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Clinical evaluation of salivary indices and levels of Streptococcus mutans and Lactobacillus in patients treated with Occlus-o-Guide

ABSTRACT

Aim *The aim of this study was to assess the changes over time associated with salivary indices and the presence of Streptococcus mutans and Lactobacillus in patients treated with Occlus-o-Guide.*

Materials and methods *Two groups of patients were evaluated: a test group of 20 patients treated with Occlus-o-Guide and a control group of 20 patients not subjected to orthodontic treatment. Both groups were homogeneous for age and sex. We examined the levels of S. Mutans and Lactobacillus, the salivary flow, the buffer capacity of saliva and the Sillness and Loe plaque index (PI). The samples were taken at baseline (T0), after 3 months (T1) and after 6 months of treatment (T2). All data were compared using Student's t test.*

Results *The percentage of patients with a level of Streptococcus mutans able to cause caries was decreased in the test group (T0 = 10%, T1 = 0%, T2 = 0%) compared to the control group (T0 = 0%, T1 = 5%, T2 = 35%), whereas the amount of Lactobacilli was different (test group T0 = 15%, T1 = 0%, T2 = 10%; control group T0 = 0%, T1 = 5%, T2 = 35%). The total salivary flow was increased in the test group (T0 = 47, T1 = 61, T2 = 61) compared to the control group, in which it had remained almost constant (T0 = 44, T1 = 45, T2 = 45). The buffer capacity of saliva was unchanged in both groups over time; the sum of PI-plaque indices was reduced in the test group (T0 = 3, T1 = 0, T2 = 2) compared to the control group (T0 = 0, T1 = 14, T2 = 27).*

Conclusion *Despite the presence of the Occlus-o-Guide device, patients are able to maintain a good level of oral hygiene, showing improvements of the examinated parameters at follow-ups.*

Keywords *Lactobacillus; Occlus-o-Guide; Salivary indices; Plaque; Streptococcus mutans.*

Introduction

The bacterial microflora of the oral cavity contributes to the health of the host by preventing infections from potentially pathogenic exogenous microorganisms, counteracting parasitic species colonisation, and by regulating the inflammatory response against commensal bacteria that inhabit the oral cavity [Marsh et al., 2010; Mummolo et al., 2008].

During caries development, the intake of dietary fermentable carbohydrates lower the oral pH, with subsequent formation of plaque, a process helped by acidogenic and acid-tolerant species as Mutans and other acidogenic streptococci, lactobacilli and bifidobacteria [Socransky et al., 1998]. Streptococcus Mutans is a Gram-positive bacterium, characterised by the following properties: ability to adhere to tooth structures, sugar transport system, production of lactic acid from sugars, production of intra- and extracellular polysaccharides, resistance to acidic environment. Streptococcus mutans colonises areas of difficult access for cleaning and oral hygiene procedures [Featherstone, 2000]. Lactobacillus is instead the main culprit for progression of caries, causing extensive damage to the tooth structure by multiplication and diffusion. Lactobacilli have the following properties: acid production, acid tolerance, resistance to substances that reduce the bacterial load such as chlorhexidine. Their presence indicates a high acid intake by the patient. Not surprisingly, there is a significant correlation between caries and amount of Lactobacilli found in both adults and children [Hardie et al., 1977].

For the prevention of dental caries during orthodontic treatment, we should consider that detection of Streptococcus mutans alone is not decisive, as the concomitant presence of Lactobacillus is required, though the latter can also exist alone [Loesche, 1986].

The aim of this study was to assess the changes associated with the salivary indices over time, and the presence of Streptococcus mutans and Lactobacilli in patients treated with Occlus-o-Guide [Saccucci et al., 2011].

Materials and methods

In this study, we evaluated the levels of Streptococcus mutans and Lactobacillus in the saliva of two groups of patients: a test group of 20 subjects treated with Occlus-o-Guide® and a control group of 20 patients who received no orthodontic treatment. The two groups were comparable for age (18-23 yeras) and sex. The stimulated salivary flows, the buffering capacity of saliva and the Silness-Löe plaque index were also analysed. All patients signed an informed consent in accordance with the ethical standards as reported in the Declaration of Helsinki of 1975, revised in 2000.

The samples were taken at baseline (T0), after three months (T1) and after six months of treatment (T2).

The data regarding the characteristics of saliva and bacterial flora were detected by the prevention system Professional Care (Ivoclar Vivadent Clinical), using the CRT® buffer to collect information from the saliva and the CRT® bacteria for the bacterial count.

All patients had been previously motivated to home oral hygiene. Patients had to refrain from eating, drinking, smoking, and brushing their teeth for at least an hour before the visit, as all these actions could alter the salivary flow.

1. To stimulate salivation, each patient was given a tablet of paraffin to chew for 30 seconds, and then the produced saliva was removed. The patient was given another paraffin tablet to chew for 5 more minutes, and the saliva was collecting as produced in a graduated glass tube. During this procedure, the quantity, in ml, of saliva collected at specific times and the salivary flow, i.e. the amount of saliva produced during the defined period of time of 5 minutes, were recorded.
2. Using the CRT® buffer the buffering capacity of saliva was calculated, and classified as low, medium or high.
3. Using the CRT® bacteria test, the count of Lactobacillus and Streptococcus mutans was performed as follows: from the graduated glass previously filled by the patient, an amount of saliva sufficient to completely wet the culture media (agar) was collected using a pipette.
4. In order to stimulate bacterial growth, a tablet of NaHCO3 was inserted in the container of the culture media.
5. The container was placed in incubator at 35-37 °C for 48 hours.
6. Evaluation of the results.

Streptococcus mutans appeared as small blue colonies with a diameter <1 mm on blue agar and Lactobacillus appeared as white colonies on clear agar. The comparison between the culture medium and a bacterial count greater than 10^5 CFU per milliliter of saliva indicated a high risk of developing caries.

Student's t-test was used for the statistical analysis of the data.

Variables	Test Group	Control Group
Age (years)	18-23	18-23
Plaque index	0	0
Salivary flow (sum of values)	47	44
Buffering capacity of saliva	Medium	Medium
For the plaque index, the scores are 0, 1, 2, 3 For the salivary flow the scores are: 1=<1; 2=1<x<1.5; 3=1.5<x<1.75; 4=1.75<x<2; 5=2<x<2.5; 6>2.5 FRANCAMENTE NON CAPISCO For the buffering capacity the scores are: 1=low; 2=medium-low; 3=medium; 4=medium-high; 5 high.		

TABLE 1

Results

Table 1 shows the distribution of values at T0 of the two groups. There were no significant differences between the two groups at T0.

For the plaque index, the scores are 0, 1, 2, 3.

For the salivary flow the scores are: 1=<1; 2=1<x<1.5; 3=1.5<x<1.75; 4=1.75<x<2; 5=2<x<2.5; 6>2.5. For the buffering capacity the scores are: 1=low; 2=medium-low; 3=medium; 4=medium-high; 5 high.

Table 2 indicates the sum of the Plaque indices (PI) and the comparison of the two groups according to Student's t-test. It can be seen that the sum of the plaque indices is by far lower than the results at T1 and T2 in the test group compared to the control group. In patients not subjected to orthodontic treatment there is a progressive increase in the plaque index over time.

Table 3 shows how the average buffering capacity of the saliva does not change over time.

Table 4 indicates the sum of salivary flow (SF) and the comparison of the two groups according to Student's t test. It can be seen that the sum of salivary flow is higher at T1 and T2 in the test group compared with the control group, where the salivary flow remains almost constant.

Table 5 shows the percentage of patients with Streptococcus mutans >105 CFU and a comparison of both groups according to the "Student's t" function.

Variables	PI (T0)	PI (T1)	PI (T2)	T0 vs T1	T0 vs T2	T1 vs T2
Test Group	3	0	2	0.082814	0.643017	0.16255
Control Group	0	14	27	2.7516×10^{-5}	1.61997×10^{-10}	0.000431873
Test Group Vs Control Group	0.082814	2.7516×10^{-5}	5.1249×10^{-11}			

TABLE 2

Average buffering capacity	T0	T1	T2
Test Group	3	3	3
Control Group	3	3	3

TABLE 3

The amount of bacteria decreases with time in the test group; where in the control group we can observe a progressive increase of the bacterial colonies over time.

Table 6 indicates the percentage of patients with Lactobacillus >105 CFU and the comparison of both groups according to the "Student's t" function. In this case, the amount of bacteria in the test group decreases at T1 (after three months), but increases at T2 (after six months). However the average values of the test group are lower than the values of the control group, in which the amount of the bacteria increases over time.

Discussion

The purpose of this study was to evaluate the microbial colonisation in the oral cavity during orthodontic treatment with Occlus-o-Guide® and compare the results with a control group of subjects not orthodontically treated, comparable for age and sex. At present, in the literature, there are no works on the CRT® bacteria protocol, used in this study, to assess the bacterial presence in saliva during removable orthodontic treatment. We evaluated also the Plaque index, the salivary flow and the buffering capacity of saliva in order to understand how these variables may affect bacterial colonisation over time. Comparing different methods of collection of S. mutans from the oral cavity, we consider that the one based on total stimulated saliva as the most suitable [Motisuki et al., 2005].

The bacterial colonisation on the Occlus-o-Guide® appliance was assumed on the basis of observations made on other removable orthodontic appliances made of resin, on which colonies of Streptococcus mutans formed a thin biofilm [Batoni et al., 2001; Mummolo et al., 2013; Rosetti Lessa et al., 2007]. As mentioned, currently there are only very few studies in the scientific literature that indicate the presence of Lactobacilli on removable orthodontic devices. Therefore, we aimed to evaluate the bacterial presence not on the resin of the Occlus-o-Guide®, but in the saliva of the patients treated with this removable appliance.

Looking at the Sillness-Löe plaque index values over time, it can be noticed that the amount of plaque at T1 (after three months) and T2 (after six months) of the test group decreases in comparison with the control group. This suggests that patients treated with this removable appliance are more meticulous regarding oral hygiene than the patients in the control group [Low et al., 2011]. As regards the buffering capacity of the saliva, we did not observe significant changes between the two groups [Lin et al., 1999; Sukontapatipark et al., 2001; Arendorf and Addy, 1985]. The salivary flow, as expected, was

Variables	SF (T0)	SF (T1)	SF (T2)	T0 vs T1	T0 vs T2	T1 vs T2
Test Group	47	61	61	0.215774	0.215774	1
Control Group	44	45	45	0.909746	0.909746	1
Test Group vs Control Group	0.769123	0.113993	0.113993			

TABLE 4

% of patients with S. mutans >10^5 CFU	T0	T1	T2	T0 vs T1	T0 vs T2	T1 vs T2
Test Group	10%	0%	0%	0.16255	0.16255	0
Control Group	0%	5%	35%	0.329877	0.004729	0.019172
Test Group vs Control Group	0.16255	0.3298768	0.00472946			

TABLE 5

% of patients with Lactobacillus >10^5 CFU	T0	T1	T2	T0 vs T1	T0 vs T2	T1 vs T2
Test Group	15%	0%	10%	0.082814	0.643017	0.16255
Control Group	0%	5%	35%	0.329877	0.004729	0.019172
Test Group vs Control Group	0.082814	0.329877	0.062005			

TABLE 6

significantly increased by the presence of a "foreign body", the removable appliance within the oral cavity which stimulated the salivary glands [Peros et al., 2011; Chang et al., 1999; Moritsuka et al., 2006; Li et al., 2009].

For the microbial counts, we relied on several researches, which state that in caries-free children the level of Streptococcus mutans is <105 CFU. Therefore, this value indicated the cut-off of reference to our observations [Krasse, 1988].

In our study, we observed a different trend of bacterial colonisation in the two groups. Referring to the above value, it can be noticed that in the test group, in which at T0 10% of patients exhibited a CFU $>10^5$, there was a total decrease of the levels Streptococcus mutans at T1 and T2 (0%). This cannot be said of the control group, in which the number of patients who had a CFU $>10^5$, at T0 was zero, after three months (T1) was equal to 5%, reaching after six months (T2) 35%. The explanation is the same as that regarding the reduction of the plaque indices: patients pay more attention to oral hygiene when wearing a removable appliance. Furthermore, from these data, it can be seen that the levels of Streptococcus mutans at the beginning of the treatment are not predictive of future bacterial colonisation, as so is the amount of plaque because over time both the microbial counts and the amount of plaque are reduced in orthodontically treated patients. This is due to the fact that the presence of an orthodontic device affects the balance of oral cavity, which leads to an increase in salivary flow and a decrease of plaque and bacteria. For the latter two conditions an important role is played by oral hygiene because otherwise the amount of plaque and the microbial presence should increase in saliva and, especially, in the biofilm present on the orthodontic device [Peixoto et al., 2011; Quivey et al., 2001]. The Lactobacillus, however, showed a particular trend in the test group, as at baseline (T0) 15% of patients had a level of bacteria $>10^5$ CFU, at T1 it was completely cleared, but after six months it stood again at 10%. Some hypotheses can be made in order to explain this phenomenon: certainly, patients wearing removable orthodontic devices are more scrupulous toward oral hygiene but we must take into account the fact that the Lactobacilli are much more resistant than the Streptococcus mutans, more acids-tolerant, colonise areas of difficult access, and substances such as chlorhexidine do not eliminate them [Cotter and Hill, 2003; Svensäter et al., 2003].

Conclusion

Comparing the data obtained we have reached the following conclusions.

- The presence of a removable orthodontic appliance helps the patient in maintaining a good level of oral hygiene, acting on the emotional component so that the patient takes better care of himself/herself.
- The increase of the salivary flow leads to an increase of the host defenses.
- The reduction of the microbial flora of the oral cavity due to the above implies a significant reduction of PI and S. mutans and Lactobacillus colonisation.
- The stability of the buffering capacity of the saliva is due to the unaltered conditions of the Occlus-o-Guide appliance over time, which is a desirable feature.

References

› Arendorf T, Addy M. Candidal carriage and plaque distribution before, during and after removable orthodontic appliance therapy. J Clin Periodontol 1985; 12: 360-8.
› Batoni G, Pardini M, Giannotti A, Ota F, Giuca MR, Gabriele M, Campa M, Senesi S. Effect of removable orthodontic appliances on oral colonisation by mutans streptococci in children. Eur J Oral Sci 2001; 109: 388-92.
› Chang HS, Walsh LJ, Freer TJ . The effect of orthodontic treatment on salivary flow, pH, buffer capacity, and levels of mutans streptococci and lactobacilli. Aust Orthod J 1999; 15:229–34.
› Cotter PD, Hill C. Surviving the acid test: responses of gram-positive bacteria to low pH. Microbiol Mol Biol Rev. 2003 Sep;67(3):429-53.
› Featherstone JD. The Science and practice of caries prevention. J Am Dent Assoc 2000; 13: 887-99.
› Hardie J, Thomson P, South R, Marsh P, Bowden G, McKee A, Fillery E, Slack G. A longitudinal epidemiological study on dental plaque and the developement of dental caries – interim results after two years. J Dent Res 1977; 56: C90-8.
› Krasse B. Biological factors as indicators of future caries. Int Dent J 1988; 38: 219-25.
› Li Y, Hu B, Liu Y, Ding G, Zhang C, Wang S. The effects of fixed orthodontic appliances on saliva flow rate and saliva electrolyte concentrations. J Oral Rehabil. 2009;36(11):781-5.
› Lin JJ, Cameron SM, Runyan DA, Craft DW. Disinfection of denture base acrylic resin. Prosthet Dent 1999; 81: 202-6.
› Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev 1986; 50: 353-80.
› Low B, Lee W, Seneviratne CJ, Samaranayake LP, Hägg U. Ultrastructure and morphology of biofilms on thermoplastic orthodontic appliances in 'fast' and 'slow' plaque formers. Eur J Orthod. 2011;33(5):577-83.
› Marsh PD. Controlling the oral biofilm with antimicrobials. J Dent 2010; 38(S1): S11-S15.
› Moritsuka M, Kitasako Y, Burrow MF, Ikeda M, Tagami J, Nomura S. Quantitative assessment for stimulated saliva flow rate and buffering capacity in relation to different ages. J Dent. 2006;34(9):716-20.
› Motisuki C, Monti Lima L, Palomari Spolidorio DM, Santos Pinto L. Influence of sample type and collection method on Streptococcus Mutans and Lactobacillus spp. counts in the oral cavity. Arch Oral Biol 2005; 50: 341-5.
› Mummolo S, Marchetti E, Giuca MR, Gallusi G, Tecco S, Gatto R, Marzo G, In-office bacteria test for a microbial monitoring during the conventional and self-ligating orthodontic treatment. Head and Face Medicine 2013, 9 (1), art.no.7.
› Mummolo, S., Marchetti, E., Di Martino, S., Scorzetti, L., Marzo, G. Aggressive periodontitis: laser Nd:YAG treatment versus conventional surgical therapy. European Journal of Paediatric Dentistry: official journal of European Academy of Paediatric Dentistry.2008
› Peixoto IT, Enoki C, Ito IY, Matsumoto MA, Nelson-Filho P. Evaluation of home disinfection protocols for acrylic base plates of removable orthodontic appliances: A randomized clinical investigation. Am J Orthod Dentofacial Orthop. 2011;140(1):51-7.
› Peros K, Mestrovic S, Anic-Milosevic S, Slaj M. Salivary microbial and nonmicrobial parameters in children with fixed orthodontic appliances. Angle Orthod 2011; 81(5): 901-6.
› Quivey RG, Kuhnert WL, Hahn K. Genetics of acid adaptation in oral streptococci. Crit Rev Oral Biol Med. 2001;12(4):301-14.
› Rosetti Lessa FC, Enoki C, Ito IY, Faria G, Matsumoto MA, Nelson-Filho P. In vivo evaluation of the bacterial contamination and disinfection of acrylic base plates of removable orthodontic appliances. Am J Orthod Dentofacial Orthop 2007; 131(6):705.e11-7.
› Saccucci, M., Tettamanti, L., Mummolo, S., (...), Festa, F., Tecco, S. Scoliosis and dental occlusion: A review of the literature Scoliosis 2011
› Socransky SS, Hafferjee AD, Cugini MA, Smith C. Kent RL. Microbial complexes in subgingival plaque. J Clin Periodontol 1998;25:134–44.
› Sukontapatipark W, el-Agroudi MA, Selliseth NJ, Thunold K, Selvig KA. Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study. Eur J Orthod 2001; 23: 475-84.
› Svensäter G, Borgström M, Bowden GH, Edwardsson S. The acid-tolerant micro biota associated with plaque from initial caries and healthy tooth surfaces. Caries Res. 2003 Nov-Dec;37(6):395-403.