically significant difference between T1 and T2 and between LAC T1 and T2 and Strp T1 and T2, for other comparisons the differences were statistically significant (as follows):

- LAC T1 - LAC T0; p=0.011;
- Strp T1 - Strp T0; p=0.005;
- LAC T2 - LAC T0; p=0.007;
- Strp T2 - Strp T0; p=0.006.

In the Test Group 2 no statistically significant difference between T1 and T2 LAC, for other comparisons the differences were statistically significant (as follows):

- LAC T2 - LAC T0; p=0.006;
- Strp T2 - Strp T0; p=0.004;
- LAC T1 - LAC T0; p=0.01;
- Strp T1 - Strp T0; p=0.006;
- Strp T1 - Strp T2; p=0.03.

Discussion

In this research, we evaluated the microbial level of Streptococcus Mutans and Lactobacillus spp. during some important phases of the rapid palatal expansion (with RPE or Mc Namara expander), and we compared the results with untreated control subjects. The plaque index, the salivary flow and the buffer capacity were taken into account in assessing a possible predisposition to caries. These bacteria tests seem to be helpful aids for the dentist. Also, these detection system are suitable for use by clinical personnel in dental clinics or other non-laboratory settings for identification of subjects potentially at risk for caries (Jordan et al., 1987). In this study it was not evaluated the adhesion of plaque and biofilm on the bands or acrylic splints of the expanders but the aim was to evaluate the changes in the levels of Streptococcus Mutans and Lactobacillus spp. in saliva. A continuous microbial monitoring, performed by dentists using in-office bacteria tests, seems to be indicated during the orthodontic treatment, in order to improve the preventive approach to oral diseases [Mummolo et al., 2013]. No statistically significant difference among the groups with the exception of Lac T2 was found between Test 2 and Control Group. In fact the high numbers of colony-forming units of Lactobacillus spp. have been associated with the use of orthodontic appliances and known to play a role in the increased levels of plaque seen in many orthodontic patients [Huser et al., 1990; Rosenbloom and Tinanoff, 1991]. Looking at the results of another study is evident that that a higher number of CFUs of LB is associated with the group wearing orthodontic appliances after two months and may play a role in the increased levels of plaque seen in many orthodontic patients [Kupietzky et al., 2005]. In this study a different trend in microbial colonisation for the two treated groups was observed. In the Group Test 1, in which patients were treated with the RPE, there is a significant difference between Strp T0 T1 and between Strp T0 and T2 (p<0.05). There is also a significant difference between LAC T1 T0 and LAC T2 and T0 (p<0.05). In the Group Test 2, in which patients were treated with Mc Namara expanders, there is a significant difference between LAC T2 T0 and LAC T1 T0. In the same group there is also a significant

<table>
<thead>
<tr>
<th>Test</th>
<th>Test2</th>
<th>Control</th>
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<tbody>
<tr>
<td>T0Lac</td>
<td>1.6±0.84</td>
<td>1.3±0.67</td>
</tr>
<tr>
<td>T1Lac</td>
<td>2.4±0.69</td>
<td>2.6±1.07</td>
</tr>
<tr>
<td>T2Lac</td>
<td>2.5±0.52</td>
<td>3±0.94</td>
</tr>
<tr>
<td>T0Strp</td>
<td>0.8±0.78</td>
<td>0.8±0.78</td>
</tr>
<tr>
<td>T1Strp</td>
<td>1.9±0.73</td>
<td>2±0.66</td>
</tr>
<tr>
<td>T2Strp</td>
<td>2.2±1.03</td>
<td>2.6±0.51</td>
</tr>
</tbody>
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TAB. 1 Values of microbial colonisation.
difference between Strp T2 T0; T1 T0; T1 T2 (p<0.05). These results are not comparable with others results in literature. There is only a study in which the authors compared Streptococcus mutans and Lactobacillus spp during orthodontic treatments with conventional or self-ligating brackets [Mummolo et al., 2013]. However the correlation between the two bacterial species is known. In fact the number of lactobacilli increases if S. mutans start to colonise in the oral cavity, since they produce a favourable acid environment for Lactobacillus spp., and the pH-value decreases. Lactobacillus spp. preferably settle in niches with a low pH-value and in the vicinity of plaque accumulation. Consequently, Lactobacillus spp. can be more easily found in cavities and carious dentin. In contrast to S. mutans, Lactobacillus spp. do not adhere to tooth surfaces on their own account, but need natural or iatrogenic retention niches, such as bands or acrylic splints, where it is usually difficult to reach and clean [Marasas et al., 1979; Takahashi and Nyvad, 2011]. The orthodontic treatment changes the oral environment: promotes a major salivary stimulated flow and increases its buffer capacity and salivary pH, which increase the antacaries activity of saliva. It is necessary to maintain the balance between protective and the caries risk factors during orthodontic treatment with a strict home care program toward correct oral hygiene procedures necessary to control plaque accumulation for caries and periodontal disease prevention [Marchetti et al., 2012; Lara-Carrillo et al., 2010].

Conclusion

The objective of this study is to inform the reader about the microbial colonisation of the oral cavity during rapid palatal expansion with RPE and Mc Namara expander. It is very important, before starting therapy with a rapid palatal expander, to assess any risk factors for caries. The use of additional aids, such as evaluation of the PI, salivary flow and assessment of microbial colonisations can help in the prevention of caries during the treatments. There is a need for further well controlled clinical trials with a longer observation period.

References