Calcium, amylase, glucose, total protein concentrations, flow rate, pH and buffering capacity of saliva in patients undergoing orthodontic treatment with fixed appliances

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Objective: To evaluate qualitative and quantitative changes in the saliva of individuals undergoing orthodontic treatment with fixed appliances.

Methods: Salivary samples were collected from 50 individuals divided in two groups: Experimental Group – patients with fixed orthodontic appliances (n=25); and Control Group – subjects with no orthodontic appliances (n=25). Salivary flow rate, pH, buffering capacity, amylase activity, concentrations of total proteins, calcium and glucose were measured in all salivary samples.

Results: There was a reduction in salivary pH and buffering capacity and an increase in the concentration of calcium ions in the experimental group ($p<0.05$); there was also an increase in glucose, amylase and protein concentrations in the saliva of the Experimental Group, but the differences were insignificant. There was insignificant correlation between calcium ion concentration and salivary flow or between buffering capacity and salivary flow.

Conclusion: The saliva of individuals with fixed orthodontic appliances had lower pH, buffering capacity and calcium concentration than that of individuals without any type of orthodontic appliance. These oral changes are enough to cause tooth demineralization. Patients with orthodontic appliances should adopt additional oral hygiene procedures.

Keywords: Saliva. Corrective orthodontics. Demineralization.

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How to cite this article: Teixeira HS, Kaulfuss SMO, Ribeiro JS, Pereira BR, Brancher JA, Camargo ES. Calcium, amylase, glucose, total protein concentrations, flow rate, pH and buffering capacity of saliva in patients undergoing orthodontic treatment with fixed appliances. Dental Press J Orthod. 2012 Mar-Apr;17(2):157-61.

Submitted: November 3, 2008 - **Revised and accepted:** March 9, 2009.

» The authors report no commercial, proprietary, or financial interest in the products or companies described in this article.

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INTRODUCTION

Saliva is a complex mixture of fluids produced by the salivary glands and the gingival crevice, as well as bacteria, leukocytes and exfoliated epithelial cells.[6,9,10,11,13,29] Its composition varies according to the flow, nature and duration of stimuli, plasma composition and time of the day when it is collected.[25]

Water (99%) is the main component of saliva, and the remaining 1% is of inorganic and organic components.[13] The inorganic elements that play the most important role in osmolarity of saliva are sodium, potassium, chloride and bicarbonate. Saliva is always hypotonic in relation to plasma. The main organic elements are salivary amylase, proteins (lysozyme, lactoferrin, secretory immunoglobulins, peroxidase and agglutinins), peroxidase complex (peroxidase and myeloperoxidase) and agglutinin. They have antimicrobial functions, inhibit calcium and phosphate precipitation, have bactericidal or bacteriostatic effects, and segregate bacteria, which facilitates their removal.[11]

The diverse composition of human saliva explains its several functions, such as the contribution of salivary amylase to digestion, protection of hard and soft tissues, cleansing of the oral cavity, neutralization and buffering of acids found in foods, and participation in bacterial metabolism, demineralization and formation of pellicle. Saliva is also responsible for maintaining the biological balance in the oral cavity since the salivary flow continuously removes bacteria from the teeth surfaces and mucosa. Another important salivary function is maintenance of a relatively neutral pH in the oral cavity what is mostly done by the bicarbonate system.[10,20,27]

Saliva is a fluid that may be easily collected by stimulation, and has been used in medicine as a diagnostic tool for certain diseases.[20] In dentistry, it has been studied as a tool for periodontal diagnosis,[15] to monitor patients with final stage kidney disease[17] and to assess the risk of caries in orthodontic patients.[30] Salivary tests are useful both in planning preventive measures and in the evaluation of the results of treatment.[25]

Orthodontic treatments using fixed appliances in a healthy oral cavity may induce the continuous accumulation of bacterial biofilm, which compromises oral hygiene, affects oral microflora, produces lesions in the oral mucosa, worsens periodontal diseases and consequently causes infection.[3] These factors may promote dental enamel demineralization and biofilm formation,[24] which increases the risk of caries.[7]

In particular, metal brackets and orthodontic bands induce specific changes in the oral environment, such as decreases in pH and increases in the amount of biofilm.[2,4,8,23]

The protective characteristics of saliva against caries are a result of the salivary flow, of its buffering capacity and of its calcium and phosphate concentrations, and well as of several antibacterial systems.[6] The process of eliminating sugar is affected by the salivary flow. The increase in salivary flow results in faster dilution of substances in the oral cavity.[18]

The evaluation of the risk of developing caries or forming dental calculi depends on the salivary composition of each individual. These diseases may lead to serious complications for the patient and may put at risk the esthetic, functional and health benefits of orthodontic treatments, requiring a premature removal of the appliances. Therefore, the characteristics of saliva of individuals under treatment using fixed orthodontic appliances should be studied.

This study evaluated whether the saliva of individuals being treated with orthodontic fixed appliances undergoes qualitative and quantitative changes. The following factors were evaluated: Salivary flow rate (SFV), salivary pH, salivary buffering capacity (SBC), amylase activity, total proteins, calcium and glucose.

MATERIAL AND METHODS

This study was evaluated and approved by the Ethics in Research Committee for Studies with Human Beings of Pontifícia Universidade Católica do Paraná (PUC-PR) under no. 1818/08.

Fifty people (18 male and 32 female) aging from 15.8 to 25.2 years were selected among individuals that sought dental care at the Dental Clinic of PUC-PR. The Experimental Group was composed of 25 individuals that were currently under treatment with fixed orthodontic appliances, and the Control Group was composed of 25 individuals with no orthodontic appliances. Patients, in the 3 months immediately before the beginning of the study, that were diagnosed with debilitating diseases, that were under current treatment with drugs, that smoked, that were users of mouthwash or that had dental prosthesis were excluded from the study.

The saliva sample was collected in the morning (between 8 and 11 a.m.) to avoid circadian rhythm variations, and was collected through the method of spitting. The saliva was collected after mechanical stimulus by chewing a piece of sterile latex tubing. During a controlled time, the subject continuously chewed and spat saliva into a sterile universal container.

The containers with the samples were placed in a Styrofoam box with ice and sent to the Biochemistry Laboratory of the Center for Biological and Health Sciences of PUC-PR for the salivary tests.

To evaluate saliva, colorimetric enzymatic kits (Labtest Diagnóstica®, Lagoa Santa, Brazil) were used according to the instructions of manufacturer to prepare biochemical samples. Readings were made using a Bio Plus spectrophotometer (Perkin-Elmer, Norwalk, CT). The following parameters were evaluated: Salivary flow rate (SFV), salivary pH, salivary buffering capacity (SBC), and concentrations of glucose, total proteins, calcium and salivary amylase.

All variables in both groups were analyzed for normality. Upon normality distribution, Student's *t* test for independent samples was used, but when at least one of the variables was not normally distributed in both groups, Mann-Whitney's *U* test was used.

RESULTS

Table 1 shows the mean values, standard deviations and the statistical analysis of the variables studied. Mean values of protein concentration, salivary flow and glucose in the control group were lower than on the experimental group.

The Kolmogorov-Smirnov test for normality was used for all variables. In the experimental group, the variable calcium was not normally distributed, and in the Control Group, the variable buffering. For the variables protein, amylase, pH, salivary flow and glucose, which had a normal distribution in the Experimental and Control groups, the Student's *t* test for independent samples was used (Table 1). The group without fixed orthodontic appliances had an average of pH greater than the group with appliances ($p<0.05$).

The Mann-Whitney's *U* test was used for the variables calcium and buffering capacity, which were not normally distributed in the experimental and control groups (Table 1). In the Control Group, mean calcium concentration and buffering capacity were greater than in the experimental group ($p<0.05$).

DISCUSSION

Saliva plays an important role on caries development because of its participation in the dilution of substances in the oral cavity, mechanical cleansing, post-eruptive maturation, demineralization and remineralization of dental enamel, pellicle formation, antimicrobial action and buffering of acids produced by biofilm and foods.[1,11,21]

Table 1 - Mean and standard deviation values of the variables of the control and experimental groups and statistical analysis.

Variable	Experimental group	Control group	p Value
Proteins	0.189 (0.076)	0.184 (0.095)	0.8307
Amylase	7.861 (0.035)	7.857 (0.045)	0.7015
Calcium	2.186 (0.734)	4.865 (1.334)	0.0000*
pH	6.885 (0.268)	7.104 (0.298)	0.0089*
Buffering capacity	2.346 (0.533)	2.698 (0.526)	0.0126*
Salivary flow	0.785 (0.353)	0.754 (0.235)	0.7207
Glucose	0.241 (0.158)	0.189 (0.064)	0.1343

* Significant differences ($p<0.05$).

The saliva buffering capacity, defined as salivary resistance to pH changes, is assigned to carbonate-bicarbonate systems, phosphates and proteins.[6,25] The bicarbonate system is responsible for about 85% of the buffering capacity in the pH range of 7.2 to 6.8. When the salivary flow rate increases, the concentration of bicarbonate ions also increases.[9,10,11,25]

In the group using orthodontic appliances, there was a significant decrease in salivary pH and buffering capacity ($p<0.05$). These results are similar to those reported by Kanaya et al,[14] who found a pH decrease in individuals that underwent orthodontic treatment. On the other hand, Chang, Walsh and Freer[7] suggested an increase on the salivary buffering capacity after the placement of orthodontic appliances, but their subjects had appliances for a shorter time.

Patients under orthodontic treatment may be more prone to tooth demineralization since the mean salivary pH and the buffering capacity were diminished on the experimental group. Consequently, preventive measures should be taken since there is a direct association between pH and salivary buffering capacity decreases and increased risk of caries.

The concentration of calcium ions in saliva was different between the groups ($p<0.05$) and lower on the Experimental group. Individuals with a low calcium concentration in saliva have a lower pH and, therefore, a greater susceptibility to demineralization than individuals that have higher concentrations of calcium.[1]

The calcium concentration in saliva is highly dependent on pH and salivary flow rate. According to Jenkins,[13] there is an increase in the concentration of saliva of calcium ions when the flow rate is higher. However, some other authors have found that the concentration of calcium ions decreases as salivary flow rates increase.[11,18] In our study, there was no significant correlation between calcium ion concentration and salivary flow rate or between salivary flow rate and buffering capacity.

Some authors[7,12,14,30] found an increase in the salivary flow rate of individuals with fixed orthodontic appliances. In our study, the experimental group also had a greater salivary flow rate, but the difference was insignificant.

A positive correlation between salivary flow rate and buffering capacity has been reported by several authors.[5,11,16,22,28] Our data differ from their results since an insignificant correlation was found between the groups studied. Laine and Pienihakkinen[19] also did not find any correlation between these two variables. Moreover, a greater glucose concentration was found in the saliva of the experimental group, even though this difference was not significant, in accordance with reports by Forsberg, Oliveby and Lagerlöf[12]. These finding may be associated with the increased salivary flow rate.

Salivary amylase, an enzyme that has the main function of starting the digestion of starch and proteins (the main organic components of saliva[11]) was elevated in the experimental group. However, when compared with the control group, this increase was insignificant.

The factors associated with the risk of caries, such as buffering capacity, concentration of the calcium ions and salivary pH, were altered in the group with fixed orthodontic appliances, what suggests that orthodontic treatment may have an effect the intraoral environment. Because of the long time that fixed appliances remain in the mouth, our findings suggest that preventive measures, such as hygiene, diet advising, topic fluoride application and prophylaxis should be adopted for orthodontic patients.

CONCLUSION

In this study, individuals with fixed orthodontic appliances had lower salivary pH, buffering capacity and calcium concentration than individuals without appliances. Patients with fixed orthodontic appliances had oral changes that might result in tooth demineralization and, therefore, should adopt additional oral hygiene procedures.

REFERENCES

1. Anderson P, Hector MP, Rampersad MA. Critical pH in resting and stimulated whole saliva in groups of children and adults. Int J Pediatr Dent. 2001;11(4):266-73.
2. Anhoury P, Nathason D, Hughes CV, Socransky S, Feres M, Chou LL. Microbial profile on metallic and ceramic bracket materials. Angle Orthod. 2001;72(4):338-43.
3. Atack NE, Sandy JR, Addy M. Periodontal and microbiological changes associated with the placement of orthodontics appliances. A review. J Periodontol. 1996;67(2):78-85.
4. Balenseifen JW, Madonia JV. Study of dental plaque in orthodontic patients. J Dent Res. 1970;49(2):320-4.
5. Bardow A, Moe D, Nyvad B, Nauntofte B. The buffer capacity and buffer systems of human whole saliva measured without loss of CO_2. Arch Oral Biol. 2000;45(1):1-12.
6. Birkhed D, Heintze U. Salivary secretion rate, buffer capacity and pH. In: Tenovuo JO. Human saliva: clinical chemistry and microbiology. Boca Raton: CRC Press; 1989. v. 1, p. 25-74.
7. Chang HG, 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(4):229-63.
8. Chatterjee R, Kleinberg I. Effect of orthodontic band placement on the chemical composition of human incisor tooth plaque. Arch Oral Biol. 1979;24(2):97-100.
9. Dowd FJ. Saliva and dental caries. Dent Clin North Am. 1999;43(4):579-97.
10. Edgar WM. Saliva: its secretion, composition and functions. Br Dent J. 1992;172(8):305-12.
11. Ericson T, Mäkinen KK. Saliva-formation, composition and possible role. In: Thylstrup A, Fejerskov O. Textbook of cariology. Copenhagen: Munksgaard; 1986. p. 28-45.
12. Forsberg C, Oliveby A, Lagerlöf F. Salivary clearance of sugar before and after insertion of fixed orthodontic appliances. Am J Orthod Dentofacial Orthop. 1992;102(6):527-30.
13. Jenkins GN. The physiology and biochemistry of the mouth. 4th ed. Oxford: Blackwell Scientific; 1978. p. 284-359.
14. Kanaya T, Kaneko N, Amaike C, Fukushima M, Morita S, Miyazaki H, et al. A study on changes in caries risk and microbial flora with the placement of Edgewise appliance. Orthod Waves; 2007;66(2):27-32.
15. Kaufman E, Lamster IB. Analysis of saliva for periodontal diagnosis. J Clin Periodontol. 2000;27(7):453-65.
16. Kedjarune U, Migasena P, Changbumrung S, Pongpaew P, Tungtrongchitr R. Flow rate and composition of whole saliva in children from rural and urban Thailand with different caries prevalence and dietary intake. Caries Res. 1997;31(2):148-54.
17. Kho H, Lee S, Chung S, Kim Y. Oral manifestations and salivary flow rate, pH, and buffer capacity in patients with end-stage renal disease undergoing hemodialysis. Oral Surg Oral Med Oral Pathol. 1999;88(3):316-9.
18. Lagerlof F, Oliveby A, Ekstrand J. Physiological factors influencing salivary clearance of sugar and fluoride. J Dent Res. 1987;66(2):430-5.
19. Laine M, Pienihakkinen K, Ojanotko-Harri A, Tenovuo J. Effects of low-dose oral contraceptives on female whole saliva. Arch Oral Biol. 1991;36(7):549-52.
20. Mandel ID. The diagnosis uses of saliva. J Oral Pathol Med. 1990;19(3):119-25.
21. Marsh PD. The significance of maintaining the stability of the natural microflora of the mouth. Br Dent J. 1991;171(6):174-7.
22. Mazengo MC, Soderling E, Alakuijala P, Tiekso J, Tenovuo J, Simell O, et al. Flow rate and composition of whole saliva in rural and urban Tanzania with special reference to diet, age, and gender. Caries Res. 1994;28(6):468-76.
23. Menzaghi N, Saletta M, Garattini G, Brambilla E, Strohmenger L. Changes in the yeast oral flora in patients in orthodontic treatment. Prev Assist Dent. 1991;17(4):26-30.
24. Mitchell L. Decalcification during orthodontic treatment with fixed appliances—an overview. Br J Orthod. 1992;19(3):199-205.
25. Newbrun E. Preventing dental caries: current and prospective strategies. J Am Dent Assoc. 1992;123(5):68-73.
26. Nikiforuk G. Understanding dental caries. New York: Karger; 1985. v. 1.
27. Papas AS, Joshi A, MacDonald SL, Maravelis-Splagounias L, Pretara-Spanedda P, Curro FA. Caries prevalence in xerostomic individuals. J Can Dent Assoc. 1993;59(2):171-4, 177-9.
28. Pienihakkinen K, Nemes J, Scheinin A, Banoczy J. Salivary buffering capacity and its relation to caries increment in children. Proc Finn Dent Soc. 1987;83(2):47-54.
29. Slavkin HC. Toward molecularly based diagnostics for the oral cavity. J Am Dent Assoc. 1998;129(8):1138-43.
30. Ulukapi H, Koray F, Efes B. Monitoring the caries risk of orthodontic patients. Quintessence Int. 1997;28(1):27-9.