Periodontitis and orthodontic tooth movement association with cigarette smoking: histologic and inflammatory response

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Abstract

Aim: This study aims to evaluate linear bone loss (LBL) and connective tissue attachment loss (CTAL) in animals suffering from ligature-induced periodontitis (P), either associated with orthodontic tooth movement (OTM) and cigarette smoking or not.

Material and methods: Fifty-six male Wistar rats were allocated into two groups in smoking and non-smoking conditions. Each group has been subdivided into the following: control (C), OTM, P, and P+OTM (POTM). Periodontitis has been induced in the animals' lower first molar by cotton ligature, and a 4 mm closed stainless steel spring was used for OTM. Animals have been exposed to the smoke of 10 cigarettes for 8 minutes, 3 times a day for 60 days before P and OTM induction, and LBL and CTAL were evaluated in the alveolar crest region of their lower first molar.

Results: There was no statistically significant difference between smoking and non-smoking groups. The POTM subgroup showed higher levels of LBL and CTAL in both groups.

Conclusion: Ligature-induced periodontitis associated with orthodontic tooth movement caused greater linear bone loss and connective tissue attachment loss in animals, whether being exposed to cigarette smoke inhalation or not.

Keywords: Biomarker. Orthodontics. Periodontal disease. Smoking.

Introduction

Induced tooth movement is a biological process characterized by sequential periodontal tissue reactions to biomechanical forces (Krishnan, Davidovitch, 2016). During orthodontic force application, there are several changes at molecular and cellular level when tissues are subjected to such forces (Isola *et al*., 2016). The initial phase of tooth movement stimulates chemical and electrical factors in the liberation of sensory nerve fibres, which triggers an inflammatory response that modifies microcirculation, accompanied by leukocyte migration from the blood capillary (Ogawa *et al*., 2002). Tissue-induced modifications in tooth movement are related to its remodelling by activation of alveolar bone resorption on the pressure side and bone apposition on the tension side. Inflammation is an important requirement for induced tooth movement (Krishnan, Davidovitch, 2016). The activation of inflammatory cells and a consequent liberation of inflammatory cytokines, such as IL-1β and TNFα, has an important role in mineralized tissues (Birkedal-Hansen, 1993).

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Periodontal disease is characterized by gingival inflammation and teeth support tissue destruction as a result of a complex interaction between a bacterial biofilm and the host's immune and inflammatory responses (Silva *et al*., 2019). In the presence of bacterial plaque, orthodontic forces may cause angular bone defects and attachment loss, mainly during inclination and intrusion movements (Ericsson *et al*., 1977). Therefore, plaque control is mandatory to prevent periodontal disease for those undergoing orthodontic treatment.

Furthermore, the periodontal disease prevalence is almost 50% in the adult population aged 30 years old or so, and many of which have harmful habits to health, such as smoking (Eke *et al*., 2015). Smoking is associated with increased longitudinal marginal bone loss (Baljoon *et al*., 2005) and it is considered a risk factor for the development of periodontal disease (Tonetti *et al*., 2018). It is known that cigarette is composed of a large variety of toxic substances (Rothem *et al*., 2009) and nicotine acts on the regulation of alkaline phosphatase activity and calcium deposition (Yuhara *et al*., 1993). Moreover, cigarette smoke components can be more damaging to bone metabolism than only nicotine (Cesar-Neto *et al*., 2003), thus affecting bone metabolism by modulation of osteoblast proliferation and formation of bone resorption cytokines, such as IL-1 and IL-6 at high levels (Hapidin *et al*., 2007). However, cigarette exerts a negative effect upon tissues, whose mechanisms are not yet completely understood. Hence, it is important to conduct Periodontology-Orthodontics-Smoking research so as to guide knowledge regarding it and aid professionals that face such an issue on a daily basis. Therefore, it has been hypothesized that there is an association of cigarette smoke inhalation during orthodontic tooth movement (OTM) in rats suffering from ligature-induced periodontitis (P), since it would generate greater bone loss in the interproximal area, and thus it was evaluated through linear bone loss (LBL).

Materials and Methods

Animals

This study has been approved by the Ethics Committee on the Use of Animals (CEUA), protocol number 1/2014-PA/CEP, and followed the ARRIVE guidelines (Kilkenny *et al*., 2010). 56 male Wistar rats (*Rattus norvegicus,* albinos), aged 12 weeks and weighing 300 g on average have been kept in plastic cages lined with pine wood, each containing 5 (one with 6) animals at room temperature for a 12- hour light cycle on a standard diet with water ad libitum.

Sample size and experimental design

The sample size was calculated using data from a previous study (Ferreira *et al*., 2018) considering power of 0.8 and α of 0.05. Fifty-six animals were simply randomized and allocated into a couple of conditions: smoking (Test group) and non-smoking (Control Group) whose subgroups have been evaluated as follows: control (C, n = 14), OTM (n = 14), ligature-induced periodontitis (P, n = 14) and P+OTM (POTM, n = 14).

Treatments

Cigarette smoke inhalation

The cigarette smoke inhalation protocol was the same as the one used by Ferreira *et al*. (2018). The animals were submitted to an adaptation period in which they have been gradually exposed to cigarette smoke inhalation for 3 days in a specific acrylic box with two compartments, one for cigarettes and another for animals. Afterwards, they have been exposed to the smoke of 10 cigarettes at the same time (MINISTER King-size Unique; Souza Cruz, Rio de Janeiro, Brazil) for 8 minutes, 3 times a day, 5 days a week. Cigarette smoking inhalation was initiated 60 days before the experimental period, which has been kept until its end. Non-smoking animals have been placed in acrylic boxes of the same size for the same period to simulate the same condition.

Ligature-induced periodontitis

The animals were anesthetized with a 2:1 mixture of ketamine hydrochloride (Dopalen; Agribrands, Paulínea, Brazil) and xylazine hydrochloride (Dopalen; Agribrands, Paulínea, Brazil), respectively, at dosage of 0.1 mL/100 g, which was administered intramuscularly. Ligature-induced periodontitis was performed with a 100% cotton thread placed on the cervical region of the animals' first lower right molar. Ligature-induced periodontitis had been initiated 7 days before the orthodontic appliance was placed, which remained in the same position until the end of the experiment.

Orthodontic tooth movement appliance placement

The OTM appliance was placed at 7 days after the beginning of P and it has been kept for the next 7 days (Ferreira *et al*., 2018). Briefly, the orthodontic appliance was placed using a 4 mm CrNi closed stainless steel helical spring (Morelli, Sorocaba, Brazil) and 0.15 mm and 0.20 mm alloy wires to attach the spring. After acid etching, a photopolymerizable composite resin was applied to prevent injuries to animals.

Force was exerted on their first lower right molar so as to allow a mesialization movement, and their lower incisors served as anchorage. 75cN of force was applied, which is equivalent to 75g.

Analysis

Histometric procedure and histomorphometric analysis

The animals were euthanized by transcardial perfusion. The samples were demineralized in a 10%

ethylene diamine tetraacetic acid solution at pH 7.8 and included in paraffin. Ten sagittal sections (5 μm) of the first lower molar were made and stained with hematoxylin and eosin. The images were captured at 25× magnification using an Axioplan 2 microscope and the software Axiovision Rel 4.7. Before initiating measurements, the images were renamed (MANJ) to properly blind the examiner (VCSL). The following histomorphometric measures were performed:

Connective tissue attachment loss (CTAL) meaning the distance between the cement enamel junction (CEJ) and the most apical portion of the junctional epithelium measured in mm;

Linear bone loss (LBL) meaning the distance between the CEJ of the first lower molar and the alveolar bone crest in the distal side measured in mm.

Inflammatory biomarkers assay

The gingival tissue around the first molar of four animals per group was collected and stored in a buffered PBS solution and kept at -80° C. IL-1β, IL-6, IL10, TNF-α and VEGF were detected by mice-kit# and analysed by Luminex system** following manufacturer's instructions. Concentration samples were estimated by standard-curve and expressed in pg/ml.

Statistical analysis

After data tabulation, mean and standard deviation values were calculated for LBL and CTAL analyses, and the Kruskal-Wallis non-parametric test was used to make a comparison between groups. Subsequently, the Mann-Whitney test was carried out to determine which group was different. The significance level was set at 5%. The SPSS V20, Minitab 16, and Excel Office 2010 software were used for the analyses. Before the histomorphometric analysis, the examiner was calibrated by the Pearson test (r = 0.93).

Results

The histomorphometric analysis results were evaluated using microscopic images (Figure 1). There was no statistically significant difference between smoking and non-smoking in the groups evaluated.

Connective tissue attachment loss (CTAL)

According to the intragroup analysis in the non-smoking group, there was a statistically significant difference between POTM versus P, OTM, and C ($p < 0.001$); P versus OTM and C ($p < 0.001$) and OTM versus C ($p = 0.032$). In the smoking group, POTM was statistically significant if compared to P ($p = 0.022$), OTM and C ($p < 0.001$); P if compared to OTM and C ($p < 0.001$) and OTM versus C ($p < 0.001$), as reported in Figure 2.

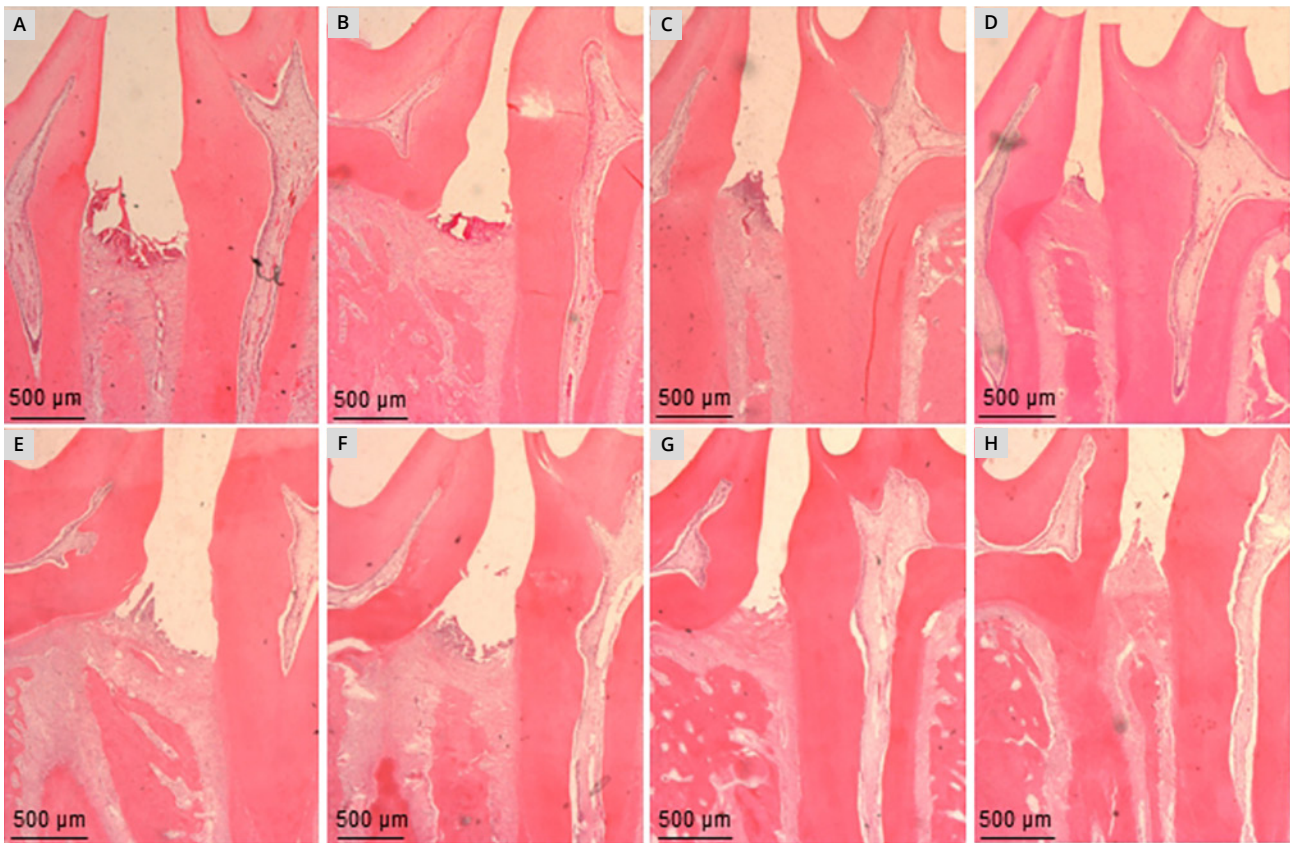

Figure 1. Histomorphometric view. Superior line: non-smoking. Inferior line: smoking. A and E= POTM; B and F= P; C and G=OTM; D and H= C. HE staining. 25x magnification.

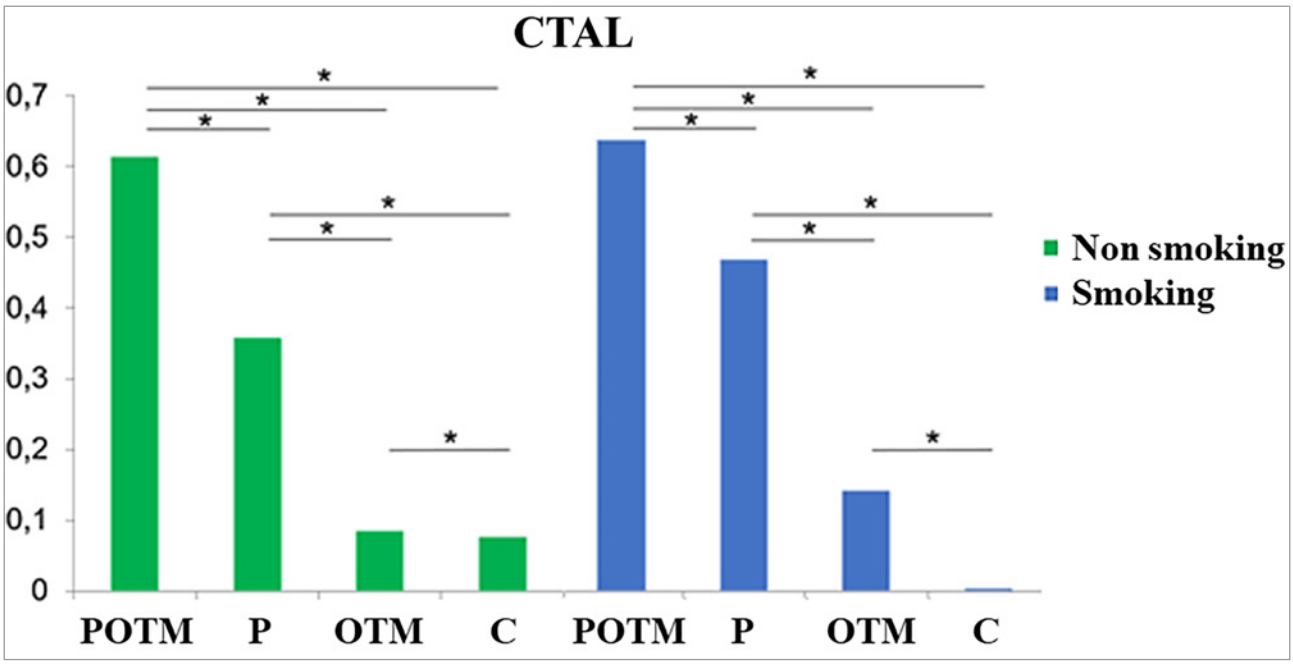

Figure 2. Connective tissue attachment loss (mm). POTM = ligature-induced periodontitis with orthodontic tooth movement; P = ligature-induced periodontitis; OTM = orthodontic tooth movement; C = control; * significant statistical difference.

Linear bone loss (LBL)

In the intragroup analysis of the non-smoking group, there was a statistically significant difference between POTM versus P, OTM and C ($p < 0.001$); P versus OTM ($p = 0.002$), and C ($p = 0.001$). However, no statistically significant difference was found in OTM versus C ($p = 0.158$). In the smoking group, POTM was statistically significant if compared to P ($p = 0.007$), OTM and C ($p < 0.001$); P if compared to OTM ($p = 0.002$) and C ($p < 0.001$) and OTM versus C ($p < 0.001$) as shown in Figure 3.

Inflammatory biomarkers

IL-1β, IL-10, and VEGF showed statistically higher levels in the non-smoking group than in the smoking one (Figure 4), but only through the POTM group analysis (IL-1β 398 ± 212,5 vs 155,6 ± 89,2; IL-10 211,1 ± 45,0 vs 136,7 ± 25,5 and VEGF 100,3 ± 35,8 vs 50,5 ± 8,2). There was no statistically significant difference for the IL-6 and TNF-α analysis.

Discussion and conclusion

Periodontal tissue reactions were analysed during induced tooth movement in the presence and absence of periodontal disease in rats, either exposed to cigarette smoke inhalation or not through histomorphometric measures and inflammatory biomarkers. In vitro studies have shown that nicotine can inhibit the defensive functions of neutrophils and monocytes (Pabst *et al*., 1995) and stimulate the activity of gingival fibroblats (James *et al*., 1999). Smoke markedly increased alveolar bone loss (Kubota *et al*., 2016). In addition, a study involving rats (Kirschneck *et al*., 2015) showed that nicotine accelerated orthodontic tooth movement.

There was no difference between smoking and non-smoking group according to CTAL and LBL analyses. It ought to be speculated that the experimental model reproduced conditions of being a light smoker rather than being a heavy one, since the used cigarette had lower concentrations of its components (Ferreira *et al*., 2016). Such lower concentration might have led to this result, since among variations of individuals who smoke, the frequency and depth of inhalation should not only be considered, but also the size of the cigarette butt, in addition to the presence or absence of a filter and its trademark (Benowitz, 1996).

The greatest loss of LBL and CTAL was found in the POTM group if compared to P, OTM and C groups, which is in accordance with some studies that showed orthodontic force application during active periodontitis in chronic inflammatory processes of the periodontium, as well as periodontal bone loss, while periodontal inflammation accelerated orthodontic tooth movement (Michelogiannakis *et al*., 2018). It is worth mentioning that OTM in the smoking group presented significant LBL if compared to the control group, as mentioned by previous studies (Michelogiannakis *et al*., 2018), thus ensuring that physiologic force was applied, since there was no difference between OTM and C in non-smoking animals.

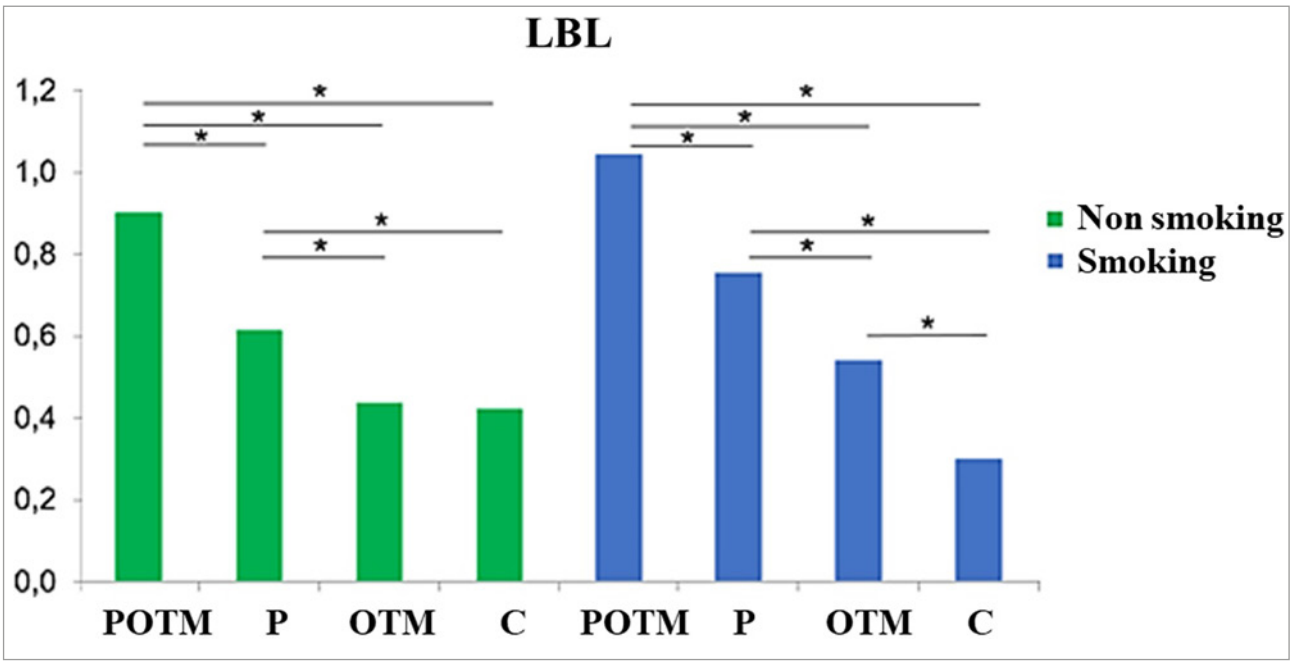

Figure 3. Linear bone loss (mm). POTM = ligature-induced periodontitis with orthodontic tooth movement; P = ligature-induced periodontitis; OTM = orthodontic tooth movement; C = control; * significant statistical difference.

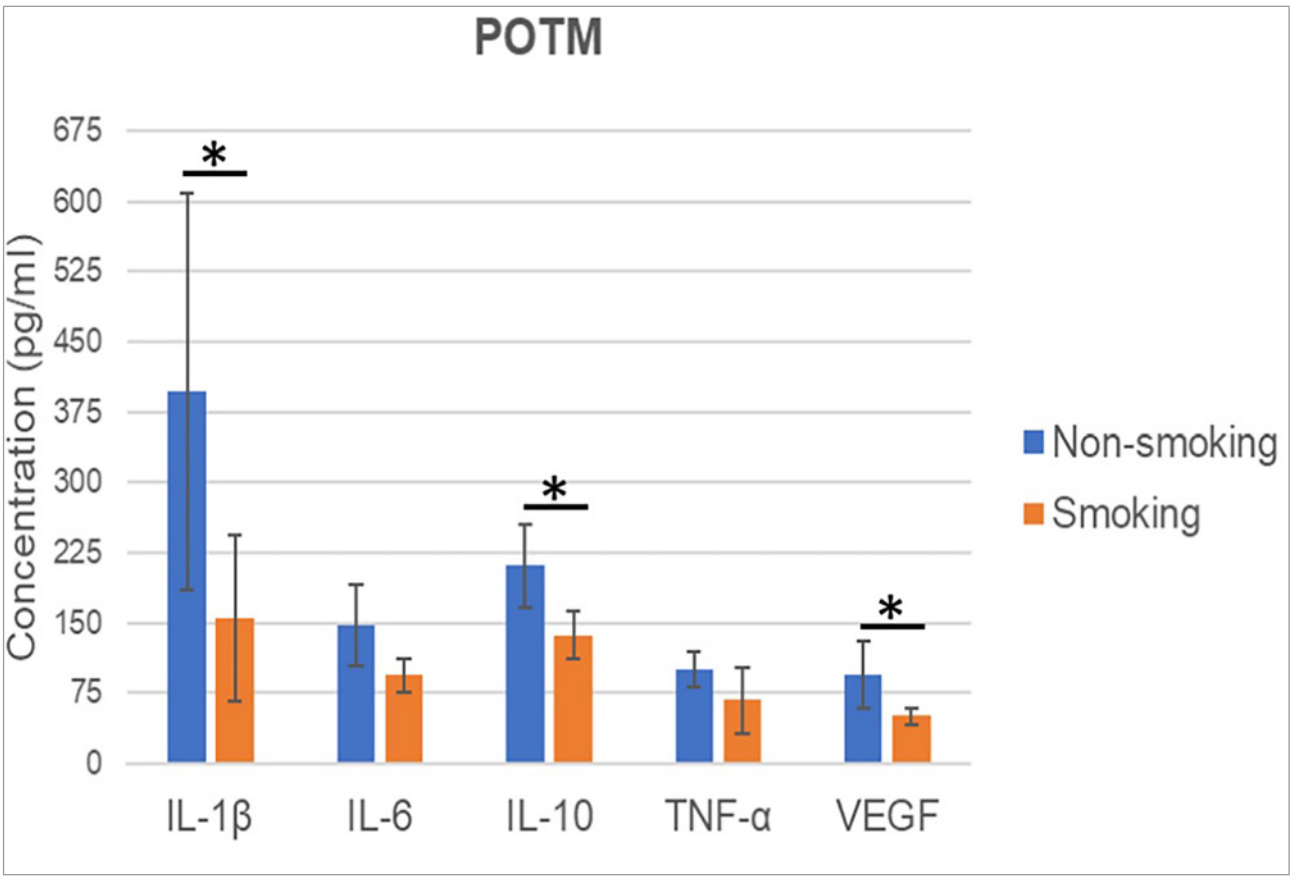

Figure 4. POTM inflammatory biomarkers. POTM = ligature-induced periodontitis with orthodontic tooth movement; *Statistically significant difference.

As regard inflammatory biomarkers, it is known that biologically active substances, such as cytokines, are expressed from periodontal cells in response to mechanical stress caused by orthodontic force application (Isola *et al*., 2016). These cytokines are only secreted by activated cells and bind specific membrane receptors, which in turn are expressed only on activated cells. In this study, the POTM group showed a difference in IL-1β, IL-10, and VEGF. Most studies found elevated values of IL-1β, IL-6, and TNF-α in periodontitis and orthodontic tooth movement combination, thus evidencing that this association can trigger a cascade of progressive tissue and bone breakdown (Ogawa *et al*., 2002). Furthermore, IL-10 plays multiple roles in restraining periodontitis severity (Garlet, 2010) and VEGF, in addition to angiogenesis and permeability vascular properties, which also has a role in bone resorption and formation due to a significant production of periodontal tissues in both conditions (Nogueira *et al*., 2017).

In corroboration with the present results, other studies have shown a reduction in inflammatory markers in smoking groups when compared to non-smokers (Timkiw *et al*., 2011; Ge *et al*., 2019). Timkiw *et al*. (2011) suggest that reduced pro-inflammatory cytokines in the smoker group could be related to lower levels of clinical inflammation in smokers with an ineffective defence against periodontal pathogens and increased susceptibility to tissue destruction. On the other hand, Kirshneck *et al*. (2015) and Ge *et al*. (2019) showed increased values of IL-6, and He *et al*. (2016) showed augmented TNF-α values by comparing smokers to non-smokers.

In addition to the evaluation of inflammatory cytokines, further studies should include other potentially useful analyses for evaluating the mechanisms involved in periodontal disease pathogenesis, such as genetic alterations which are possibly due to failure at epithelial cell adhesion (Currò *et al*., 2014), for instance.

Although a translation of animal model results for a scenario involving humans can be explained by similarities regarding the periodontal anatomy of the molar, biofilm development and composition, and histopathology of periodontal lesions, results of studies must be carefully evaluated, since there is a wide variation in study models, simulation or exposure to smoke (nicotine injection or smoke inhalation, for instance), and the source for detecting biomarkers (serum, gingival crevicular fluid or tissue).

In conclusion, the present study found that ligature-induced periodontitis associated with orthodontic tooth movement caused greater linear bone loss and connective tissue attachment loss in animals, regardless of whether they have been exposed to cigarette smoke inhalation or not.

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