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**CÁRIE DENTÁRIA E AUMENTO DE VOLUME GENGIVAL EM  
PACIENTES ORTODÔNTICOS**

Santa Maria, RS  
2017

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ORTODÔNTICOS**

Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Ciências Odontológicas, Área de Concentração em Odontologia, ênfase em Dentística, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Doutor em Ciências Odontológicas**.

Orientador: Prof. Dr. Júlio Eduardo do Amaral Zenkner

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**Aprovado em 22 de junho de 2017:**

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Santa Maria, RS  
2017

## DEDICATÓRIA

*Dedico essa Tese às minhas filhas **Elizza e Lívia** por representarem o mais gratificante motivo para eu acordar todos os dias com gratidão à Vida e a Deus e por me proporcionarem vontade sincera e insistente de aprender, de crescer e de me tornar alguém melhor e mais útil a tudo e a todos.*

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*Tão bom sermos lembrados pelo que somos e não pelo que temos. De nada adianta acumularmos ganhos, bens materiais e aprendizados intelectuais na Terra se não soubermos espalhar o conhecimento e oportunizarmos uma vida melhor ao próximo. Saímos melhores dessa experiência corpórea quando aprendemos que nossos títulos são consequências de oportunidades bem aproveitadas e não decorrentes de maior elevação ou superioridade frente aos demais.*

*Alice Souza Pinto*

## RESUMO

### CÁRIE DENTÁRIA E AUMENTO DE VOLUME GENGIVAL EM PACIENTES ORTODÔNTICOS

AUTORA: Alice Souza Pinto

ORIENTADOR: Júlio Eduardo do Amaral Zenkner

Esta tese é composta por três artigos, cujos principais objetivos foram: 1. Determinar a associação entre o tempo de uso de aparelho ortodôntico fixo e a ocorrência de aumento de volume gengival (artigo 1) e atividade de cárie dentária (artigo 2) entre pacientes ortodônticos; 2. Avaliar a incidência/incremento de cárie dentária ativa em pacientes ortodônticos após um ano de acompanhamento (artigo 3). A amostra foi composta por indivíduos que buscaram e/ou estavam em tratamento ortodôntico em uma Escola de Especialização em Ortodontia de Santa Maria-RS. Nos estudos transversais (artigos 1 e 2), 260 pacientes foram divididos em quatro grupos distintos: G0, sem aparelho (grupo controle); G1, com aparelho fixo há um ano (10-14 meses); G2, com aparelho fixo há dois anos (22-26 meses) e G3, com aparelho fixo há três anos (34-38 meses). No estudo longitudinal (artigo 3), a amostra foi composta por 135 pacientes divididos em dois grupos distintos: G0, sem aparelho (grupo controle); G1, indivíduos usando o aparato ortodôntico fixo há um ano (10-14 meses). Inicialmente, os indivíduos responderam a um questionário sobre características sócio demográficas e hábitos de higiene bucal. Em seguida, foram submetidos ao exame clínico, que incluiu índice de acúmulo de biofilme bacteriano, índice gengival, limpeza profissional, secagem, índice de cárie dentária, e avaliação do aumento de volume gengival. A associação entre os desfechos e as variáveis independentes foi avaliada através de modelos de regressão de Poisson. Nos artigos 1 e 2, observou-se, respectivamente, ocorrência crescente de aumento de volume gengival e de atividade de cárie entre os grupos G0, G1 e G2 ( $p < 0,05$ ), não tendo sido detectada diferença entre os grupos G2 e G3 ( $p > 0,05$ ). O artigo 1 demonstrou, ainda, que indivíduos submetidos a tratamento ortodôntico apresentaram risco 20-28 vezes maior de apresentar aumento de volume gengival do que aqueles sem aparelhos ortodônticos (G1, razão de taxas [RT] = 20,2, IC 95% = 9,0-45,3; G2, RT = 27,0, IC 95% = 12,1-60,3; G3, RT = 28,1, IC 95% = 12,6-62,5). A incidência de lesões de cárie ativa, ilustrada no artigo 3, foi de 4,8% no G0 e de 39,6% no G1. Pacientes com aparelho ortodôntico fixo há um ano apresentaram um risco aproximadamente 9 vezes maior de desenvolver pelo menos uma lesão de cárie ativa quando comparados ao G0 (razão de incidência [RI] ajustada = 9,47 [IC95% = 2,62-34,30]). O incremento médio de lesões ativas foi de 0,14 no G0 e 0,61 no G1, indicando que pacientes usando aparelho fixo apresentaram um risco aproximadamente 4 vezes maior de desenvolver uma lesão ativa adicional do que pacientes do grupo controle (RI ajustada = 4,13 [1,94-8,79]). Assim, observou-se uma associação significativa entre o tempo de uso de aparelho ortodôntico fixo e a prevalência de aumento de volume gengival e de cárie dentária ativa, e também entre o uso do aparato fixo e a incidência e o incremento de lesões ativas de cárie após um ano de acompanhamento.

**Palavras-chave:** Aparelho ortodôntico. Cárie Dentária. Aumento de Volume Gengival. Ortodontia. Epidemiologia.



## ABSTRACT

### DENTAL CARIES AND GINGIVAL ENLARGEMENT IN ORTHODONTIC PATIENTS

AUTHOR: Alice Souza Pinto

ADVISOR: Júlio Eduardo do Amaral Zenkner

This thesis is composed by three articles, whose main objectives were: 1. To determine the association between the duration of fixed orthodontic treatment and the occurrence of gingival enlargement (paper 1) and dental caries activity (paper 2) among orthodontic patients; 2. To evaluate the incidence/increment of active caries lesions in orthodontic patients after a 1-year follow-up (paper 3). The sample consisted of individuals who sought and/or were undergoing orthodontic treatment at an orthodontic graduate program in Santa Maria-RS (Brazil). In the cross-sectional studies (papers 1 and 2), 260 patients were divided into four distinct groups: G0, without the orthodontic device (control group); G1, using fixed apparatus for one year (10-14 months); G2, using fixed apparatus for two years (22-26 months), and G3, using fixed apparatus for three years (34-38 months). In the longitudinal study (paper 3), the sample consisted of 135 patients divided into two distinct groups: G0, without the orthodontic device (control group); G1, individuals using the fixed orthodontic appliance for one year (10-14 months). Initially, the subjects answered a questionnaire on socio-demographic characteristics and oral hygiene habits. Then, individuals were submitted to clinical examination, which included bacterial biofilm accumulation index, gingival index, professional cleaning, drying, dental caries index, and evaluation of gingival enlargement. The association between the outcomes and the independent variables was assessed using Poisson regression models. In papers 1 and 2, respectively, it was found an increasing occurrence of gingival enlargement and caries activity between groups G0, G1 and G2 ( $p < 0.05$ ), with no difference between G2 and G3 groups ( $P > 0.05$ ). It was also observed (paper 1) that individuals submitted to orthodontic treatment had a 20-28-fold increased risk of presenting a gingival enlargement than those without orthodontic appliances (G1, rate ratio [RR] = 20.2, 95% CI = 9.0-45.3; G2, RR = 27.0, 95% CI = 12.1-60.3; G3, RR = 28.1, 95% CI = 12.6-62.5). As shown in paper 3, the incidence of active caries lesions was 4.8% in G0 and 39.6% in G1. Patients with a fixed orthodontic appliance for one year presented an approximately 9-fold increased risk of developing at least one active caries lesion when compared to G0 (adjusted incidence rate ratio [IRR]= 9.47 [95% CI = 2.62-34.30]). The mean increment of active lesions was 0.14 in G0 and 0.61 in G1, indicating that patients using fixed appliance had an approximately 4-fold greater risk of developing an additional active lesion than patients in the control group (adjusted IRR = 4.13 [1.94-8.79]). This study observed a significant association between the duration of fixed orthodontic treatment and the prevalence of gingival enlargement and active dental caries, as well as between the use of the fixed apparatus and the incidence and increase of active caries lesions after one year of monitoring.

**Keywords:** Orthodontic appliance. Dental Caries. Gingival Enlargement. Orthodontics. Epidemiology.

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## 1 INTRODUÇÃO

Tem sido sugerido na literatura que a utilização de aparelhos ortodônticos fixos está associada a uma maior ocorrência de cárie dentária (SUNDARARAJ et al., 2015; AHMED et al., 2011; TUFEKCI et al., 2011), bem como de gengivite e aumento de volume gengival (ZANATTA et al., 2014; EID et al., 2014; ZANATTA et al. 2011; GOMES et al. 2007; ELLIS; BENSON, 2002; SADOWSKY; BEGOLE, 1981; ALSTAD; ZACHRISSON, 1979). Isto pode ser atribuído à sua superfície retentiva de biofilme dentário, dificultando uma higiene bucal adequada e, por consequência, exigindo do paciente maior tempo e cuidados diários com a sua saúde bucal (SUNDARARAJ et al., 2015; AKIN et al., 2013; RICHTER et al., 2011; ZANATTA et al., 2011; ELLIS; BENSON, 2002).

Lesões não cavitadas ativas de cárie (LUCCHESI; CHERLONE, 2013; SAGARIKA et al., 2012; ENAIA et al., 2011; TUFEKCI et al., 2011; MIZRAHI, 1982) o aumento de volume gengival (ZANATTA et al., 2014; EID et al., 2014; ZANATTA et al. 2011; GOMES et al. 2007; ELLIS; BENSON, 2002; SADOWSKY; BEGOLE, 1981; ALSTAD; ZACHRISSON, 1979) da face vestibular dos dentes que recebem os braquetes estão entre as consequências mais comuns associadas à terapia ortodôntica fixa. No entanto, pode-se dizer que ainda não está claramente elucidado na literatura se o tempo de uso do aparato ortodôntico contribuiria para uma maior manifestação dessas alterações.

Para isto, se faz necessário o desenvolvimento de pesquisas cujos exames clínicos sejam realizados de maneira criteriosa, após limpeza profissional e secagem das superfícies dentárias, e que sejam realizados por examinadores calibrados e reprodutíveis. Além disso, em diversos trabalhos, a detecção de lesões cariosas é feita por meio do uso de fotografias digitais tomadas para fins documentais, usualmente não padronizadas, e sem a prévia realização de limpeza e secagem das superfícies. Estas condições tendem a subestimar o número de lesões cariosas iniciais em esmalte (ASSAF et al., 2004).

Portanto, essa tese objetiva estudar a cárie dentária e o aumento de volume gengival em pacientes ortodônticos, por meio da avaliação: 1. da prevalência e da extensão de aumento de volume gengival (artigo 1) e atividade de cárie (artigo 2) após diferentes períodos de tempos de tratamento ortodôntico fixo corretivo; 2. da incidência e do incremento de lesões cariosas ativas após um ano de tratamento ortodôntico fixo corretivo (artigo 3).

**2 ARTIGO 1 – GINGIVAL ENLARGEMENT AMONG ORTHODONTIC PATIENTS:  
THE EFFECT OF TREATMENT DURATION**

Este artigo foi submetido e aceito para publicação no periódico *American Journal of Orthodontics & Dentofacial Orthopedics*, Elsevier, ISSN:0889-5406. Fator de impacto = 1.981; Qualis A1. As normas para publicação estão descritas no Anexo A.

## **Gingival enlargement among orthodontic patients: the effect of treatment duration**

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## ABSTRACT

**Introduction:** This study aimed to assess the effect of the duration of fixed orthodontic treatment on gingival enlargement (GE) among adolescents and young adults.

**Methods:** The sample consisted of 260 10-30-year-old individuals divided in 4 groups: patients with no fixed orthodontic appliances (G0) and patients undergoing orthodontic treatment for 1 year (G1), 2 years (G2) or 3 years (G3). Participants completed a structured questionnaire on socio-demographic characteristics and oral hygiene habits. Clinical examinations were conducted by a single calibrated examiner and included the plaque index, the gingival index and the Seymour index. Poisson regression models were used to assess the association between group and GE.

**Results:** It was observed increasing means of plaque, gingivitis, and GE in G0, G1 and G2. No significant differences were observed between G2 and G3. Adjusted Poisson regression analysis showed that individuals undergoing orthodontic treatment presented a 20-28-fold increased risk for GE than those without orthodontic appliances (G1, RR=20.2, 95%CI=9.0-45.3; G2, RR=27.0, 95%CI=12.1-60.3; G3=28.1; 95%CI= 12.6-62.5).

**Conclusions:** The duration of orthodontic treatment significantly influenced the occurrence of GE. Oral hygiene instructions and motivational activities should target adolescents and young adults undergoing orthodontic treatment.

## **INTRODUCTION**

The effect of fixed orthodontic appliances on periodontal parameters has been shown previously.<sup>1,2,3,4,5</sup> In general, favorable conditions for plaque stagnation as well as the difficulty to perform usual oral hygiene measures have been associated with a poorer periodontal health among orthodontic patients.<sup>1,2</sup> Nevertheless, some studies have suggested that gingival changes during the use of fixed orthodontic appliance do not cause permanent aggression to periodontal support tissues.<sup>3,4,5</sup>

Gingival enlargement (GE) is an excessive growth of the gums where the inflammatory tissue may be in a limited region or it may be generalized.<sup>1,3</sup> The mechanism by which it occurs during orthodontic treatment is not fully understood, wherein artificially deep periodontal pockets are established.<sup>6,7,8,9,10</sup> Few studies have assessed the occurrence of GE during orthodontic treatment. In 2014, Eid et al<sup>11</sup> and Zanatta et al<sup>12</sup> have found a positive association between the use of fixed orthodontic appliances and gingivitis and GE. However, both studies had no control groups (without the use of brackets) and combined in the same category orthodontic patients undergoing treatment during 12 months or more or during 18 months or more.<sup>11,12</sup> This way, the effect of longer periods of orthodontic treatment was not studied.

A previous study has shown that anterior GE promotes a negative impact on oral health-related quality of life of orthodontic patients, thus emphasizing the need for further investigations on this issue.<sup>13</sup> Therefore, the present study aimed to assess the effect of the duration of fixed orthodontic treatment on gingival enlargement among adolescents and young adults.

## **MATERIALS AND METHODS**

This cross-sectional study selected participants who sought or were undergoing fixed orthodontic treatment in an orthodontic graduate program in Santa Maria, South Brazil. The study protocol was approved by the Ethics Committee of the Federal University of Santa Maria, Brazil (approval protocol number 0109/2013). Patients or their legal guardians were informed about the study objectives and authorized their participation by signing a written informed consent form.

The required number of individuals was estimated based on an expected difference among groups of 20%. Considering a power of 80% and a confidence interval of 95%, 65 individuals per group were required. Such sample was stratified into four groups according to



the duration of fixed orthodontic treatment, namely: G0 (control) composed by individuals candidates for corrective orthodontic treatment, examined previously to the installation of the fixed apparatus; G1 composed by participants undergoing fixed orthodontic treatment for 1 year (10-14 months); G2 composed by participants undergoing fixed orthodontic treatment for 2 years (22-26 months); and G3 composed by patients undergoing fixed orthodontic treatment for 3 years (34-38 months).

Individuals aged from 10 to 30 years were considered eligible for this study. To be included in Groups 1, 2 and 3, participants should be using fixed orthodontic appliances for a specific period of time, as previously described. Fixed corrective orthodontic treatment was carried out with conventional metal brackets, straight wire technique, with orthodontic arches fixed with simple elastic bandages, without the use of metal ligature, elastic chain or proximal enamel stripping execution. Orthodontic rings (bands) were adapted to the molars with glass ionomer cement. Initially, orthodontic movement of alignment and leveling were performed in order to correct horizontal and vertical discrepancies with subsequent space closure movement, when necessary, and finishing movement which included compensatory folds in the arches, such as torque, intrusion and extrusion. Patients in the need of traction of impacted teeth and of the repositioning of teeth widely lingually/buccally (>2mm) were not included in the sample. To be included in G0, individuals should not be using nor have previously used fixed orthodontic appliances. Patients suffering from congenital abnormality, systemic illness, special needs, cysts, crevices or using systemic medication for the treatment of chronic diseases which may interfere on gingival overgrowth were excluded from the sample. Patients who required chemoprophylaxis before clinical examination were also excluded.

Initially, individuals answered a structured questionnaire on socio-demographic characteristics and oral hygiene habits. Clinical examinations were performed in a dental unit, using a dental mirror, a periodontal probe type Williams (Golgran, Brasil) and a World Health Organization Probe.

Clinical examination included the assessment of the Löe and Silness plaque index (PI),<sup>14</sup> evaluation of the Löe gingival index (GI),<sup>15</sup> professional prophylaxis with sodium bicarbonate spray (Jet Laxis Uno, Schuster, Brazil), tooth drying with air-water syringe and relative isolation with cotton rolls, assessment of the excess of composite resin at the cervical side of the brackets, where 0 was considered absent and 1 present, adapted from Zanatta et al,<sup>1</sup> assessment of the Seymour index<sup>16</sup> to record the occurrence of GE in the anterior segment by visual inspection. Buccal and lingual papillae of the six anterior teeth, upper and lower, were examined. Gingival thickening and gingival encroachment onto adjacent crowns were

graded. The sum of both scores (thickening and encroachment) resulted in an enlargement score for each gingival unit. The maximum score obtainable using this method is 5 and the sum of all papillae provides a single score per patient (ranging from 0 to 100). The assessed indexes are described in Table I.

### **Calibration and Reproducibility**

A single examiner (ASP) performed the clinical examinations. Training sessions were performed to ensure examiner reliability in regards to the used indexes. Repeated examinations were performed in 16 individuals (6%) and a kappa value of 0.95 was obtained for the Seymour index. Considering that plaque accumulation and gingival bleeding are variable conditions, examiner calibration was not assessed for these indexes.

### **Statistical analysis**

The main outcome of this study was anterior GE. The prevalence of anterior GE was defined as the proportion of individuals presenting a Seymour index  $\geq 30$  based on the cutoff proposed by the index for the definition of clinically relevant GE.<sup>16</sup> Additionally, the Seymour index was also treated as a counting variable and referred to in the manuscript as the extent of GE.

Individuals were classified in three categories regarding age group:  $\leq 15$ , 15-20,  $>20$ . Mother's education was classified as primary school, high school and university. Family income was classified in  $\leq 3$  and  $>3$  Brazilian minimum wages (1 BMW corresponded to around 295 US dollars during the period of data collection). Tooth brushing frequency was dichotomized into  $\leq 2$  times/day and  $\geq 3$  times/day. The use of dental floss was classified as no use, non-daily use, and daily use. The excess of composite resin on all assessed surfaces was summed and an overall score per patient were obtained, ranging from 0 to 12. The plaque index and the gingival index were calculated as the mean score of all assessed sites.

Chi-square test was used to compare groups G0, G1, G2, and G3 according to age, gender, mother's education, tooth brushing frequency, and use of dental floss. The plaque index, the gingival index and the Seymour index were compared among groups using the Wald test. The relationship between the presence/absence of clinically relevant GE and groups was assessed using the Fischer exact test.

Poisson regression analysis with robust variance (unadjusted and adjusted models) was used to assess the association between group and the extent of GE. Estimates were adjusted for socio-demographic characteristics, self-reported oral hygiene habits, and clinical variables

(plaque index, gingival index and the excess of composite resin). All variables were maintained in the adjusted model irrespective of their P-values.

Data analysis was performed using STATA software (Stata 11.1 for Windows; Stata Corporation, College Station, TX, USA). The significant level was set at 5%.

## RESULTS

A total of 260 individuals were included in the study (n per group = 65). Around 5% of the sample (n=14) presented incomplete permanent dentition, aged from 10 to 16 years. The number of absent teeth ranged from 1 to 5 teeth, being second molars and upper canines the most commonly absent teeth.

Table II shows the sample distribution according to group, socio-demographic characteristics and oral hygiene habits. There was no difference among groups in regards to gender, mother's education and tooth brushing. The proportion of individuals undergoing orthodontic treatment for 3 years (G3) was reduced in the age category  $\leq 15$  years ( $P=0.03$ ). The number of individual receiving  $\leq 3$  BMW and the use of dental floss decreased as the duration of orthodontic treatment increased ( $P=0.004$  and  $P=0.04$ , respectively).

Table III shows the Plaque, Gingival and Seymour indexes according to the duration of orthodontic treatment. It was observed increasing means from G0 to G1 and G2. No significant difference was observed between G2 and G3. The presence of clinically relevant GE was significantly associated with the duration of orthodontic treatment (Table IV).

Table V shows the association between independent variables and the extent of GE. Group was significantly associated with the outcome in the unadjusted analysis. Compared with individuals without fixed orthodontic appliances, those undergoing orthodontic treatment for 1, 2 and 3 years presented increased risks for GE [G1, RR (95% CI) = 22.9 (10.2-51.4); G2, RR (95% CI) = 32.5 (14.6-72.2); G3, RR (95% CI) = 35.2 (15.9-78.0)]. A slight decrease in the GE risk estimates was observed when socio-demographic characteristics, self-reported oral hygiene habits, and clinical variables were added to the model. Adjusted estimates showed that individuals undergoing orthodontic treatment for 1, 2 and 3 years presented a 20-28-fold increased risk for GE than their counterparts without orthodontic appliances. Furthermore, age ( $\geq 20$  years, RR=0.83, 95%CI=0.70-0.98) and the gingival index (RR=1.43, 95%CI=1.02-2.02) were significantly associated with the extent of anterior GE in this population.

Due to the lack of individuals presenting clinically relevant GE in G0, it was not possible to assess the association between group and the prevalence of GE.

## DISCUSSION

This study aimed to assess the effect of the duration of fixed orthodontic treatment on GE in adolescents and young adults. The time under fixed orthodontic treatment strongly influenced the extension of GE, with severer manifestations of this condition among patients using orthodontic appliances for longer periods of time. To the best of our knowledge, this is the first study evaluating the long-term effect of orthodontic treatment on GE and the first one including a control group composed of patients without orthodontic appliances.

As shown in two recent studies<sup>11,12</sup>, an association between GE and the use of fixed orthodontic appliances was observed in this study. We have also observed an increasing occurrence of GE as the duration of orthodontic treatment increased. Even after the adjustment for important co-factors, individuals using fixed orthodontic appliances for 1, 2 and 3 years presented a 20-28-fold increased risk for GE. It was observed that the inclusion of behavioral and clinical variables in the adjusted model promoted a slight decrease in the magnitude of the association between the duration of orthodontic treatment and the extent of GE, suggesting that a small portion of the effect of “group” on GE was mediated by some of these variables. For example, dental floss and clinical variables showed significant associations with the outcome in the unadjusted models ( $p < 0.05$ ). The association between the duration of orthodontic treatment and GE is in disagreement with those by Zanatta et al.<sup>12</sup>. Although the authors have found a significant higher frequency of GE scores 2 and 3 in individuals using orthodontic appliances for >12 months compared with those using the apparatus for 6-12 months, no association between the duration of treatment and GE was found in the risk assessment analysis.

GE is characterized as an inflammatory response to the plaque microbiota and related by-products.<sup>19,20</sup> The placement of orthodontic brackets influences the accumulation of biofilm and colonization of bacteria, thereby being more prone to inflammation and bleeding.<sup>21,22</sup> Previous studies have shown a high prevalence/extension of gingival inflammation among orthodontic patients,<sup>2,10,17,18</sup> which is in agreement with the present findings. The greater the GE, the greater the difficulty to access tooth surfaces, inhibiting good oral hygiene and resulting in more inflammation and bleeding. Our data seems to support the plaque-related reaction of GE due to the linear increase in plaque and gingival inflammation overtime and because of the significant association between GE and gingival inflammation found in the adjusted analysis. Furthermore, no additional effect of time on the prevalence of GE was observed from 2 to 3 years of orthodontic treatment, which is in

accordance with the similar plaque and gingival patterns observed in these groups, thus pointing to the inflammatory nature of GE.

Despite this inflammatory factor, other causal factors have been proposed to explain the association between orthodontic treatment and GE. In recent years, it has been suggested that a continuing low-dose of nickel released to epithelium due corrosion of orthodontic appliances may be the initiating factor of GE among orthodontic patients.<sup>23-26</sup> Another factor that may be associated with the occurrence of GE is the hormonal changes that occur during puberty. Sexual maturation during puberty is related to increased levels of the steroid sex hormones. As a result, subclinical inflammatory changes may modulate periodontal tissues to be more sensitivity to small amounts of plaque and a hyperplastic reaction of the gingiva may occur.<sup>27-29</sup> This influence of sex hormone levels may explain, at least in part, the higher likelihood of younger participants to present GE. Furthermore, older individuals are more likely to have preventive attitudes and habits due to a greater interest in health than do younger individuals.<sup>11,12,30</sup>

In order to investigate the effect of time under orthodontic treatment, characteristics that could act as confounding variables were also collected. The use of dental floss was significantly different among groups, with a lower proportion of individuals reporting its daily use as the duration of orthodontic treatment increased. Evidence supports that the compliance rate for daily flossing is low, especially among subjects with orthodontic appliances, because orthodontic apparatus increases difficulties of flossing technique.<sup>31-33</sup> In our sample, less than 20% of participants undergoing orthodontic treatment for 2 or 3 years reported daily use of dental floss, contrasting with the other 2 groups (32%-37%). This finding may point to a decreasing motivation of orthodontic patients in the performance of interproximal cleanness as the treatment time increases. Notwithstanding, our data showed that the use of dental floss was not associated with GE extent in the adjusted model, which corroborates the findings of Zanatta et al.<sup>12</sup> This lack of association may be due tooth brushing alone can result in low levels of dental plaque in the anterior segments and by the determination of dental floss use which may have been subject to information bias, leading to a possible overestimation of frequency of dental floss habit.<sup>12</sup>

Since this study has been conducted in a Post-graduate orthodontic school, the variability of professionals in charge of orthodontic treatments and the possible variations of techniques could be seen as limitations of this study. However, it increases its external validity. Another fragility of our study is its cross-sectional design. We acknowledge that to really verify if GE increases with time, we should have monitored the same patient during the

course of orthodontic treatment. Notwithstanding, this is the first study comparing subjects under fixed orthodontic treatment for different periods of time with those without orthodontic appliances. Thus, we cautiously considered generalizations to patients under orthodontic treatment, between 10 and 30 years of age, with characteristics similar to our sample. Among the strengths of this study we could emphasize the single examiner assessing all clinical parameters (which may increase the quality of data collection and results reliability) and the presence of a control group composed of patients without orthodontic appliance. Furthermore, patient selection strategy excluding cases of large space closure as those in the need of traction of impacted teeth and of >2mm of buccal/lingual repositioning may also be seen as a strength of this study. As previously shown in the literature, significant alterations in the widths of the keratinized and attached gingiva occur after facial or lingual movements.<sup>34</sup>

## CONCLUSIONS

- This study showed an increasing occurrence of GE as the duration of orthodontic treatment increased.
- Oral hygiene instructions and motivational activities should target adolescents and young adults undergoing orthodontic treatment.
- Further longitudinal studies may elucidate the association between the use of fixed orthodontic appliances and GE.

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## TABLES

Table I. Description of the assessed indexes.

Plaque index (PI) <sup>14</sup>	
0	Surface without plaque
1	Plaque at the gingival margin and the tooth, visible only after probe use
2	Moderate accumulation of plaque at the gingival margin and the tooth, visible to the naked eye
3	Abundant accumulation of plaque at the gingival margin and the tooth
Gingival index (GI) <sup>15</sup>	
0	Normal gum
1	Mild inflammation, with a slight change in color and slight swelling but no bleeding on probing
2	Moderate inflammation with redness, swelling and bleeding on probing
3	Severe inflammation with redness and severe edema, tending to ulceration and spontaneous bleeding
Seymour index <sup>16</sup>	
Gingival thickening	
0	Normal
1	Thickening $\leq 2\text{mm}$
2	Thickening $> 2\text{mm}$
Gingival encroachment	
0	Normal
1	Papilla involving 1/3 of adjacent tooth crown half
2	Papilla involving 2/3 of adjacent tooth crown half
3	Papilla involving $> 2/3$ of adjacent tooth crown half

Table II. Sample distribution according to socio-demographic characteristics, oral hygiene habits, and the duration of orthodontic treatment. N.

	G0	G1	G2	G3	Total	P <sup>‡</sup>
Age (years)						0.03
≤15	17	21	20	7	65	
15-20	26	18	23	35	102	
>20	22	26	22	23	93	
Gender						0.78
Male	28	29	25	24	106	
Female	37	36	40	41	154	
Mother's education*						0.15
Primary school	29	22	24	13	88	
High school	19	20	20	25	84	
University	17	22	21	27	87	
Family income						0.004
≤3 BMW	47	41	29	31	148	
> 3 BMW	18	24	36	34	112	
Tooth brushing						0.37
≤2 times/day	20	19	27	19	85	
≥3 times/day	45	46	38	46	175	
Dental floss*						0.04
No	17	11	16	24	68	
Non-daily	27	30	35	30	122	
Daily	21	24	13	11	69	
TOTAL	65	65	65	65	260	

BMW = Brazilian minimum wage (equivalent to around 295 US dollars during the period of data gathering).

\* Missing value. ‡Chi-square test.

Table III. Plaque Index, Gingival Index and Seymour Index according to the duration of orthodontic treatment. Mean ( $\pm$  standard deviation).

	Plaque Index	Gingival Index	Seymour Index
G0	0.18 (0.14) <sup>a</sup>	0.18 (0.14) <sup>a</sup>	0.81 (2.66) <sup>a</sup>
G1	0.31 (0.18) <sup>b</sup>	0.34 (0.22) <sup>b</sup>	18.71 (12.83) <sup>b</sup>
G2	0.50 (0.28) <sup>c</sup>	0.50 (0.27) <sup>c</sup>	26.51 (12.51) <sup>c</sup>
G3	0.56 (0.25) <sup>c</sup>	0.54 (0.26) <sup>c</sup>	28.73 (11.17) <sup>c</sup>

Different letters in columns indicate statistically significant differences among categories (P<0.05, Wald test).

Table IV. Relationship between clinically relevant gingival enlargement and the duration of orthodontic treatment. N.

	Clinically Relevant Gingival Enlargement		P <sup>‡</sup>
	Absent	Present	
G0	65	0	<0.001
G1	49	16	
G2	37	28	
G3	36	29	

Absence of clinically relevant GE = Seymour Index <30;

Presence of clinically relevant GE = Seymour Index ≥30.

<sup>‡</sup>Fischer exact test

Table V. Association between independent variables and the extent of gingival enlargement. (Seymour index) Unadjusted and adjusted Poisson regression models.

	Unadjusted		Adjusted	
	RR (95% CI)	P	RR (95% CI)	P
<b>Group</b>				
G0	1.00		1.00	
G1	22.9 (10.2-51.4)	<0.001	20.2 (9.0-45.3)	<0.001
G2	32.5 (14.6-72.2)	<0.001	27.0 (12.1-60.3)	<0.001
G3	35.2 (15.9-78.0)	<0.001	28.1 (12.6-62.5)	<0.001
<b>Age (years)</b>				
≤15	1.00		1.00	
15-20	0.99 (0.77-1.26)	0.93	0.89 (0.76-1.0)	0.13
>20	0.79 (0.61-1.02)	0.07	0.83 (0.70-0.98)	0.03
<b>Gender</b>				
Male	1.00		1.00	
Female	0.95 (0.78-1.17)	0.66	0.96 (0.85-1.09)	0.55
<b>Mother's education</b>				
Primary school	1.00		1.00	
High school	1.18 (0.91-1.53)	0.21	1.02 (0.87-1.20)	0.78
University	1.24 (0.97-1.59)	0.09	1.00 (0.86-1.19)	0.91
<b>Family income</b>				
≤3 BMW	1.00		1.00	
> 3 BMW	1.21 (0.99-1.47)	0.06	1.02 (0.89-1.17)	0.75
<b>Tooth brushing</b>				
≤2 times/day	1.00		1.00	
≥3 times/day	0.85 (0.69-1.05)	0.13	0.89 (0.78-1.02)	0.09
<b>Dental floss</b>				
No	1.00		1.00	
Non-daily	1.01 (0.80-1.28)	0.13	1.01 (0.87-1.17)	0.88
Daily	0.73 (0.54-0.98)	0.04	1.02 (0.84-1.24)	0.84
Excess of resin	1.10 (1.07-1.14)	<0.001	1.00 (0.98-1.03)	0.92
Plaque index	4.79 (3.46-6.63)	<0.001	1.35 (0.94-1.94)	0.10
Gingival index	4.88 (3.75-6.35)	<0.001	1.43 (1.02-2.02)	0.04

RR = Rate Ratio; CI = Confidence Interval.

**3 ARTIGO 2 – DOES THE DURATION OF FIXED ORTHODONTIC TREATMENT AFFECT CARIES ACTIVITY AMONG ADOLESCENTS AND YOUNG ADULTS?**

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**Does the duration of fixed orthodontic treatment affect caries activity among adolescents and young adults?**

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**Short title**

Fixed orthodontic treatment affects caries activity

**Key words**

Dental caries, orthodontic appliances, prevalence, clinical study.

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**Declaration of interest**

The authors declare no conflict of interest related to this study.



**Abstract**

This cross-sectional study assessed the relationship between fixed orthodontic treatment duration and caries activity. Two hundred and sixty patients with 10-30 years-olds were divided into 4 groups (n=65/each): no fixed orthodontic appliances (G0), orthodontic treatment for 1 year (G1), 2 years (G2) or 3 years (G3). Orthodontic treatment duration was significantly associated with active caries: prevalence increased from 1.5% for G0 to 27.7% for G1 to 72.3% for G2 and G3; median number of lesions increased from 0 for G0 and G1 to 2 for G2 and G3. Statistically significant differences were observed among G0, G1 and G2 ( $p < 0.001$ ), whereas no significant differences were observed between G2 and G3.

## Introduction

Enamel demineralization, clinically diagnosed as the presence of white spot lesions (WSL), is one of the most common adverse effects associated with fixed orthodontic treatment [Mizrahi, 1982; Enaia et al., 2011; Tufekci et al., 2011; Sagarika et al., 2012; Lucchese and Gherlone, 2013]. A recent meta-analysis estimated that the prevalence of WSL for patients undergoing fixed orthodontic treatment for  $\geq 12$  months was 68.4% [Sundararaj et al., 2015].

Whereas it is well-known that the prevalence of WSL increases during orthodontic therapy, it is unclear the effect that the duration of fixed orthodontic treatment has on caries activity. Few studies have shown that individuals undergoing fixed orthodontic therapy for 12 months had higher prevalence of WSL than those using the fixed apparatus for 6 months [Ahmed et al., 2011; Tufekci et al., 2011; Lucchese and Gherlone, 2013]. However, these studies have important shortcomings, such as lack of a control group [Ahmed et al., 2011], exclusion of upper molars and lower teeth on caries examination [Tufekci et al., 2011], and limited follow-up [Ahmed et al., 2011; Tufekci et al., 2011; Lucchese and Gherlone, 2013]. Studies with longer follow-up periods have used pre- and post-treatment photographs for caries assessment, which greatly limit caries detection [Richter et al., 2011; Khalaf, 2014].

This study was undertaken to assess the relationship between fixed orthodontic treatment duration and caries activity among adolescents and young adults undergoing fixed orthodontic treatment for up to 3 years. Our hypothesis was that the longer the duration of the treatment, the higher the prevalence/extent of active caries lesions.

## Methods

### *Study design and sample*

This cross-sectional study included participants who sought or were undergoing fixed orthodontic treatment in an orthodontic graduate program in Santa Maria, South Brazil. Data were collected between November/2013 and January/2015. Two hundred and sixty participants were included into four groups (n=65/group): G0 (control) – individuals who were seeking orthodontic treatment, but had not received the orthodontic appliance; G1 – individuals undergoing fixed orthodontic treatment for 1 year (10-14 months); G2 – individuals undergoing fixed orthodontic treatment for 2 years (22-26 months); and G3 – individuals undergoing fixed orthodontic treatment for 3 years (34-38 months).

Individuals aged between 10 and 30 years were considered eligible for this study. To be included in Groups 1, 2 or 3 participants should have metal brackets bonded with

composite resin on the buccal surfaces of permanent teeth. To be included in Group 0, individuals should not be using nor have previously used fixed orthodontic appliances. Individuals suffering from congenital abnormality, systemic illness, special needs, cysts, crevices, or using systemic medication which may induce gingival overgrowth were excluded from the sample. Furthermore, individuals who required chemoprophylaxis before clinical examination as well as those exposed to daily supplemental fluoride regimen were also excluded. All participants were exposed to fluoridated water (0.7-0.8 ppm F) and used fluoridated toothpaste (1000-1500 ppm F) regularly.

Based on a power of 80% and a confidence interval of 95%, a sample size of 65 individuals per group was considered necessary to detect a difference of 20% in WSL prevalence among groups.

#### *Data collection*

A structured questionnaire was used to collect socio-demographic characteristics and oral hygiene habits. Clinical examinations were performed in a dental unit using a dental mirror, Williams periodontal probe (Golgran, São Caetano do Sul, Brasil) and WHO probe. After professional prophylaxis with sodium bicarbonate spray (Jet Laxis Uno, Schuster, Santa Maria, Brazil), teeth were dried with air-water syringe and isolated with cotton rolls. Caries examination included the assessment of all dental surfaces, which were classified based on their clinical characteristics (surface texture and brightness), as follows: non-cavitated inactive lesions, surface areas presenting a shiny appearance with different degrees of brownish discoloration; non-cavitated active lesions, opaque enamel with a dull-whitish surface; cavitated inactive lesions, localized surface destruction with active characteristics (dull-whitish enamel and soft dentin with light brown color); cavitated active lesions, localized surface destruction with arrested characteristics (shiny, hard surfaces with different degrees of brownish discoloration) [adapted from Maltz et al., 2003] .

#### *Reproducibility*

All clinical examinations were performed by a single examiner (ASP), who was trained and calibrated for caries examination by a reference examiner (JEAZ). After intensive training sessions, repeated examinations were performed in 10 individuals with a minimal time interval of 7 days. Intra-examiner unweighted kappa value of 0.92 and inter-examiner kappa value of 0.87 were obtained. Examiner reproducibility was also assessed after the repeated

examination of individuals using fixed orthodontic appliances, and an intra-examiner kappa value of 0.89 was obtained.

#### *Data analysis*

The main outcomes of this study were the prevalence and extent of caries activity. Prevalence of caries activity was defined as the percentage of participants having at least one active caries lesion (either non-cavitated or cavitated). Extent of caries activity was defined as the number of active lesions (either non-cavitated or cavitated).

Individuals were classified in three categories regarding age group:  $\leq 15$ , 15-20,  $> 20$ . Mother's education was classified as primary school, high school, and university. Family income was classified in  $\leq 3$  and  $> 3$  Brazilian minimum wages (1 BMW corresponded to approximately 295 US dollars during the period of data collection). Tooth brushing frequency was dichotomized into  $\leq 2$  times/day and  $\geq 3$  times/day. The use of dental floss was classified as no use, non-daily use, and daily use.

Chi-square test was used to compare groups G0, G1, G2, and G3 according to age, gender, mother's education, tooth brushing frequency, and use of dental floss. Kruskal-Wallis test followed by Dunn test adjusted for multiple comparisons (Bonferroni) was used to compare prevalence and extent of caries activity among groups. Unadjusted and adjusted median regression models were performed to assess the effect of socio-demographic characteristics and oral hygiene habits on the association between the duration of orthodontic treatment and the extent of caries activity. Data analysis was performed using STATA software (Stata 14.2 for Windows; Stata Corporation, College Station, TX, USA). The significant level was set at 5%.

#### *Ethical aspects*

The study protocol was approved by the Ethics Committee of the Federal University of Santa Maria, Brazil (number 0109/2013). Patients or their legal guardians signed a written informed consent form.

## **Results**

The sample distribution according to socio-demographics, oral hygiene habits, and duration of orthodontic treatment is shown in Table 1. Significant differences among groups were observed for age, family income, and use of dental floss ( $p < 0.05$ ). The proportion of individuals 15 years old and younger, receiving  $\leq 3$  BMW, and reporting the daily use of dental floss decreased as the duration of orthodontic treatment increased.

Prevalence and extent of caries activity are reported in Table 2. Significant differences in prevalence and extent of caries activity were observed among groups; estimates increased significantly from G0 to G1 to G2, but no significant differences were observed between G2 and G3.

A multivariable median regression model was used to clarify the possible influence of socio-demographics and oral hygiene habits in the detected differences (Table 3). After adjusting for age, sex, mother's education, tooth brushing frequency, and use of dental floss, duration of fixed orthodontic treatment was still significantly associated with caries activity.

## **Discussion**

This study assessed the relationship between the duration of fixed orthodontic treatment and caries activity. A significant association was observed with higher prevalence and extent of active caries among individuals undergoing fixed orthodontic therapy for longer periods of time. To the best of our knowledge, this is the first study evaluating the long-term effect of fixed orthodontic therapy on caries activity using proper clinical detection criteria.

Long-term optimal oral hygiene is a cornerstone for oral health. Fixed orthodontic appliances act as an important biofilm retentive factor, which not only may compromise the maintenance of biofilm levels compatible with oral health, but also decrease the patient's long-term motivation and commitment to oral hygiene. Our study observed higher caries activity among patients undergoing fixed orthodontic treatment, and caries activity increased with treatment length leveling off after 2 years. This last finding is in disagreement with previous long-term follow-up studies [Richter et al., 2011; Khalaf, 2014]. Richter et al. [2011] found that the number of WSL increased from 3.0 to 5.3 after 22 and 33 months of treatment, respectively. Similarly, Khalaf et al. [2014] found a higher likelihood of developing WSL when the treatment length increased from <24 months to 24-36 months or to >36 months. Possible differences in caries experience/risk among study samples and the different caries detection methods may explain, at least in part, these differences. It is important to point out that our sample is derived from a population with low caries experience and widely exposed to fluoridated products as well as to fluoridated water (0.7-1.0 ppm F), which may have contributed to the stabilization of caries activity between the two last observational periods.

Strengths of this study include the clinical examination protocol (tooth cleaning and drying, single calibrated examiner, and assessment of all tooth surfaces), inclusion of individuals without the fixed appliance as well as individuals undergoing fixed orthodontic treatment for 2 and 3 years. Weaknesses include the cross-sectional study design and

differences in socio-demographics and oral hygiene habits among groups. Longitudinal studies assessing caries incidence over long periods of time are the most adequate design to investigate the issue on caries activity during orthodontic therapy; however, longitudinal studies are difficult and expensive to execute. With regards to confounding, a multivariable analysis was performed and no major differences in the estimates of caries activity were observed after adjustment.

In conclusion, this cross-sectional study showed that the duration of fixed orthodontic treatment significantly affected caries activity among adolescents and young adults. Orthodontics patients should be target for preventive strategies to control caries development.

**Table 1**

Table 1. Sample distribution according to socio-demographics, oral hygiene habits, and duration of orthodontic treatment. N (%).

	No treatment	1 year	2 years	3 years	Total	P <sup>‡</sup>
<b>Age (years)</b>						0.03
≤15	17 (26)	21 (32)	20 (31)	7 (11)	65 (25)	
15-20	26 (40)	18 (28)	23 (35)	35 (54)	102 (39)	
>20	22 (24)	26 (40)	22 (34)	23 (35)	93 (36)	
<b>Gender</b>						0.78
Male	28 (43)	29 (45)	25 (38)	24 (37)	106 (41)	
Female	37 (57)	36 (55)	40 (62)	41 (63)	154 (59)	
<b>Mother's education</b> *						0.15
Primary school	29 (45)	22 (34)	24 (37)	13 (20)	88 (34)	
High school	19 (29)	20 (31)	20 (31)	25 (38)	84 (32)	
University	17 (26)	22 (34)	21 (32)	27 (42)	87 (34)	
<b>Family income</b>						0.004
≤3 BMW	47 (72)	41 (63)	29 (45)	31 (48)	148 (57)	
> 3 BMW	18 (28)	24 (37)	36 (55)	34 (52)	112 (43)	
<b>Tooth brushing</b>						0.37
≤2 times/day	20 (31)	19 (29)	27 (42)	19 (29)	85 (33)	
≥3 times/day	45 (69)	46 (71)	38 (58)	46 (71)	175 (67)	
<b>Dental floss</b> *						0.04
No	17 (26)	11 (17)	16 (25)	24 (37)	68 (26)	
Non-daily	27 (42)	30 (46)	35 (55)	30 (46)	122 (47)	
Daily	21 (32)	24 (37)	13 (20)	11 (17)	69 (27)	
<b>TOTAL</b>	65 (100)	65 (100)	65 (100)	65 (100)	260 (100)	

BMW = Brazilian minimum wage (equivalent to around 295 US dollars during the period of data gathering).

\* Missing value. <sup>‡</sup>Chi-square test.

**Table 2**

Table 2. Prevalence (percentage of subjects with active lesions) and extent (number of active lesions) of caries activity according to duration of fixed orthodontic treatment (n=260).

	<b>Prevalence (95% CI)</b>	<b>Extent Mean (95% CI) [median; 25%-75%]</b>
<b>No treatment</b>	1.5 (0.1-8.3) <sup>a</sup>	0.15 (0.1-0.86) <sup>a</sup> [0; 0-0]
<b>1 year</b>	27.7(17.3-40.2) <sup>b</sup>	0.61 (0.44-0.84) <sup>b</sup> [0; 0-1]
<b>2 years</b>	72.3 (59.8-82.7) <sup>c</sup>	2.14 (1.80-2.53) <sup>c</sup> [2; 0-3]
<b>3 years</b>	72.3 (59.8-82.7) <sup>c</sup>	1.95 (1.63-2.32) <sup>c</sup> [2; 0-3]

Different letters in columns indicate statistically significant differences among categories (Dunn test adjusted for multiple comparisons, p<0.05).



**Table 3**

Table 3. Crude and adjusted estimates of caries activity. Median regression on number of active lesions (n=260).

	<b>Unadjusted</b>	<b>Adjusted<sup>‡</sup></b>
<b>No treatment</b>	Reference	Reference
<b>1 year</b>	0 (-0.43 – 0.53)	0 (-0.43 – 0.43)
<b>2 years</b>	2 (1.58 – 2.43)*	2 (1.56 – 2.44)*
<b>3 years</b>	2 (1.58 – 2.43)*	2 (1.60 – 2.44)*

<sup>‡</sup>Estimates are adjusted for age, gender, mother's education, tooth brushing frequency, and use of dental floss.

\* p< 0.05

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**4 ARTIGO 3 – ASSOCIATION BETWEEN FIXED ORTHODONTIC TREATMENT AND DENTAL CARIES AMONG ADOLESCENTS AND YOUNG ADULTS: A 1-YEAR LONGITUDINAL STUDY**

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**Association between fixed orthodontic treatment and dental caries among adolescents and young adults: a 1-year longitudinal study**

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## ABSTRACT

**Objective:** To assess the association between the use of fixed orthodontic appliance and the incidence/increment of active caries lesions in adolescents and young adults over 1 year.

**Methods:** This longitudinal study included individuals aged 10-30 years divided in two groups: Group G0, individuals who have not undergone fixed orthodontic treatment over the study period (n=70); and Group G1, individuals who used the fixed orthodontic apparatus for 1 year (n=65). Data collection included a questionnaire and clinical examinations, performed at baseline and after 1 year by a single calibrated examiner. Examinations included the plaque index, the gingival index, professional prophylaxis, tooth drying, and detection of caries lesions. The association between group and incidence/increment of caries activity was assessed using Poisson regression. **Results:** Incidence of active caries lesions was 4.8% for Group G0 and 39.6% for Group G1. Group 1 showed an 8-9-fold increased risk for developing at least one active caries lesion when compared with Group 0 (adjusted IRR=9.47 [95% CI=2.62-34.30]). The mean increment of active lesions was 0.14 for Group G0 and 0.61 for Group G1. Group G1 presented approximately a 4-fold higher risk for developing an additional active lesion than did Group G0 (adjusted IRR=4.13 [1.94-8.79]). **Conclusion:** Individuals undergoing fixed orthodontic therapy over 1 year had a significantly higher incidence and increment of active caries lesions than did those without the fixed apparatus. **Clinical relevance:** These findings may bring light to the issue of caries activity among orthodontic patients, pointing to the need for monitoring caries activity during the fixed therapy.

## KEYWORDS

Orthodontic appliance, Incidence, Dental caries.

## INTRODUCTION

Even considering the evident reduction in the occurrence of dental caries at the population level and the rate of progression of active lesions towards cavitation [1-4], some factors contribute for a higher risk of developing the disease. The use of fixed orthodontic appliances has been suggested as a risk factor for dental caries since it promotes favorable conditions for biofilm accumulation and it hampers the execution of usual oral hygiene practices [5-7]. In a recent systematic review with meta-analysis, Sundararaj et al [5] summarized prevalence and incidence rates of active non-cavitated caries lesions during orthodontic treatment. A total of 14 studies were included. The authors found a mean prevalence rate of 68.4% (data derived from 9 studies) and a mean incidence rate of 45.8% (data derived from 7 studies).

An important limitation of the longitudinal studies available in the literature refers to the method of detecting caries lesions during orthodontic therapy. In these studies, the occurrence of non-cavitated caries was investigated by means of photographs taken before and after orthodontic treatment for documentary purposes [6-12]. Although Chapman et al [11] reported that the use of digital photographs is a valid method for examining the incidence and severity of such lesions, there is no study comparing this method of detection with conventional clinical examinations. Considering that photographs for orthodontic documentation usually do not adopt cleaning and drying of dental surfaces, this method can be presumed to underestimate the number of initial caries lesions in enamel, as previously described in the literature [13]. Although the purpose of these studies has been limited to the evaluation of caries on buccal surfaces associated with orthodontic brackets, it is notorious that they do not express the overall situation of the patient in regards to caries activity.

To the best of our knowledge, there is no longitudinal study adopting clear and rigorous clinical criteria for the detection of non-cavitated caries lesions among orthodontic

patients. Therefore, this study aimed to assess the association between the use of fixed orthodontic appliance and the incidence/increment of active caries lesions in adolescents and young adults over 1 year.

## **METHODS**

### **Sample**

The sample consisted of patients aged 10-30 years who sought orthodontic treatment in an orthodontic graduate program in Santa Maria, South Brazil. Individuals were divided in two groups, as follows: Group G0 (n=70), composed by individuals who have not undergone fixed orthodontic treatment over the study period; Group G1 (n=65), composed by individuals who used the fixed orthodontic apparatus for 1 year (10-14 months of treatment).

Sample size calculation used the following parameters: difference of 20% between the comparison groups, power of 80% and level of significance of 95%. This resulted in a required sample size of 65 individuals per group. Patients who had congenital anomalies, special needs, or were under the use of systemic medication for the treatment of chronic diseases that could interfere with caries activity were excluded from the sample.

### **Data collection**

Initially, the subjects answered a questionnaire on socio-demographic characteristics and oral hygiene habits.

Clinical examinations were conducted in a dental unit, with a clinical mirror and a WHO probe, according to the following systematic: 1) Evaluation of the plaque index [14]; 2) Evaluation of the gingival index [15]; 3) Professional prophylaxis with bicarbonate jet (Jetlaxis Uno, Schuster, Santa Maria, Brazil); 4) Tooth drying with air-water syringe and isolation with cotton rolls; and 5) Detection of the presence and activity of caries lesions,

adapted from Maltz et al [16]. Active non-cavitated lesion was defined as an opaque enamel with a dull-whitish surface; inactive non-cavitated lesion defined as a shiny appearance of the surface area with a white or different degrees of brownish discoloration; active cavitated lesion defined as a localized surface destruction with active characteristics (dull-whitish enamel and soft dentin with light brown color); inactive cavitated lesion defined as a localized surface destruction with arrested characteristics (shiny, hard surfaces with different degrees of brownish discoloration). Sealed, filled and missed surfaces were also recorded.

Clinical examinations were performed at baseline and after 1 year according to the same protocol.

### **Reproducibility**

All clinical examinations were conducted by a single examiner (ASP). After intensive training sessions supervised by a reference examiner (JEAZ), repeated examinations were performed in 10 individuals with a minimal time interval of 7 days. Intra-examiner kappa value (ASPxASP) of 0.92 and inter-examiner kappa value (ASP x JEAZ) of 0.87 were obtained. Examiner reproducibility was also assessed after the repeated examination of individuals using fixed orthodontic appliances, and an intra-examiner kappa value (ASPxASP) of 0.89 was obtained. Considering that plaque accumulation and gingival bleeding are variable conditions, examiner calibration was not assessed for these indexes.

### **Data analysis**

Incidence of caries activity was defined as the proportion of individuals who developed at least one active caries lesions over the study period. Increment of caries activity was defined as the difference between the number of active lesions at follow-up and the number of active lesions at baseline.



The evaluated independent variables were age, gender, mother's education, socioeconomic status, family income, tooth brushing frequency, dental flossing, plaque index and gingival index.

Subjects were classified into three groups according to age:  $\leq 15$  years, 15-20 years,  $> 20$  years. Mother's education was classified as primary school, high school and university. Socioeconomic status was collected as a 4-categories variable (high, mid-high, mid-low, and low), and grouped to compose a binary variable: high/mid-high and mid-low/low. Family income was classified in  $\leq 3$  and  $> 3$  Brazilian minimum wages (1 BMW corresponded to around 295 US dollars during the period of data collection). Tooth brushing frequency was dichotomized as  $\leq 2$  times/day and  $\geq 3$  times/day. The use of dental floss was classified as daily and non-daily. Plaque index and gingival index were calculated as the mean score of all evaluated sites.

Group 0 and Group 1 were compared according to baseline characteristics using the chi-square test (categorical variables) and the Wald test (counting variables).

The association between group and the outcomes (incidence and increment of caries activity over the study) was assessed using Poisson regression models. Age, gender, mother's education, socioeconomic status, family income, tooth brushing, dental floss, dental plaque, and gingival bleeding were included and maintained in the adjusted model irrespective of their p-values.

## **RESULTS**

Sample distribution according to baseline characteristics and group is presented in Table 1. Groups 0 and 1 were similar in regards to all evaluated variables, with the exception of age. Group 1 presented a higher proportion of individuals aged  $\leq 15$  years than Group 0. It is important to emphasize that the prevalence of caries activity, the mean number of active

caries lesions, the plaque index, and the gingival index were similar in both groups at the baseline examination.

Out of the 135 individuals included in the sample, 14 already presented caries activity at baseline. Therefore, 121 participants were considered at risk for developing the disease and included in the incidence analyses. Incidence of caries activity by group and the Poisson regression models are shown in Table 2. It was observed an incidence of caries activity of 4.8% for Group 0 and 39.6% for Group 1. Individuals undergoing fixed orthodontic treatment showed an 8-fold increased risk for developing at least one active caries lesion when compared with individuals without the fixed apparatus (unadjusted IRR=8.38 [95%CI=2.50-27.73]). After the inclusion of socio-demographic, behavioral, and clinical variables in the adjusted model, this estimate presented a slight increase (adjusted IRR=9.47 [95%CI=2.62-34.30]).

Table 3 presents the increment of active caries lesions according to group and the risk assessment analysis. Individuals without fixed orthodontic appliance present a mean increment of active lesions of 0.14 whereas those using the apparatus developed, on average, 0.61 new active lesions. Poisson regression models showed that individuals composing Group 1 presented approximately a 4-fold higher risk for developing an additional active lesion than participants of Group 0 (unadjusted IRR=4.31 [95%CI=2.15-8.61]; adjusted IRR=4.13 [1.94-8.79]).

## **DISCUSSION**

This study assessed the association between the use of fixed orthodontic appliance and the occurrence of active caries lesions over 1 year. It was observed a significantly greater risk for the development of active caries in patients submitted to orthodontic therapy than individuals without the apparatus. This is the first longitudinal study to assess this relationship adopting

proper clinical criteria and conditions to detect non-cavitated caries in both observational periods.

Previous studies have demonstrated higher caries incidence among orthodontic patients by using photographs as the diagnostic tool [6-12]. The present study, using rigorous clinical examinations including tooth cleaning and drying and evaluating all tooth surfaces corroborated these previous findings. Fixed orthodontic appliance users had a higher incidence of active caries (39.6%) than did the control group (4.8%). In the risk assessment analysis, it was found that using a fixed orthodontic apparatus conferred around an 8-9-fold higher risk to develop at least one active caries lesion in a period of 1 year in comparison with the group without the fixed appliance. In order to remove the possible confounding effect of other variables, the adjusted analysis included all the independent variables in the model. The adjusted estimate was slightly higher than the crude estimate, which reinforces the effect of the use of the orthodontic device on the association.

In addition to the incidence analysis, the increment of active caries lesions was also evaluated. It was found a greater caries increment in Group 1 compared to Group 0, which is in agreement with the incidence data. In the risk assessment analysis, the use of fixed orthodontic appliance resulted in an approximately 4-fold increased risk for developing an additional active lesion than individuals without the appliance, even after the adjustment for possible confounding factors. There is no previous study in the literature evaluating caries increment in orthodontic patients.

Noteworthy strengths of our study include its clinical examination protocol encompassing tooth cleaning and drying for better detection of non-cavitated lesions, and the high reproducibility of the examiner. Furthermore, through a risk assessment analysis, adjusted for socio-demographic, behavioral, and intraoral factors, our study was able to more accurately estimate the effect of fixed orthodontic treatment on caries activity over a period of

1 year. Considering the number of individuals included in previous studies [6, 9, 10, 17], a possible limitation of our study might be its sample size. Notwithstanding, the number of patients examined in our study conferred it sufficient statistical power to detect the aimed differences between both groups. The similarities between the comparison groups were checked at baseline. As the existence of age differences between the comparison groups, even slight, could impact the outcome, the factor “age” was included in the adjusted models.

In conclusion, this longitudinal study showed that individuals undergoing fixed orthodontic therapy over 1 year had a significantly higher incidence and increment of active caries lesions than did those without the fixed apparatus. These findings may bring light to the issue of caries activity among orthodontic patients, pointing to the need for monitoring caries activity during the fixed therapy.

## **COMPLIANCE WITH ETHICAL STANDARDS**

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: The study protocol was approved by the Ethics Committee of the Federal University of Santa Maria, Brazil (protocol number 0109/2013). All procedures were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: All patients or their legal guardians signed a written informed consent form.

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## TABLES

Table 1. Sample distribution according to baseline characteristics and group.

	<b>Group 0</b> <b>n (%)</b>	<b>Group 1</b> <b>n (%)</b>	<b>P<sup>‡</sup></b>
<b>Age (years)</b>			
≤15	19 (27.1)	35 (53.8)	0.006
15-20	26 (37.1)	14 (21.5)	
>20	25 (35.7)	16 (24.6)	
<b>Gender</b>			
Male	28 (40.0)	27 (41.5)	0.86
Female	42 (60.0)	38 (58.5)	
<b>Mother's education<sup>*</sup></b>			
Primary school	27 (39.1)	25 (38.5)	0.68
High school	24 (34.8)	19 (29.2)	
University	18 (26.1)	21 (32.3)	
<b>Socioeconomic status</b>			
High/Mid-high	37 (52.9)	36 (55.4)	0.77
Mid-low/Low	33 (47.1)	29 (44.6)	
<b>Family income</b>			
≤3 BMW	46 (65.7)	36 (55.4)	0.22
> 3 BMW	24 (34.3)	29 (44.6)	
<b>Tooth brushing</b>			
≤2 times/day	27 (38.6)	16 (24.6)	0.08
≥3 times/day	43 (61.4)	49 (75.4)	
<b>Dental floss<sup>*</sup></b>			
Non-daily	40 (58.0)	31 (47.7)	0.23
Daily	29 (42.0)	34 (52.3)	
<b>Prevalence of active caries</b>			
0 active caries	63 (90.0)	58 (89.2)	0.88
≥1 active caries	7 (10.0)	7 (10.8)	
	<b>Mean (±SD)</b>	<b>Mean (±SD)</b>	<b>P<sup>‡‡</sup></b>
<b>Plaque index</b>	0.20 (0.16)	0.22 (0.18)	0.62
<b>Gingival index</b>	0.20 (0.19)	0.20 (0.21)	0.96
<b>Active caries lesions</b>	0.24 (0.94)	0.31 (0.63)	0.63

BMW = Brazilian minimum wage (equivalent to around 295 US dollars during the period of data gathering). SD = Standard deviation.

\* Missing value. <sup>‡</sup>Chi-square test. <sup>‡‡</sup>Wald test.



Table 2. Incidence of caries activity over the study period and its association with independent variables. Unadjusted and adjusted Poisson regression models (n=121).

	<b>Incidence</b>	<b>Unadjusted</b>		<b>Adjusted*</b>	
	<b>% (95% CI)</b>	<b>IRR (95% CI)</b>	<b>P</b>	<b>IRR (95% CI)</b>	<b>P</b>
<b>Group</b>					
<b>0</b>	4.8 (-0.6-10.1)	1.00		1.00	
<b>1</b>	39.6 (26.8-52.5)	8.38 (2.50-27.73)	0.001	9.47 (2.62-34.30)	0.001

\* Estimates adjusted for age, gender, mother's education, socioeconomic status, family income, tooth brushing, dental floss, plaque index, and gingival index.  
IRR = Incidence risk ratio; CI = Confidence Interval.

Table 3. Increment of caries activity over the study period and its association with independent variables. Unadjusted and adjusted Poisson regression models (n=135).

	<b>Increment</b>	<b>Unadjusted</b>		<b>Adjusted*</b>	
	<b>Mean (95% CI)</b>	<b>IRR (95% CI)</b>	<b>P</b>	<b>IRR (95% CI)</b>	<b>P</b>
<b>Group</b>					
<b>0</b>	0.14 (-0.05-0.33)	1.00		1.00	
<b>1</b>	0.61 (0.33-0.89)	4.31 (2.15-8.61)	<0.001	4.13 (1.94-8.79)	<0.001

\* Estimates adjusted for age, gender, mother's education, socioeconomic status, family income, tooth brushing, dental floss, plaque index, and gingival index.

IRR = Incidence risk ratio; CI = Confidence Interval.

## 5 DISCUSSÃO

Esta tese apresentou os resultados de dois estudos que avaliaram o efeito da duração do tratamento ortodôntico fixo no aumento de volume gengival e na atividade de cárie em indivíduos com idade entre 10 e 30 anos, bem como um terceiro estudo investigando a associação entre o uso do aparato ortodôntico fixo e a incidência e o incremento de lesões cáries ativas após um ano. A partir dos dados do primeiro estudo pode-se concluir que o tempo sob tratamento ortodôntico fixo influenciou fortemente a extensão do aumento de volume gengival, com manifestações mais severas desta condição entre os pacientes que usavam aparelhos ortodônticos por períodos mais longos de tempo. Adicionalmente, o segundo estudo demonstrou que a duração do tratamento ortodôntico fixo também afetou significativamente a atividade de cárie, de forma que quanto maior o tempo em tratamento, maior foi a prevalência/extensão de lesões de cárie ativas. Em ambos os estudos transversais, não foi observada diferença entre os indivíduos que utilizavam o aparato ortodôntico fixo por 2 anos ou por 3 anos. Finalmente, no terceiro estudo, de natureza longitudinal, o acompanhamento de pacientes submetidos à terapia ortodôntica ao longo de um ano indicou um risco significativamente maior para o desenvolvimento de lesões ativas de cárie, quando comparado a pacientes sem aparelho e monitorados pelo mesmo período de tempo.

Dois estudos recentes (ZANATTA et al., 2014; EID et al., 2014) observaram associação entre uso de aparelhos ortodônticos fixos e aumento de volume gengival. Zanatta et al. (2014) avaliaram alterações periodontais, características sociodemográficas e frequência do uso do fio dental em 330 indivíduos, com aparelho ortodôntico fixo há, pelo menos, 6 meses. A ausência do uso diário do fio dental e a ocorrência de sangramento gengival proximal anterior apresentaram associação com o aumento de volume gengival anterior. EID et al. (2014) encontraram uma alta frequência (48%) de gengivite e aumento de volume gengival em adolescentes (10-19 anos de idade) durante a terapia ortodôntica fixa, cuja frequência de escovação e uso do fio dental era restrito a uma vez ao dia ( $p < 0,001$ ). Indivíduos cuja prática de higienização dos dentes era superior a três vezes por dia não apresentaram aumento de volume gengival.

No presente estudo (artigo 1), também foi observada uma crescente ocorrência de aumento de volume gengival à medida que a duração do tratamento ortodôntico aumentou. Indivíduos que usavam aparelhos ortodônticos fixos por 1, 2 e 3 anos apresentaram um risco 20-28 vezes maior de apresentar aumento de volume gengival em comparação ao grupo

controle, sem o uso do aparato ortodôntico. A associação entre duração do tratamento ortodôntico e aumento de volume gengival está em desacordo com os achados de Zanatta et al. (2014). Embora os autores tenham encontrado uma maior frequência de aumento de volume gengival severo (escores 2 e 3 do índice de Seymour) em indivíduos que usam aparelhos ortodônticos por > 12 meses em comparação com aqueles que usam o aparato por 6-12 meses, não houve associação significativa entre a duração do tratamento e o aumento de volume gengival na análise de risco.

A alta prevalência/extensão de inflamação gengival entre pacientes ortodônticos tem sido relatada na literatura (ZACHRISSON; ZACHRISSON, 1972; POLSONET al., 1988; ELLIS; BENSON, 2002; LEVIN et al. 2008) e está de acordo com achados do estudo 1. Quanto maior o aumento de volume gengival, maior a dificuldade de acesso às superfícies dos dentes, inibindo a boa higiene bucal e resultando em mais inflamação e sangramento. Apesar disso, outros fatores podem estar associados ao aumento de volume gengival durante o tratamento ortodôntico como a baixa dose de níquel liberada continuamente para o epitélio, devido à corrosão de aparelhos ortodônticos (JIA et al, 1999; PAZZINI et al, 2009) e alterações hormonais que ocorrem durante a puberdade, modulando os tecidos periodontais para serem mais sensíveis a pequenas quantidades de placa e desencadeando uma reação hiperplásica da gengiva (BOYD et al., 1989; MARKOU et al., 2009).

A relação existente entre uso de aparelho ortodôntico fixo e prevalência/incidência de cárie tem sido investigada na literatura odontológica há décadas. Sundararaj et al. (2015) sumarizaram tais achados por meio de uma meta-análise. Após avaliar 14 estudos, foi encontrada uma taxa de prevalência de cárie de 68,4% (dados derivados de 9 estudos) e uma taxa de incidência de cárie de 45,8% (dados derivados de 7 estudos). Segundo os autores, esse problema generalizado de desenvolvimento de lesões ativas de cárie durante a terapia ortodôntica é um desafio, justificando a atenção de pacientes e profissionais, os quais devem atuar em conjunto para prevenir sua ocorrência.

No estudo 2 desta tese, também foi observada uma maior prevalência e extensão de cárie ativa entre os pacientes que utilizaram o aparelho ortodôntico por 1, 2, e 3 anos quando comparados ao grupo controle. Estes achados sugerem que quanto maior o tempo de tratamento, mais frequentes se tornam tais alterações, sem diferença estatística entre os indivíduos que usam o aparelho fixo por 2 ou 3 anos.

Tufekci et al. (2011) e Lucchese e Gherlone (2013) também encontraram um aumento na prevalência de lesões não cavidadas ativas de cárie em pacientes submetidos a terapia ortodôntica por 6-12 meses, avaliados por meio do exame clínico visual, quando comparados

a um grupo controle sem aparelho ortodôntico. Em ambos estudos, os autores relataram a rápida progressão da desmineralização do esmalte em torno dos braquetes, amplamente visível já nos primeiros 6 meses de uso do aparato ortodôntico, mostrando uma taxa de evolução menos lenta aos 12 meses, sem diferença estatística entre tais períodos de avaliação. Outros estudos disponíveis na literatura reportando a ocorrência de cárie por períodos mais longos de terapia ortodôntica estão em desacordo com os resultados do artigo 2, no que concerne à falta de diferença entre os grupos G2 e G3. Richter et al. (2011) encontraram maior número de lesões não cavitadas ativas de cárie em indivíduos sob tratamento ortodôntico fixo por 33 meses em comparação àqueles sob tratamento por 22 meses, com médias de 5,3 e 3,0 superfícies afetadas, respectivamente. Em outro estudo, foi encontrada maior probabilidade de desenvolver lesões ativas de cárie quando o tempo de tratamento ortodôntico foi de 24-36 meses ou >36 meses, com estimativas de risco de 1,65 e 3,65, respectivamente, quando comparados a um grupo tratado por <24 meses (KHALAF, 2014).

As possíveis diferenças na experiência/risco de cárie entre as amostras dos estudos e os diferentes métodos de detecção de cárie podem explicar, pelo menos em parte, esses achados controversos. É importante salientar que as amostras incluídas nos estudos que compõe esta tese são provenientes de uma população de baixa prevalência de cárie, amplamente exposta a produtos fluoretados e à água fluoretada (0,7-1,0 ppm F), o que pode ter contribuído para a estabilização das estimativas de cárie nos dois últimos períodos observacionais. Ao avaliar o aumento de volume gengival no mesmo grupo de pacientes, o artigo 1 indicou o mesmo padrão de aumento acentuado até o ano 2 e uma taxa estável do ano 2 ao ano 3, o que reforça o papel do controle do biofilme em ambas as condições bucais, cárie dentária e aumento de volume gengival.

No artigo 3 da presente tese, usuários de aparelhos ortodônticos fixos apresentaram maior incidência de cárie ativa (39,6%) do que o grupo controle (4,8%). Na análise de risco, verificou-se que a utilização do aparelho ortodôntico fixo conferiu um risco 8-9 vezes maior de desenvolver pelo menos uma lesão de cárie ativa em um período de um ano em comparação com o grupo sem o aparelho. No mesmo estudo, a análise de risco indicou que o uso do aparato ortodôntico aumentou, aproximadamente, em 4 vezes a chance de desenvolver uma lesão ativa adicional do que indivíduos sem o aparelho, mesmo após o ajuste para possíveis fatores de confusão.

Estudos longitudinais prévios, mesmo dispondo de amostras maiores de pacientes, apresentam importantes limitações no que concerne ao método de detecção das lesões cariosas. Enquanto alguns utilizam fotografias pré e pós-tratamento ortodôntico (KHALAF et

al., 2014; AKIN et al., 2013; JULIEN et al., 2013; ENAIA et al., 2011; RICHTER et al., 2011; CHAPMAN et al., 2010; GOLERICK et al., 1982), outros restringem a avaliação clínica às superfícies vestibulares dos dentes ântero-superiores (SAGARIKA et al. 2012; HADLER-OLSEN et al., 2011; MARTIGNON et al, 2010; MIZRAHI, 1982). Assim, apesar da possível limitação do tamanho de amostra incluída no artigo 3, o número de pacientes examinados conferiu poder estatístico suficiente para garantir a detecção das diferenças existentes entre os dois grupos de comparação.

Nos três artigos apresentados nesta tese, o protocolo de exame clínico incluiu limpeza e secagem prévias bem como a avaliação de todas as superfícies dentárias, por um único examinador calibrado. Além disso, a inclusão de grupos controles sem o aparelho fixo permitiu uma adequada análise da relação existente entre o uso do aparato ortodôntico fixo e suas consequências para a saúde bucal, diante da falta de orientação e limpeza profissionais frequentes e rotineiras.

## **6 CONCLUSÃO**

A utilização de aparelho ortodôntico fixo por diferentes períodos de tempo (1, 2 ou 3 anos) promoveu maior ocorrência de aumento de volume gengival e de atividade de cárie em pacientes jovens em comparação a indivíduos sem o aparato ortodôntico. Não foi observada diferença entre os grupos que utilizaram aparelho fixo por 2 ou 3 anos.

Indivíduos sob tratamento ortodôntico fixo por um ano apresentaram maior incidência e incremento de cárie ativa quando comparados a pacientes sem aparelho.

Esses achados indicam a necessidade de monitoramento da atividade de carie durante a terapia ortodôntica, por meio de instruções de higiene bucal rotineiras e controles profissionais frequentes.

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Before you begin, please review the guidelines below. To view a 7-minute video explaining how to prepare your article for submission, go to [Video on Manuscript Preparation](#).

1. *Title Page*. Put all information pertaining to the authors in a separate document. Include the title of the article, full name(s) of the author(s), academic degrees, and institutional affiliations and positions; identify the corresponding author and include an address, telephone and fax numbers, and an e-mail address. This information will not be available to the reviewers.

2. *Abstract*. Structured abstracts of 200 words or less are preferred. A structured abstract contains the following sections: Introduction, describing the problem; Methods, describing how the study was performed; Results, describing the primary results; and Conclusions, reporting what the authors conclude from the findings and any clinical implications.

3. *Manuscript*. The manuscript proper should be organized in the following sections: Introduction and literature review, Material and Methods, Results, Discussion, Conclusions, References, and figure captions. Express measurements in metric units, whenever practical. Refer to teeth by their full name or their FDI tooth number. For style questions, refer to the *AMA Manual of Style, 10th edition*. Cite references selectively, and number them in the order cited. Make sure that all references have been mentioned in the text. Follow the format for references in "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Ann Intern Med 1997;126:36-47); <http://www.icmje.org>. Include the list of references with the manuscript proper. Submit figures and tables separately (see below); do not embed figures in the word processing document.

4. *Figures*. Digital images should be in TIF or EPS format, CMYK or grayscale, at least 5 inches wide and at least 300 pixels per inch (118 pixels per cm). Do not embed images in a word processing program. If published, images could be reduced to 1 column width (about 3 inches), so authors should ensure that figures will remain legible at that scale. For best results, avoid screening, shading, and colored backgrounds; use the simplest patterns available to indicate differences in charts. If a figure has been previously published, the legend (included in the manuscript proper) must give full credit to the original source, and written permission from the original publisher must be included. Be sure you have mentioned each figure, in order, in the text.

5. *Tables*. Tables should be self-explanatory and should supplement, not duplicate, the text. Number them with Roman numerals, in the order they are mentioned in the text. Provide a brief title for each. If a table has been previously published, include a footnote in the table giving full credit to the original source and include written permission for its use from the copyright holder. Submit tables as text-based files (Word is preferred, Excel is accepted) and not as graphic elements. Do not use colors, shading, boldface, or italic in tables. Do not submit tables as parts A and B; divide into 2 separate tables. Do not "protect" tables by making them "read-only." The table title should be put above the table and not as a cell in the table. Similarly, table footnotes should be under the table, not table cells.

6. *Model release and permission forms*. Photographs of identifiable persons must be accompanied by a release signed by the person or both living parents or the guardian of minors. Illustrations or tables that have appeared in copyrighted material must be accompanied by written permission for their use from the copyright owner and original author, and the legend must properly credit the source. Permission also must be obtained to use modified tables or figures.

7. *Copyright release*. In accordance with the Copyright Act of 1976, which became effective February 1, 1978, all manuscripts must be accompanied by the following written statement, signed by all authors: *"The undersigned author(s) transfers all copyright ownership of the manuscript [insert title of article here] to the American Association of Orthodontists in the event the work is published. The undersigned author(s) warrants that the article is original, does not infringe upon any copyright or other proprietary right of any third party, is not under consideration by another journal, has not been previously published, and includes any product that may derive from the published journal, whether print or electronic media. I (we) sign for and accept responsibility for releasing this material."* Scan the printed [copyright release](#) and submit it via EES.

8. Use the *International Committee of Medical Journal Editors Form for the Disclosure of Conflict of Interest (ICMJE Conflict of Interest Form)*. If the manuscript is accepted, the disclosed information will be published with the article. The usual and customary listing of sources of support and institutional affiliations on the title page is proper and does not imply a conflict of interest. Guest editorials, Letters, and Review articles may be rejected if a conflict of interest exists.

9. *Institutional Review Board approval*. For those articles that report on the results of experiments of treatments where patients or animals have been used as the sample, Institutional Review Board (IRB) approval is mandatory. No experimental studies will be sent out for review without an IRB approval accompanying the manuscript submission.

## ANEXO B – NORMAS PARA PUBLICAÇÃO NO PERIÓDICO *CARIES RESEARCH*

### Guidelines for Authors

#### Aims and Scope

'Caries Research' is an international journal, the aim of which is to promote research in dental caries and related fields through publication of original research and critical evaluation of research findings. The journal will publish papers on the etiology, pathogenesis, prevention and clinical control or management of dental caries. Papers on health outcomes related to dental caries are also of interest, as are papers on other disorders of dental hard tissues, such as dental erosion. Aspects of caries beyond the stage where the pulp ceases to be vital are outside the scope of the journal. The journal reviews papers dealing with natural products and other bacterial inhibitors against specific criteria, details of which are available from the Editor.

#### Submission

Manuscripts written in English should be submitted online:

Should you experience problems with your submission, please contact:

Prof. David Beighton

(Editor-in-Chief, Caries Research)

Department of Microbiology

The Henry Wellcome Laboratories for Microbiology and Salivary Research

KCL Dental Institute, Floor 17, Guys Tower

London Bridge SE1 9RT (UK)

Tel. +44 2071887465

Fax +44 2071887466

[cre@karger.com](mailto:cre@karger.com)

During the online submission you will be asked to list complete mailing addresses, including e-mail addresses of three potential reviewers for your manuscript.

Copies of any 'in press' papers cited in the manuscript must accompany the submission. Manuscripts reporting on clinical trials must be accompanied by the CONSORT checklist (see below).

#### Plagiarism Policy

Whether intentional or not, plagiarism is a serious violation. We define plagiarism as a case in which a paper reproduces another work with at least 25% similarity and without citation.

If evidence of plagiarism is found before/after acceptance or after publication of the paper, the author will be offered a chance for rebuttal. If the arguments are not found to be satisfactory, the manuscript will be retracted and the author sanctioned from publishing papers for a period to be determined by the responsible Editor(s).

#### Conditions

All manuscripts are subject to editorial review. Manuscripts are received with the explicit understanding that the data they contain have not previously been published (in any language) and that they are not under simultaneous consideration by any other publication.

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#### Types of Papers

*Original papers or Short Communications* are reports of original work (including systematic reviews and meta-analyses). Both have the structure outlined below but for Short Communications the abstract should be less than 100 words and the manuscript should not exceed 3 printed pages, equivalent to about 9 manuscript pages (including tables, illustrations and references).



*Reviews* can have a freer format but should nevertheless commence with a Title page, an Abstract and an Introduction defining the scope. Reviews are not subject to page charges. *Current topics* are concise articles that present critical discussion of a topic of current interest, or a fresh look at a problem, and should aim to stimulate discussion.

*Letters to the Editor*, commenting on recent papers in the journal, are published occasionally, together with a response from the authors of the paper concerned.

Preparation of Manuscripts Text should be one-and-a-half-spaced, with wide margins. All pages and all lines must be numbered, starting from the title page. A conventional font, such as Times New Roman or Arial, should be used, with a font size of 11 or 12. Avoid using italics except for Linnaean names of organisms and names of genes.

Manuscripts should be prepared as a text file plus separate files for illustrations. The text file should contain the following sequence of sections: Title page; Declaration of interests; Abstract; Introduction; Materials and Methods; Results; Discussion; Acknowledgements; References; Legends; Tables. Each section should start on a new page, except for the body of the paper (Introduction to Acknowledgements), which should be continuous. Lines in the manuscript must be numbered consecutively from the title page until the last page. Submissions which do not conform to these simple guidelines will be returned to the author.

**Title page:** The first page of each manuscript should show, in order:

- the title, which should be informative but concise;
- the authors' names and initials, without degrees or professional status, followed by their institutes;
- a short title, maximum length 60 characters and spaces, for use as a running head;
- a list of 3-10 key words;
- the name of the corresponding author and full contact details (postal address, telephone and fax numbers, and e-mail address).

**Declaration of Interests:** Potential conflicts of interest should be identified for each author or, if there are no such conflicts, this should be stated explicitly. Conflict of interest exists where an author has a personal or financial relationship that might introduce bias or affect their judgement. Examples of situations where conflicts of interest might arise are restrictive conditions in the funding of the research, or if an author or their employer holds patent(s) on a product used in the study, or payment to an investigator from organisations with an interest in the study (including employment, consultancies, honoraria, ownership of shares, travel grant). Investigators should disclose potential conflicts to study participants and should state whether they have done so.

The possible existence of a conflict of interest does not preclude consideration of a manuscript for publication, but the Editor might consider it appropriate to publish the disclosed information along with the paper.

**Abstract:** The abstract should summarise the contents of the paper in a single paragraph of no more than 250 words (to ensure that the abstract is published in full by on-line services such as PubMed). No attempt should be made to give numerical results in detail. References are not allowed in the abstract.

**Introduction:** This section should provide a concise summary of the background to the relevant field of research, introduce the specific problem addressed by the study and state the hypotheses to be tested.

**Materials and Methods (or Subjects and Methods):** All relevant attributes of the material (e.g. tissue, patients or population sample) forming the subject of the research should be provided. Experimental, analytical and statistical methods should be described concisely but in enough detail that others can repeat the work. The name and brief address of the manufacturer or supplier of major equipment should be given.

Statistical methods should be described with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, findings should be quantified and appropriate measures of error or uncertainty (such as confidence intervals) given. Sole reliance on statistical hypothesis testing, such as the use of P values, should be avoided. Details about eligibility criteria for subjects, randomization and the number of observations should be included. The computer software and the statistical methods used should be specified. See Altman et al.: Statistical guidelines for contributors to medical journals [Br Med J 1983;286:1489-93] for further information.

Manuscripts reporting studies on human subjects should include evidence that the research was ethically conducted in accordance with the Declaration of Helsinki (World Medical Association). In particular, there must be a statement in Materials and Methods that the consent of an appropriate ethical committee was obtained prior to the start of the study, and that subjects were volunteers who had given informed, written consent.

Information detailing the power and sample size calculations must be included in the manuscript. Randomized clinical trials should be reported according to the standardised protocol of the CONSORT Statement. The CONSORT checklist must be submitted together with papers reporting clinical trials. Randomized clinical trials must be registered at clinicaltrials.gov or similar national authority and the trial number included in the manuscript.

Trials beginning after 1 July 2012 must be registered before recruitment of the first patient. Caries Research will accept 'retrospective registration' of trials that began before 1 July 2012 (retrospective meaning registration occurs after patient enrolment begins). When submitting a paper on a clinical trial, the trial registration number should be stated at the end of the abstract in the following format: Trial registration: [name of the trial registry, the registry URL and the trial registration number].

In studies on laboratory animals, the experimental procedures should conform to the principles laid down in the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and/or the National Research Council Guide for the Care and Use of Laboratory Animals.

Unless the purpose of a paper is to compare specific systems or products, commercial names of clinical and scientific equipment or techniques should only be cited, as appropriate, in the 'Materials and Methods' or 'Acknowledgements' sections. Elsewhere in the manuscript generic terms should be used.

In any manuscript involving microradiography, the following information must be included: the radiation source and filters used and the kV used (this determines the wavelength of radiation and hence the validity of using Angmar's equation).

Manuscripts on experimental enamel caries should show that the lesions retain a relatively well-preserved surface layer, i.e. are not surface-softened lesions. Proof of surface integrity can be provided either as illustrations in the paper or as supplementary material for the reviewers. Transverse microradiography, polarized light microscopy of a section immersed in water or backscattered scanning electron microscopy of a polished cross-section can be used to provide the necessary proof. To allow the nature of experimental changes to be assessed, microradiographs or micrographs should be provided to show part of the experimental lesion and the adjacent control (e.g. figure 2 of Zaura et al.: *Caries Res* 2007;41:489–492). Again, these images can be provided as part of the paper or as supplementary material for review purposes.

**Results:** Results should be presented without interpretation. The same data should not be presented in both tables and figures. The text should not repeat numerical data provided in tables or figures but should indicate the most important results and describe relevant trends and patterns.

**Discussion:** This section has the functions of describing any limitations of material or methods, of interpreting the data and of drawing inferences about the contribution of the study to the wider field of research. There should be no repetition of preceding sections, e.g. reiteration of results or the aim of the research. The discussion should end with a few sentences summarising the conclusions of the study. However, there should not be a separate 'Conclusions' section.

**Acknowledgements:** Acknowledge the contribution of colleagues (for technical assistance, statistical advice, critical comment etc.) and provide the position(s) of author(s) employed by commercial firms. This section should describe the source(s) of funding that have supported the work including relevant grant numbers. Please also include this sentence: "The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript." If this statement is not correct, you must describe the role of any sponsors or funders, and amend the sentence as needed. Additionally, the roles of all authors must be described (For example: Conceived and designed the experiments: AA, BB. Performed the clinical examination: AA, CC. Performed the experiments: DD, FF. Analyzed the data: BB, FF. Wrote the paper: AA, CC, FF, EE).

**Legends:** The table headings should be listed first, followed by the legends for the illustrations.

**Tables:** Tables should be numbered in Arabic numerals. Each table should be placed on a separate

page. Tables should not be constructed using tabs but by utilising the table facilities of the word-processing software.

**Illustrations:**

- Illustrations should be numbered in Arabic numerals in the sequence of citation. Figure numbers must be clearly indicated on the figures themselves, outside the image area.
- Black and white half-tone illustrations must have a final resolution of 300 dpi after scaling, line drawings one of 800-1200 dpi.
- Figures with a screen background should not be submitted.
- When possible, group several illustrations in one block for reproduction (max. size 180 x 223 mm).

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**Online edition:** Color illustrations are reproduced free of charge. In the print version, the illustrations are reproduced in black and white. Please avoid referring to the colors in the text and figure legends.

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References

Reference to other publications should give due acknowledgement to previous work; provide the reader with accurate and up-to-date guidance on the field of research under discussion; and provide evidence to support lines of argument. Authors should select references carefully to fulfil these aims without attempting to be comprehensive.

Cited work should already be published or officially accepted for publication. Material submitted for publication but not yet accepted should be cited as 'unpublished results', while unpublished observations communicated to the authors by another should be cited as 'personal communication', with credit in both cases being given to the source of the information. Neither unpublished nor personally communicated material should be included in the list of references. Abstracts more than 2 years old and theses should not be cited without a good reason, which should be explained in the covering letter accompanying the paper.

References should be cited by naming the author(s) and year. Where references are cited in parenthesis, both names and date are enclosed in square brackets. Where the author is the subject or object of the sentence, only the year is enclosed in brackets.

One author: [Frostell, 1984] or Frostell [1984].

Two authors: [Dawes and ten Cate, 1990] or Dawes and ten Cate [1990].

More than two authors: [Trahan et al., 1985] or Trahan et al. [1985].

Several references cited in parenthesis should be in date order and separated by semi-colons: [Frostell, 1984; Trahan et al., 1985; Dawes and ten Cate, 1990].

Material published on the World Wide Web should be cited like a reference to a print publication, and the URL included in the reference list (not in the text), together with the year when it was accessed. The reference list should include all the publications cited in the text, and only those publications. References, formatted as in the examples below, should be arranged in strict alphabetical order. All authors should be listed. For papers by the same authors, references should be listed according to year. Papers published by the same authors in the same year should be distinguished by the letters a, b, c, ... immediately following the year, in both the text citation and the reference list. For abbreviation of journal names, use the Index Medicus system. For journals, provide only the year, volume number and inclusive page numbers.

*Examples*

(a) *Papers published in periodicals:* Lussi A, Longbottom C, Gyax M, Braig F: Influence of professional cleaning and drying of occlusal surfaces on laser fluorescence in vivo. *Caries Res* 2005;39:284-286.

(b) *Papers published only with DOI numbers:* Theoharides TC, Boucher W, Spear K: Serum interleukin-6 reflects disease severity and osteoporosis in mastocytosis patients. *Int Arch Allergy Immunol* DOI: 10.1159/000063858.

(c) *Monographs:* Matthews DE, Farewell VT: *Using and Understanding Medical Statistics*. Basel, Karger, 1985.

(d) *Edited books*: DuBois RN: Cyclooxygenase-2 and colorectal cancer; in Dannenberg AJ, DuBois RN (eds): COX-2. ProgExp Tum Res. Basel, Karger, 2003, vol 37, pp 124-137.

(e) *Patents*: Diggins AA, Ross JW: Determining ionic species electrochemically. UK Patent Application GB 2 064 131 A, 1980.

(f) *World Wide Web*: Chaplin M: Water structure and behavior. [www.lsbu.ac.uk/water](http://www.lsbu.ac.uk/water), 2004.

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For papers published online first with a DOI number only, full citation details must be added as soon as the paper is published in its final version. This is important to ensure that citations can be credited to the article.

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No page proofs are supplied to the author.

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## **ANEXO C – NORMAS PARA PUBLICAÇÃO NO PERIÓDICO *CLINICAL ORAL INVESTIGATIONS***

### Instructions for Authors

#### Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

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#### TITLE PAGE

The title page should include: The name(s) of the author(s); A concise and informative title; The affiliation(s) and address(es) of the author(s); The e-mail address, telephone and fax numbers of the corresponding author.

#### Abstract

Please provide a structured abstract of 150 to 250 words which should be divided into the following sections: Objectives (stating the main purposes and research question); Materials and Methods; Results

Conclusions; Clinical Relevance; These headings must appear in the abstract.

#### Keywords

2017-5-15 Clinical Oral Investigations – incl. option to publish open access

[http://www.springer.com/medicine/dentistry/journal/784?print\\_view=true&detailsPage=plctci\\_0606983/16](http://www.springer.com/medicine/dentistry/journal/784?print_view=true&detailsPage=plctci_0606983/16)

#### TEXT

##### Text Formatting

Manuscripts should be submitted in Word. Use a normal, plain font (e.g., 10-point Times Roman) for text. Use italics for emphasis. Use the automatic page numbering function to number the pages. Do not use field functions. Use tab stops or other commands for indents, not the space bar. Use the table function, not spreadsheets, to make tables. Use the equation editor or MathType for equations. Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

##### Headings

Please use no more than three levels of displayed headings.

##### Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

##### Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables. Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols. Always use footnotes instead of endnotes.

##### Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

#### REFERENCES

##### Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

1. Negotiation research spans many disciplines [3].
2. This result was later contradicted by Becker and Seligman [5].
3. This effect has been widely studied [1-3, 7].

#### Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works

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[http://www.springer.com/medicine/dentistry/journal/784?print\\_view=true&detailsPage=pltdci\\_10606984/16](http://www.springer.com/medicine/dentistry/journal/784?print_view=true&detailsPage=pltdci_10606984/16)

#### TABLES

All tables are to be numbered using Arabic numerals. Tables should always be cited in text in consecutive numerical order. For each table, please supply a table caption (title) explaining the components of the table. Identify any previously published material by giving the original source in the form of a reference at the end of the table caption. Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

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[http://www.springer.com/medicine/dentistry/journal/784?print\\_view=true&detailsPage=pltdci\\_10606985/16](http://www.springer.com/medicine/dentistry/journal/784?print_view=true&detailsPage=pltdci_10606985/16)

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#### Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware) Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)

Any figure lettering has a contrast ratio of at least 4.5:1

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#### Submission

Supply all supplementary material in standard file formats. Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author. To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.

#### Text and Presentations

Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability. A collection of figures may also be combined in a PDF file.

#### Spreadsheets

Spreadsheets should be submitted as .csv or .xlsx files (MS Excel).

#### Specialized Formats

Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

#### Collecting Multiple Files

It is possible to collect multiple files in a .zip or .gz file.

#### Numbering

If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables. Refer to the supplementary files as “Online Resource”, e.g., “... as shown in the animation (Online Resource 3)”, “... additional data are given in Online Resource 4”. Name the files consecutively, e.g. “ESM\_3.mpg”, “ESM\_4.pdf”.

#### Captions

For each supplementary material, please supply a concise caption describing the content of the file.  
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#### Accessibility

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