A high salivary calcium concentration is a protective factor for caries development during orthodontic treatment

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Received: 22/09/2019
Accepted: 08/01/2020

Abstract
Background: This research aimed to evaluate the salivary concentrations of fluoride (F), calcium (Ca$^{2+}$), and phosphate (Pi) after brackets bonding, and to identify the role of [F$^-$], [Ca$^{2+}$], and [Pi] on the development of active caries lesion (ACL) in individuals under fixed orthodontic treatment.

Material and Methods: A longitudinal investigation with twenty-two individuals from 11 to 22 years of age was performed in four phases (baseline and after 1, 3, and 6 months). Analyses were carried out considering the salivary concentration of [F$^-$], [Ca$^{2+}$], and [Pi], as well as the caries index. Data were analyzed using the Friedman test, followed by the Wilcoxon test and the multivariate Cox model ($p \leq 0.05$).

Results: 1 and 3 months after appliance bonding, the [Ca$^{2+}$] was statistically lower than after 6 months ($p < 0.0083$). On the other hand, salivary [F$^-$] and [Pi] did not show any significant difference during the follow-up. The Cox model demonstrated that the increase of 1 µg/mL in Ca$^{2+}$ decreased the risk of ACL development by 27%. In conclusion, the levels of Ca$^{2+}$ changed during orthodontic treatment.

Conclusions: A high Ca$^{2+}$ level in the saliva is a protective factor for ACL development over time.

Key words: Adolescents, bioinorganic chemistry, dental caries, orthodontic appliances.
Introdução

O acúmulo de biofilme, facilitado por áreas de stagnação aumentadas, torna indivíduos submetidos a tratamento ortodôntico mais suscetíveis ao desenvolvimento de lesões ativas de cáries (ACL) (1). Nesse contexto, modificações relevantes no ambiente oral podem facilitar o desenvolvimento de ACL, especialmente em relação às propriedades salivares como a taxa de fluxo saliva e capacidade de proteção (2). No entanto, a influência de eletrólitos salivares como F-, Ca²⁺, e Pi no desenvolvimento de ACL em indivíduos com tratamento ortodôntico com capas fixas deve ser explorada.

No interior da cavidade oral, os eletrólitos salivares desempenham um papel relevante no proteção individual contra a cárie. Esses eletrólitos mantêm o saliva supersaturado em relação à hidroxiapatita e fornecem um influência positiva na reparação de esmalte dental. Teoricamente, há evidência robusta sobre as propriedades físico-químicas do saliva e seu efeito anticárie (3). No entanto, há evidência limitada de estudos clínicos que apoiam o efeito protetor dos eletrólitos salivares naturalmente ocorrentes (4).

Assim, este estudo de cohortes de 6 meses objetivou 1) avaliar as concentrações salivares de F-, Ca²⁺, e Pi antes e após o implante de aplicações ortodônticas fixas, e 2) identificar a influência de F-, Ca²⁺, e Pi no desenvolvimento de ACL em indivíduos submetidos ao tratamento ortodôntico.

Material e Métodos

-Ethical Considerations

Este estudo foi aprovado por um Comitê de Ética de Pesquisa de uma Universidade do Brasil sob o protocolo No. 37395514.1.0000.5418. Os guardiões de participantes de 18 anos ou menos e todos os outros indivíduos assinaram um formulário de consentimento informado.

-Study Design and Sample

Um estudo de cohortes prospectivo cego foi realizado em uma amostra de 23 adolescentes e jovens adultos submetidos a tratamento ortodôntico fixo. Análises foram realizadas em quatro momentos distintos de coleta: 1) Uma semana antes (baseline) do implante de aplicações ortodônticas, 2) 1 mês após o implante de aplicações ortodônticas, 3) 3 meses após o implante de aplicações ortodônticas, 4) 6 meses após o implante de aplicações ortodônticas (Fig. 1).

O protocolo de pesquisa considerou o estudo clínico do tratamento ortodôntico (Nyvad index) e da coleta saliva. As metodologias detalhadas do estudo e informações sobre o sistema de aplicações foram publicadas anteriormente (2).

O tamanho da amostra foi calculado para testar a diferença na média de lesões de cárie branca entre indivíduos submetidos ao tratamento ortodôntico e aqueles que não foram submetidos a ele (baseline). O tamanho da amostra foi calculado considerando o hipótese bidirecional e foi realizado seguindo esta fórmula (5), (Fig. 2):

\[ n = \left( \frac{Z_{\alpha/2} + Z_{\beta}}{\theta (1 - \theta)} \right)^2 \]

Fig. 2: Formula.

Onde, \( \alpha \) foi a probabilidade de erro tipo I (0.05), \( \beta \) foi a probabilidade de erro tipo II (0.20), \( \theta \) foi a proporção de lesões de cárie branca em indivíduos expostos (0.43) e \( \theta_0 \) foi a proporção de lesões de cárie branca em indivíduos não expostos (0.13) indivíduos. Os valores de referência de lesões de cárie branca foram baseados em Lucchese e Gherlone (6). O tamanho da amostra calculado foi de 22. Este estudo começou com 23 voluntários para compensar possíveis perdas.

Os participantes foram recrutados de acordo com prioridade de uma lista de espera para tratamento ortodôntico no Departamento Pediatría da Pontifícia Universidade Católica de Piracicaba.
School, University of Campinas (Piracicaba, SP, Brazil). The inclusion criteria considered individuals of both sexes, aged from 11 to 22 years, with no primary teeth, with Angle Class I malocclusion, and absence of ACL, dental fluorosis, systemic diseases, dental hypoplasia, and severe dental cro
ing. The exclusion criteria were a history of antibiotic use in the 3 months prior to the beginning of the treatment, use of drugs that cause dry mouth for an extended amount of time, smoking, poor oral hygiene, periodontal disease, pregnancy, neuromotor disabilities, communication difficulties, and previous dental caries experience.

During every research protocol, the individuals were submitted to a comprehensive caries prophylactic program on a monthly basis. This program consisted of instructions (verbal and visual) regarding mechanical biofilm control, professional prophylaxis and daily use of the same fluoride dentifrice with 1.100 ppm of F. Besides this, the volunteers have access to 0.71 ppm of F in drinking water.

-Fixed Orthodontic Treatment

For the orthodontic treatment, we used the Portia self-ligating fixed orthodontic appliances system (Abzil, 3M, São José do Rio Preto, SP, Brazil), with Roth prescription and straight wire of 0.022″ x 0.028″ slot. The appliances were bonded using a light-cured Transbond XT adhesive (3M Unitek, Monrovia, California, USA), according to the manufacturer’s instructions. The brackets were applied to both arches on the buccal surface of incisors, canines, premolars and first permanent molars. The same orthodontist (first author) performed all treatment.

-Examiner Training and Dental Caries Assessment

Before the beginning of the experiment, a training exercise regarding the clinical examination of caries lesions was carried out in two steps (theoretical and clinical).

- Theoretical: Discussion of Nyvad index and analysis of clinical images.
- Clinical: The examiner and a gold standard (a researcher calibrated to the Nyvad index) performed clinical evaluations in a sample of 20 adolescents who were not included in the main sample. The Inter-rater reliability was assessed by the kappa test (κ= 0.89) and the intra-rater reliability was checked one week after the first exam in 20% of the adolescents.

Clinical examinations were performed after the good reliability of training exercise. Individuals were examined at the dental office by a calibrated dentist (the first author), in a face-to-face position, using artificial light, a sterilized mouth mirror, and a WHO probe. A ball-ended dental probe explorer was used to remove debris and to enhance visualization and to confirm questionable findings, checking loss of structure (cavitation) and surface texture (hard or rough/soft). Dental caries diagnosis was carried out according to Nyvad’s caries detection criteria, after the dental prophylaxis (7). This method consists of visual and tactile inspection of caries lesions, considering caries activity (active or inactive), extension and depth (intact, microcavities or cavitated surfaces) of the lesions, including all stages of the disease from white spot lesion to cavitation of dental surface. During the examination of each child, personal protective equipment as well as sterilized and individual clinical material were used.

-Saliva collection

For significant expression of electrolytes, stimulated saliva samples were collected in the morning, between 9:00 and 10:30 hours to avoid variations in the circadian rhythm, at least 1 hour before feeding. Participants were instructed to chew a piece of Parafilm® (Sigma Chemical Company, Chicago, Illinois, USA) and deposit the saliva in a Falcon® tube (BD Biosciences, Bedford, Massachusetts, USA) for 5 minutes. (Flow rate at baseline = 0.97 (0.50) mL/min, 1 month after orthodontic appliances placement = 1.17 (0.59) mL/min, 3 months after orthodontic appliances placement = 1.10 (0.53) mL/min, 6 months after orthodontic appliances placement = 1.06 (0.50) mL/min, ANOVA test with p-value = 0.66) Saliva samples were kept under refrigeration (2 to 8°C) in an ice container. The samples were centrifuged at 16097.2 g for 15 minutes and then stored at -40°C until analysis.

-Assessment of Saliva Inorganic Composition

Calcium and inorganic phosphate concentrations in saliva were analyzed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) (ICap 7400, Duo, Thermo Scientific). Fluoride concentration in centrifuged saliva samples was measured by the direct method, using an ion-selective electrode (8). The values obtained were expressed in µg/mL. Analyses were performed in duplicate.

-Statistical analysis

Statistical analysis was performed using SPSS software, version 21.0 (SPSS, Inc., Chicago, IL, USA). The dependent variables were F, Ca²⁺, and P concentration in saliva. The independent variable was the time of orthodontic treatment follow-up. Data were analyzed by the Friedman test, followed by the Wilcoxon test after non-Gaussian distribution. The level of significance established was 0.0083 for the two-tailed hypothesis, considering the Bonferroni adjustment of p-value to control the family-wise error rate. Multivariate Cox regression was carried out to determine the effect of risk factors (covariates: Salivary F, Ca²⁺, and P) on ACL development. The Enter Method was employed to test covariates considering a p-value lower than 0.05.

Results

At baseline, the caries experience of volunteers was 4.0 of restored surfaces (Interquartile Range: 8.0) without caries activity. One volunteer dropped out the study,
thus, the final sample size was 22. The mean (standard deviation) age was 14 (2.9). The sex ratio was 0.83 male: 1.00 female volunteers. During the follow-up, it was observed that 59% of the sample (n=13) develop white spot lesions around the brackets after 3 months (40 dental surfaces) and 6 months (55 dental surfaces) from fixed orthodontic appliance placement. No cavity was identified in the follow-up period – Table 1. Table 2 shows that the Ca\(^{2+}\) concentrations 1 and 3 months after the beginning of the orthodontic treatment were statistically lower than after 6 months (\(p<0.0083\)). However, when the Ca\(^{2+}\) concentration was compared with the baseline values, no statistical difference at 1, 3, and 6 months could be evidenced (\(p>0.0083\)). Regarding salivary concentrations of F\(^{-}\) and Pi, no significant change was demonstrated (\(p>0.05\)). The Cox model demonstrated that an increase of 1 unit (\(\mu\)g/mL) of Ca\(^{2+}\) decreased the risk of ACL development by 27%. – Table 3.

### Discussion

In the oral cavity, calcium and phosphate ions are responsible for saliva supersaturation, cooperating to inhibit demineralization and enhance remineralization (9,10). Thus, salivary calcium and phosphate form a natural defense mechanism against dental caries (3,11). However, in the presence of a constant cariogenic challenge, it is possible that the frequent pH falls disrupt the

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**Table 1:** Number of dental surfaces during the follow-up periods according to Nyvad’s diagnostic criteria.

|                | Baseline | 1 Month | 3 Months | 6 Months |
|----------------|----------|---------|----------|----------|
| Sound          | 19.82    | 18.09   | 17.23    | 16.27    |
| Active, non-cavitated | 0.00   | 0.00    | 1.82     | 2.50     |
| Active, cavitated          | 0.00   | 0.00    | 0.00     | 0.05     |
| Inactive, non-cavitated     | 5.00   | 6.64    | 5.95     | 6.05     |
| Inactive, cavitated         | 0.00   | 0.00    | 0.00     | 0.00     |
| Filled (sound and inactive) | 3.23   | 3.23    | 3.09     | 3.00     |
| Filled (active)             | 0.00   | 0.00    | 0.09     | 0.23     |

**Table 2:** Medians and interquartile ranges of F\(^{-}\), Ca\(^{2+}\), and Pi concentrations in saliva at baseline and after the beginning of orthodontic treatment.

|                | Baseline | 1 Month | 3 Months | 6 Months |
|----------------|----------|---------|----------|----------|
| F\(^{-}\) (\(\mu\)g/mL) | 0.012\(^a\) (0.0106) | 0.022\(^a\) (0.022) | 0.018\(^a\) (0.012) | 0.018\(^a\) (0.037) |
| Ca\(^{2+}\) (\(\mu\)g/mL) | 50.30\(^b\) (11.70) | 43.80\(^b\) (15.70) | 39.00\(^b\) (26.50) | 55.00\(^a\) (14.60) |
| Pi (\(\mu\)g/mL) | 109.41\(^a\) (57.80) | 108.81\(^a\) (53.53) | 112.99\(^a\) (48.56) | 124.53\(^a\) (58.59) |

\(^a\)-value of Friedman test was significant and Kendall’s W test was equal to 0.29; n=22. Different letters indicate statistically significant differences among groups by Wilcoxon test after Bonferroni adjustment (\(p\)-value has to be smaller than .05/6 = .0083 to be significant). Chi-Square over the mean ranks of F\(^{-}\) (5.182), Ca\(^{2+}\) (18.873) and Pi (1.964).

**Table 3:** Cox’s regression of risk factors for the development of active caries.

|                | HR  | 95% CI for HR | \(p\)  |
|----------------|-----|---------------|--------|
| F\(^{-}\)      | 0.99| 0.98 – 1.02   | 0.90   |
| Ca\(^{2+}\)   | 0.73| 0.54 – 0.98   | 0.04   |
| Pi            | 0.99| 0.98 – 1.00   | 0.31   |

HR: Hazard ratio; Interpretation: HR corresponds to the risk of suffering from active caries lesion per one unit of time (month), considering an increase of one unit of the predictor variable.

For interpretation, we computed the hazard ratios by exponentiating the coefficient for the constant Beta of the regression.

Overall Chi-square score: 5.34 (\(p=0.021\))
equilibrium of these ions on the enamel surface (12,13). Regarding the relevance of these salivary ions on the dental caries development during the orthodontic treatment, our data demonstrated that the calcium concentration was lower after 1 and 3 months, whereas the inorganic phosphate concentration did not change during the follow-up period.

It is interesting to note that, as previously demonstrated by our group, the first clinical evidence of ACL was seen 3 months after the placement of orthodontic appliances (2). In line with these findings, this investigation pointed out that the deficiency in calcium concentration can be considered a risk factor (HR of 0.73) associated with ACL development in individuals submitted to orthodontic treatment. Thus, the results of this study emphasize a possible effect of the concentration of calcium ions on ACL development and its predictive value for carries activity in individuals under orthodontic treatment. In this context, it is hypothesized that the decrease in calcium concentration in saliva during the follow-up (1 and 3 months) may be a consequence of the demineralization and remineralization dynamic that is more pronounced in individuals at high risk of dental caries development and with frequent pH fluctuations during the day (14). More importantly, considering that the ion activity product (IAP) for hydroxyapatite is a function of the calcium concentration potentiated by $5 \text{(IAP} = \text{Ca}^{2+})^2 \times (\text{PO}_4^{3-})^3 \times \text{OH}^-)$ it is possible that a decrease in the calcium concentration may provide a deeper effect on the degree of saliva saturation in respect to hydroxyapatite and an increase in the critical pH for enamel dissolution, which would turn the enamel surface more susceptible to dental caries (4). In addition, there is also a possibility that salivary calcium may be complexed by acids, mainly lactate (15), reducing the saturation of saliva and increasing the driving force for demineralization, and consequently, the likelihood for the development of ACL. In regards to fluoride salivary levels, although concentration increased during orthodontic treatment, no significant differences from baseline values were found. This can be considered a paradox, mainly because the salivary concentration of fluoride did not change enough to explain the development of ACL. It is well-known that available fluoride in saliva can be taken up into dental plaque, thus enhancing the process of teeth remineralization (16,17). However, our finding suggests that the severe cariogenic challenge around orthodontic appliances appears to require more fluoride than that delivered by the low fluoride concentrations in water and by the 1,100-ppm fluoride dentifrice. In fact, it is evidenced that the use of professional methods of topical fluoride application, overcomes the preventive effects of the single-use of self-administered (fluoride toothpaste) method in patients wearing orthodontic appliances; however, the level of evidence regarding the fluoride professional therapies against ACL is low (18). With this in mind, it must be pointed out that the diffusion and dissolution processes of this salivary ion on the tooth-pellicle-biofilm-saliva interface in individuals who do and who do not develop ACL should be investigated in detail considering the fluoride therapy. The results of this investigation have clinical implications because they provide a broader comprehension concerning the behavior of salivary electrolytes over time in a sample of individuals under fixed orthodontic treatment. Besides this, this research highlights the placement of fixed orthodontic appliances as a high-risk situation for ACL development, as well as the limited effectiveness of the preventive effect of mechanical biofilm removal and daily use of fluoride dentifrice at 1.100 ppm and fluoridated water. However, to extrapolate these findings, more clinical studies are needed to determine the exact importance of salivary ions such as $F^-$, $Ca^{2+}$, and $Pi$ on the physicochemical properties of saliva and, consequently, their influence on the development of ACL in individuals undergoing orthodontic treatment.

**Conclusions**

$Ca^{2+}$ levels changed during orthodontic treatment. These changes were more pronounced when the salivary $Ca^{2+}$ composition at 6 months was compared with those at 1 and 3 months after the placement of orthodontic appliances. Conversely, no change could be noted regarding the salivary concentrations of $F^-$ and $Pi$.

The increasing of 1 µg/mL in $Ca^{2+}$ decreased the risk of ACL development by 27%. Thus, an increased salivary concentration of $Ca^{2+}$ may be considered a protective factor for ACL development over time in individuals undergoing orthodontic treatment.

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Acknowledgments
The authors thank Espaço da Escrita – Pró-Reitoria de Pesquisa - UNICAMP - for the language review services provided. We especially thank all volunteers for their valuable participation in this study.

Disclosure statement
The authors declare no potential conflict of interest concerning the authorship and/or the publication of this article.

Funding
This work was supported by FAPESP [grant number 2015/24600-2]; and by FAPEPEX/UNICAMP [grant number 0617/15].

Conflict of interest
The authors have declared that no conflict of interest exist.