UNIVERSIDADE FEDERAL DE UBERLÂNDIA

FACULDADE DE ODONTOLOGIA

FILIPE GONTIJO SILVA

IMPACTO DA INALAÇÃO DE CIGARRO NOS TECIDOS PERIODONTAIS EM PERIODONTITE APICAL: ESTUDO IN VIVO

UBERLÂNDIA

2024

FILIPE GONTIJO SILVA

IMPACTO DA INALAÇÃO DE CIGARRO NOS TECIDOS PERIODONTAIS EM PERIODONTITE APICAL: UM ESTUDO IN VIVO

Trabalho de Conclusão de Curso apresentado à Faculdade de Odontologia da Universidade Federal de Uberlândia, como requisito parcial para obtenção do título de Graduado em Odontologia

Orientadoa: Prof. Dra. Priscilla Barbosa Soares

UBERLÂNDIA 2024

RESUMO

O objetivo deste estudo foi avaliar o impacto da fumaça ambiental do tabaco (FAT) nos tecidos periodontais associado ao dente com à periodontite apical (PA) e seu adjacente. Vinte e oito ratos foram divididos em quatro grupos: controle (sem PA e FAT), PA (com PA e sem FAT), FAT (com FAT e sem PA) e FAT+PA (com FAT e com PA). Após a indução da PA e a exposição ao FAT os animais foram eutanasiados e as regiões dos primeiros e segundos molares, mostrando espessamento do ligamento periodontal (LP) e perda óssea região das mandibulas foram analisadas por microtomografia computadorizada. A análise do LP foi realizada usando ANOVA unidirecional, enquanto os valores da fração de volume ósseo (BV/TV) foram analisados usando ANOVA bidirecional. Os resultados não mostraram diferença significativa no LP entre os primeiros e segundos molares nos grupos sem PA, independentemente da exposição ao FAT. O LP do primeiro molar foi maior no grupo FAT+PA quando comparado ao grupo ETS ($p<0,05$). O grupo primeiro molar FAT+PA apresentou taxa BV/TV significativamente menor ($p<0,05$) em comparação todos outros grupos nas regiões analisadas. Nas regiões de furca e apical adjacentes à lesão do primeiro molar, os valores foram menores nos grupos FAT, PA e FAT+PA em relação ao controle ($p<0,05$). Conclui-se que fumantes passivos, expostos a baixos níveis de fumaça ambiental de tabaco, tendem a apresentar comprometimento ósseo e comprometimento periodontal sutil quando associados a uma endodontia.

Palavra-chaves: Microtomografia por Raio-X; Periodontite Periapical; Poluição por Fumaça de Tabaco.

ABSTRACT

The aim of this study was to evaluate the impact of environmental tobacco smoke (ETS) on periodontal tissues associated with teeth with apical periodontitis (AP) and their adjacent teeth. Twenty-eight rats were divided into four groups: control (no AP and no ETS), AP (with AP and no ETS), ETS (with ETS and no AP), and ETS+AP (with ETS and AP). After AP induction and ETS exposure, the animals were euthanized, and the regions of the first and second molars, showing thickening of the periodontal ligament (PL) and bone loss in the mandibular region, were analyzed by micro-computed tomography. The PL analysis was performed using one-way ANOVA, while the bone volume fraction (BV/TV) values were analyzed using two-way ANOVA. The results showed no significant difference in the PL between the first and second molars in the groups without AP, regardless of ETS exposure. The PL of the first molar was greater in the ETS+AP group compared to the ETS group ($p<0.05$). The first molar ETS+AP group showed a significantly lower BV/TV ratio ($p<0.05$) compared to all other groups in the analyzed regions. In the furcation and apical regions adjacent to the first molar lesion, the values were lower in the ETS, AP, and ETS+AP groups compared to the control ($p<0.05$). It was concluded that passive smokers, exposed to low levels of environmental tobacco smoke, tend to present bone impairment and subtle periodontal impairment when associated with endodontic treatment.

Key-words: Periapical Periodontitis; Tobacco Smoke Pollution; X-Ray Microtomography.

SUMÁRIO

Artigo a ser submetido para Scientic Report

Effects of Cigarette Smoke Inhalation on Bone Tissue in Apical Periodontitis: *In Vivo* Study

INTRODUCTION

Smoking is considered a public health problem that negatively impacts systemic health (Gupta *et al.*, 2019), and also oral health (Leite *et al.,* 2018). The deleterious effects of cigarettes on periodontal tissues and their relationship with a higher prevalence of periodontal diseases have been widely studied (Leite *et al.,* 201; Chaffee *et al.*, 2021; Pesce *et al*., 2022). The similarity between the pathophysiological pathways of periodontal and periapical disease has led, in recent years, to study the impact of smoking on the development of apical periodontitis (AP) (Segura-Egea *et al.*, 2015; Pinto *et al.*, 2020a; Pinto *et al.,* 2020b). A recent systematic review demonstrated that smokers were twice as likely to have apical periodontitis and/or root canal treatment compared to nonsmokers (Pinto *et al.*, 2020a)

It has been postulated that smoking can impact the periradicular tissues, reducing the blood supply and consequently the arrival of nutrients and oxygenation (Segura-Egea *et al*., 2015), which potentially can contribute to necrosis of the pulp tissue, and a loss of defense capacity against bacterial infections (Krall *et al.,* 2006). The role of smoking in this process has gained attention as it can induce a worse result in endodontic treatment when compared to non-smokers (Kirkevang & Wenzel 2003; Segura-Egea *et al.*, 2008). In addition, smoking seems to alter the immune response, (Gomes *et al.*, 2013; Segura-Egea *et al.*, 2015; Pinto et al. 2020a), accelerate bone loss and alter collagen synthesis by fibroblasts, interfering with the repair of periapical lesions (Bergström *et al.,* 2004, López-López *et al.*, 2012, Paljevic *et al.*, 2023).

While active smoking is known to negatively affect the periodontal and (Leite *et al.,* 2018, Chaffee *et al.*, 2021) periapical (Pinto *et al.,* 2020a) health of smokers, there are scarce data on the effect of exposure to ETS through “passive” or “involuntary” exposure on the development of AP (Vasques *et al.* 2023). Some studies have addressed potential deleterious effects of ETS on the periodontium of non-smokers (Akinkugbe *et al*., 2016, Li *et al.*, 2021). ETS is composed of a mixture of secondhand smoke emitted by a lit cigarette and the smoke exhaled by a smoker, having similar chemical constituents such as nicotine, and other compounds such as ammonia, nitrogen and sulfur oxides (Akinkugbe *et al*., 2016, Oliveira *et al.,* 2022). A recent systematic review found a

positive epidemiological association between exposure to ETS and periodontitis among non-smokers (Sahni & Gupta, 2022; Oliveira *et al.,* 2022). Based on these findings, and on the scarcity of clinical data that support a similar statement regarding the AP, the present *in vivo* study aimed to evaluate the impact of "involuntary" exposure to cigarette smoke isolated or associated to AP on the impairment of periodontal ligament space (PDL) and alveolar bone volume through new method for computed microtomography analysis (μCT).

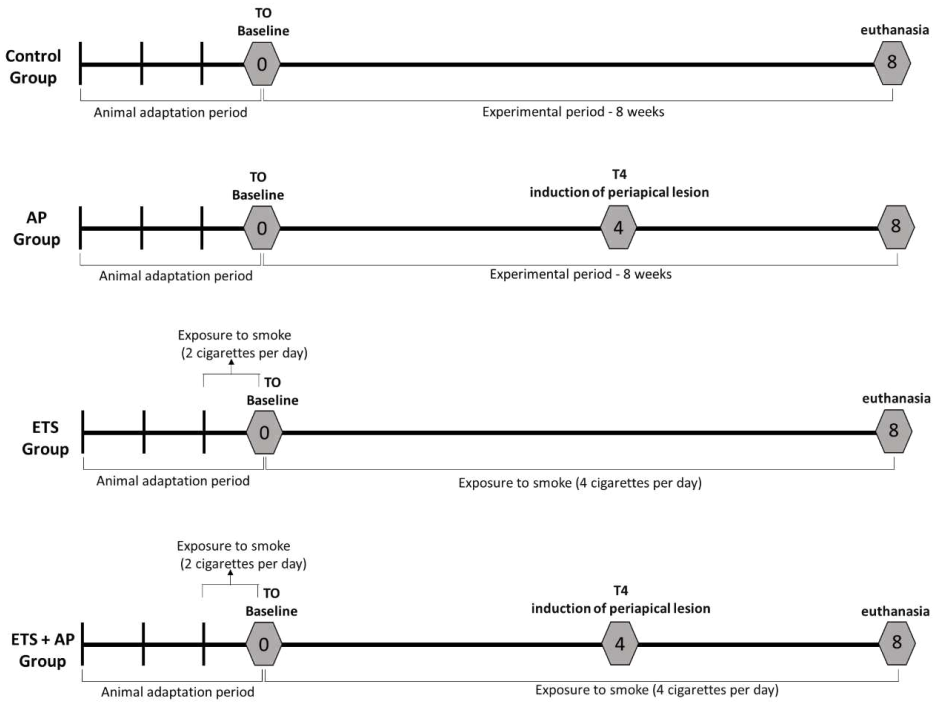
METHODOLOGY

Sample size calculation

The sample size was estimated based on data from Pinto *et al.* (2020)b. Considering an alpha error of 5% and 95%, and ability to recognize a significant difference of 1 in median scores, a minimum of seven animals per group was required.

Animals

This study was conducted in accordance with the ARRIVE guidelines for preclinical studies and the normative guidelines of the National Council for Animal Control and Experimentation. The Animal Ethics Committee of the institution Federal University of Uberlândia (Protocolo nº: CEUA-23117.043409/2023-11), which is affiliated to the Council of Animal Experiments (CONCEA), following international norms of animal care and maintenance, approved the procedures. Twenty-eight rats (Rattus norvegicus albinus, Wistar) with an average initial body mass of 250g (±10g) were included in the study. The animals were kept at room temperature (20°C to 24°C), with light/dark: 12 x 12 hours control, and free access to food and water. Water and food intake was controlled daily, with assessment of average feed and water consumption. The animals were randomly distributed into 4 equal groups (n=7 per group): Control: not exposed to tobacco smoke and without inducing AP; ETS: exposed to tobacco smoke inhalation and without AP induction; AP: not exposed to tobacco smoke and with AP induction; ETS+AP: exposed to tobacco smoke inhalation and AP induction. The total experimentation time was 8 weeks in each group (Figure 1).



Exposure to ETS

Equipment previously standardized by Amaral *et al.* (2020), which consists of a rectangular box with a transparent acrylic lid and four cylindrical tubes (10 cm in diameter x 25 cm in length) with openings at the ends for introducing the animals were used. At the funneled end, there is a connection with an orifice connected to a peristaltic pump, through hoses in which cigarette smoke is pumped intermittently in a closed system, being distributed equally to the four tubes of the equipment. All equipment connections are connected to an exhaust fan for aspiration and cleaning of excess smoke in the system at each cycle, as well as from the external environment.

The animals from the ETS and ETS+AP groups were exposed to cigarette smoke. The animals stayed for 01 week in acclimatization, simply being placed in the device without burning the cigarette. In the second week (adaptation), they were exposed to 02 cigarettes/day with a six-hour interval between exposures. In the following week, the animals were exposed to 04 cigarettes per day (two in the morning and two in the afternoon), following the same time interval throughout the 8 weeks. Cigarette brand Marlboro (Phillip Morris) was used, containing 0.8 mg of nicotine, 10 mg of tar and 10 mg of carbon monoxide.

Induction of AP

After 4 weeks of exposure to cigarette smoke in the animals of the AP and ETS+AP groups the AP was induced by occlusal pulpal exposure of the mandibular right first molars. Pulp exposure was performed under anesthesia (ketamine/xylazine, 70 and 6 mg/kg, in intramuscular injection) with a spherical drill 1011 (KGSorensen® Ind. Com. Ltda, Brasil) at high speed, under constant irrigation as previously described (Pinto *et al.*, 2020b). After pulp exposure, the pulp chambers were left exposed to the oral environment for 4 weeks to allow the establishment of AP.

Euthanasia and Preparation of samples

After the experimental period (8 weeks), all animals were euthanized. The jaws were collected and fixed in a 4% paraformaldehyde solution at neutral pH for 24 hours. The specimens were washed in running water for 12 hours and transferred to sodium phosphate buffer (PBS) and stored in the refrigerator until they were scanned in a microtomography device (Skyscan 1176, Bruker, Kontich, Bélgica).

MicroCT scanning, reconstruction and analysis

The experimental steps were carried out by three independent operators to avoid bias in the analyses: a single operator scanned the samples, which were previously randomized by the software (www.randon.org); a second operator performed the reconstructions, and a third operator executed the customized (manual) and predefined ROI segmentation for all samples.

Hemimandibles were scanned using identical acquisition settings: 80 kV, 125 μA, isotropic voxel size of 12 μm, Al 1.0-mm filter, 0.2° rotation step, image pixel size 12.000 μm, 360º rotation. Using NRecon reconstruction software (Buker-microCT, Kontich, Belgium), it was reconstructed the mandibular molar region with the following parameters: ring artifact correction: 0% beam-hardening correction, 0 smoothing, 4 ring artifact reduction. Reconstructions were performed in the region of the first and second lower molars.

The DataViewer® software (version 1.5.1.2, Bruker, Kontich, Belgium) was used to adjust the hemimandible images for better positioning of the region of interest. Images in the transaxial plane with standard orientation were exported to CT-Analyzer® software (version 1.14.4.1, SkyScan, Bruker, Belgium). Cuts including the first molar

with a periapical lesion and the second molar were selected to delineate the region of interest (ROI). All analyses were performed by a single trained operator.

For two-dimensional linear analysis, a total of 40 slices were defined, from a transaxial cut at three different levels: cervical, midroot, and apical, where PDL width was measured with the line tool from the mesial root of the first and second molars. For each root, measurements were taken every 5 slices, totaling 6 measurements per group through manual measurements in 7 linear lines between the dental root and surrounding bone (M1, M2, M3, M4, M5, M6, M7), as demonstrated in Figure 2A.

In the mesial root of the first molar, the 7 analysis areas were established and standardized as follows: M1 - cervical region near the cement-enamel junction; M2 - below M1, in the middle of the root; M3 - below M2, more apical; M4 - root apex; M5 - more apical region relative to the furcation, closer to the lesion; M6 - adjacent to the mesial face, in the middle of the root; M7 - region closest to the furcation. In the mesial root of the second molar, the 7 analysis areas were established and standardized as follows: M1 - cervical region near the cement-enamel junction; M2 - below M1, in the middle of the root; M3 - below M2, more apical and closer to the lesion; M4 - root apex, M5 - more apical region relative to the furcation, away from the lesion; M6 - middle of the root, in the furcation region; M7 - region closest to the furcation.

Three-dimensional analysis of the ROI was performed to examine the characteristics of bone microarchitecture (with the bone volume fraction (BV/TV %) as the selected parameter. The top and bottom of a transaxial cut, totaling 40 slices, were defined, in which the total percentage of bone volume (BV/TV; %) was evaluated by manual selection using a computer mouse to delimit the regions of interest through a predefined circular ROI with a diameter of 0.4920 x 0.4920 mm. Predefined ROI segmentation between the roots was performed individually, selected, and positioned at 4 points in the mesial root of the first and second molars, defining 4 ROIs in the mesial root of the first molar and 4 ROIs in the mesial root of the second molar [VOI-cervical (Cr), VOI- distant apical from the lesion (Da), VOI-furcation (F), VOI-near apical to the lesion (Na)] . The entire regions were defined by interpolating the ROIs for the 40 slices, after this delineation, a global threshold was used for all samples (Figure 2B).

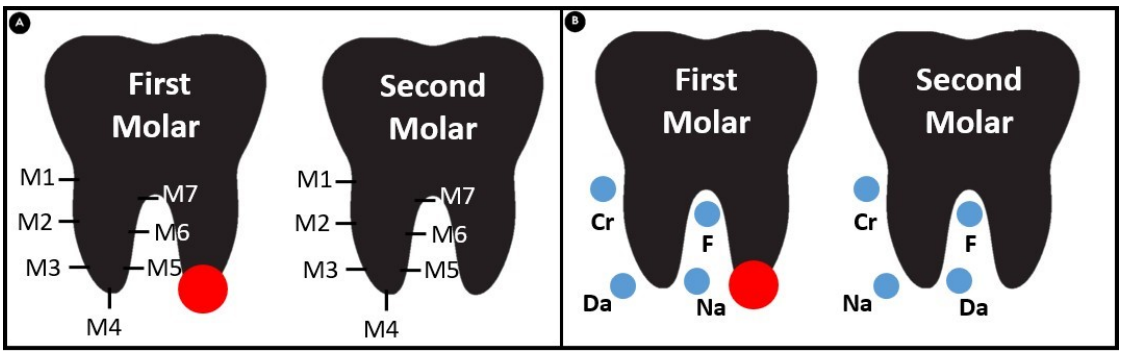


Figure 2. MicroCT analisys. **A:** Two-dimensional linear analysis measurements in 7 analysis areas - M1 - cervical region near the cement-enamel junction; M2 - below M1, in the middle of the root; M3 - below M2, more apical; M4 - root apex; M5 - more apical region relative to the furcation, closer to the lesion; M6 - adjacent to the mesial face, in the middle of the root; M7 - region closest to the furcation; **B:** Three-dimensional analysis in 4 points in the mesial root of the first and second molars – Cr: cervical; Da: distant apical from the lesion; F: furcation; An: near apical to the lesion.

Statistical analyses

SigmaPlot® (SigmaPlot v13.1; Systat Software Inc., San Jose, CA, EUA) was used to compare the four groups. Analysis of PDL space in each level, in each root, was performed with one-way analysis of variance (ANOVA). BV/TV values was assessed within each group with a two-way ANOVA. The variables for the alveolar process and root regions were averaged for analysis. Tukey's post hoc test with a significance level of α=0.05 was applied to the tested groups

RESULTS

Bidimensional analysis of PDL space

The evaluation of the thickness of PDL in the first and second molars is shown in Table 1 and Table 2, respectively. In the first molar, there was no difference in the thickness of the PDL among the groups without AP, exposed or not to cigarette smoke at any evaluated level (ETS=Control; P>0.05). In the ETS+AP group, the mean thickness of the PDL was greater than in the ETS group at all levels (ETS+AP > ETS; $p < 0.001$). There was no cumulative effect of AP and cigarette smoke when compared to the AP group in the evaluated regions (p>0.05), except in M1 (ETS+AP >AP; $p < 0.001$).

On the second molar, no difference was detected in the thickness of the PDL among the groups without AP, whether exposed or not to cigarette smoke at any level (Control = ETS, P>0.05). On the other hand, the difference was significant between control and AP group at M2, M3, M4 and M7 levels (AP > C; $p < 0.001$). AP associated

with cigarette smoke resulted in a thicker PDL compared to control and tobacco groups at all levels (ETS+AP > Control, $p < 0.001$;ETS+AP > ETS). The PDL in the AP group were smaller on M1, M5, M6 and M7 levels compared to the group ETS+AP (AP< ETS+AP; $p < 0.001$).

Tridimensional analysis of bone volume fraction (BV/TV, %)

The BV/TV means and standard deviations are presented in Table 3 and 4. Two-way ANOVA showed that BV/TV values were significantly influenced by the presence of AP ($P<0.001$), analysis region ($P<0.001$), and the interaction between these factors ($P<0.001$).

Intragroup analysis in the first molar region revealed lower BV/BT values in the furcation and apical regions near the lesion compared to the cervical and apical regions distant from the lesion, both in the ETS group ($p<0.05$) and the ETS+AP group ($p<0.05$).

Intergroup analysis in the first molar region showed that VOI values in the furcation and the apical region near the lesion gradually decreased in the ETS, AP, and ETS+AP groups compared to the control. In the control x ETS ($p=0.342$) and AP x ETS+AP ($p=0.311$) groups, no differences were found in the cervical region. The apical region distant from the lesion had lower BV/TV values in the ETS+AP group compared to the control, AP, and ETS groups ($p<0.003$). Statistically significant differences were found in the furcation and apical regions near the lesion between groups, except between control x ETS ($p=0.216$) in the furcation and ETS x AP in the near apical ($p=0.247$) .

Intragroup analysis in the second molar region showed lower BV/BT values in the cervical and apical regions near the lesion compared to the apical regions distant from the lesion and furcation, both in the AP group and the ETS+AP group ($p<0.001$). Intergroup analysis demonstrated that lower BV/BT values did not differ between groups in the apical region distant and furcation ($p>0.05$). With the presence of the lesion, whether associated with cigarette smoke inhalation or not, there was an impact only in the apical region near the lesion and cervical, which showed lower BV/BT values than the ETS and control groups.

DISCUSSION

The μCT analysis revealed that AP alone or associated with ETS affected the thickness of the PDL and the volume of bone adjacent to the lesion in rats, rejecting the null hypothesis. Although it is well-established that smoking has deleterious effects on

the skeletal system, promoting imbalance in bone remodeling mechanisms, reducing bone mass and bone mineral density (Al-Bashaireh *et al.*, 2018; Moon *et al.*, 2018), the impacts of ETS on bone metabolism and its influence on the repair capacity in the oral cavity have been studied in recent years (Apatzidou, 2022).

In dentistry, most studies on the effects of ETS are related to periodontics (Akinkugbe *et al*., 2016; Javed *et al.*, 2018; Li *et al.*, 2021) or implantology (Javed *et al.*, 2018), although there has recently been a study on the effects of cigarette smoke on the development of AP, its inflammatory profile, and immune response in rats (Vasques *et al.*, 2023). However, to our knowledge, this is the first research in which the effect of ETS and AP on the periodontal ligament space and bone volume of the affected tooth and adjacent tooth was evaluated using μCT in a rat animal model. For this purpose, AP was induced in the lower right first molar of the rat, and the analyses were performed at pre-established points on the affected tooth, outside the lesion area, and at equivalent points on the adjacent tooth.

Radiographs are commonly used tools in clinical practice for evaluating periapical lesions in AP, but they have many limitations in the diagnosis and monitoring of these lesions, especially in multi-rooted teeth (Leonardi *et al.*, 2016). In small animal models like rats, μCT is an important tool for research (Kalatzis-Sousa *et al.*, 2017), providing high-resolution, two-dimensional (2D), and three-dimensional (3D) images of the sample in a multiplanar fashion (von Stechow *et al.*, 2003; Kalatzis-Sousa *et al.*, 2017). Several studies have used micro-CT to assess the area and volume of periapical lesions in rats and mice (Kang *et al.*, 2013; Sun *et al.*, 2014; Tang *et al.*, 2014; Yang *et al.*, 2014; Pinto *et al.*, 2020), although none have performed a similar evaluation to that proposed in this study.

The use of pre-established regions of interest (ROI) allowed the application of the same parameters in both study groups, with AP, and the control group (without lesion). The use of standardized ROI in 3D analyses was previously discussed by Borges *et al.* (2023), demonstrating that the determination of ROI significantly influences the results when analyzing alveolar bone repair.

Considering that the method used for ROI delineation can affect morphometric results of micro-CT (Lazenby *et al.*, 2011; Kalatzis-Sousa *et al.*, 2017), as well as its positioning (Borges *et al.,* 2023), we opted to use a predetermined circular ROI with a diameter of 0.4920 x 0.4920 mm, pre-established in four regions of the mesial root of the

affected tooth and in four regions of the mesial root of the adjacent tooth. The positioning was always the same in the assessments of bone loss determined by the total percentage of bone volume (BV/TV; %). In general, the results showed lower BV/TV percentages near the lesion in the first molar both in the AP and ETS+AP groups, which was not observed in points distant from the lesion and in the second molar. It was expected that the inflammatory factors from cigarette smoke inhalation and periapical lesion could influence bone volume in the region adjacent to the lesion in the proposed model, which did occur. However, ETS alone was not able to statistically significantly alter these parameters compared to the control, except in the apical region near the lesion, which may be related to the ETS induction protocol used in rats.

In this study, the rats were subjected to cigarette smoke inhalation for a total period of 8 weeks, according to a previously established protocol (Santiago *et al.*, 2017; Amaral *et al.*, 2020). However, there is a wide variety of ETS induction protocols in rats, with different daily exposure times of the animal to smoke and the number of cigarettes used, which can impact the bone changes found (César-Neto *et al.*, 2003; Li *et al.*, 2021; Vasques *et al.*, 2023). Although there is no standardized model of ETS in rats, obviously, models with higher exposures (César-Neto *et al.,* 2003; Vasques *et al.*, 2023) can result in greater tissue damage to bone tissue and more pronounced differences compared to the control. However, it was not the aim of this study to mimic the behavior of heavy smokers but rather passive smokers who are exposed to environmental smoke attributable to active smokers, as previously established (Santiago *et al.*, 2017).

Bidimensional analyses evaluated the same roots as the three-dimensional BV/TV analyses, but 7 linear measurement points of the PDL were determined in the mesial root of the first molar , and 7 equivalent points in the second molar, in order to identify possible thickening due to cigarette smoke inhalation, inflammation from AP, or associations. Although there was an increase in PDL thickness in the AP and ETS+AP groups compared to the control in the first molar, there was no significant difference between these two groups, supporting the hypothesis that the ETS model used was not able to generate a significant cumulative effect on the damage already produced by AP, which is the most relevant factor in the observed periodontal and bone compromise. The results of the group exposed only to ETS support this hypothesis since no statistically significant changes were identified compared to the control. Indeed, the damage caused by smoking seems to be dose-dependent, as studies using nicotine injections (Pinto *et al.*, 2020; da Silva *et al.*, 2023) or a number of cigarettes per day greater than 10 (; Vasques

et al., 2023) resulted in greater damage to bone tissue than the control group, not exposed. However, it is not possible to compare such data to the present study since these studies did not perform PDL assessments. Regarding the second molar, within the conditions studied, there seems to be little, but still significant, influence of inflammation promoted by AP alone or associated with ETS on the adjacent tooth. Future studies that gradually increase the number of cigarettes used in the ETS model are necessary to validate the present findings. Although there are several studies on the deleterious effects of cigarette smoke on the periodontium, in animals or humans, to our knowledge, there is no similar measurement method in the literature using μCT, making it difficult to compare the results of periodontal changes from different studies.

CONCLUSION

Despite the limitations of this study, we can conclude that passive smokers, subjected to low doses of ETS, tend to present bone compromised and a subtle periodontal compromised when associated with an endodontic lesion. Additionally, AP should be carefully considered as a compromising factor for periodontal health.

ACKNOWLEDGMENT

This study was supported by grants from the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), Rede Mineira em Saúde Oral e Odontologia (#RED-00204-23), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior- Brazil (CAPES # Finance Code 001) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) - INCT Saúde Oral e Odontologia - Grants n. 406840/2022-9)

REFERENCES

Akinkugbe, Aderonke A et al. “Systematic Review and Meta-analysis of the Association Between Exposure to Environmental Tobacco Smoke and Periodontitis Endpoints Among Nonsmokers.” Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco vol. 18,11 (2016): 2047-2056. doi:10.1093/ntr/ntw105

Al-Bashaireh AM, Haddad LG, Weaver M, Chengguo X, Kelly DL, Yoon S. The Effect of Tobacco Smoking on Bone Mass: An Overview of Pathophysiologic Mechanisms. J Osteoporos. 2018 Dec 2;2018:1206235. doi: 10.1155/2018/1206235.

Amaral, Eleonora de Paula et al. “Overexpression of HIF-1α and Morphological Alterations in the Tongue of Rats Exposed To Secondhand Smoke.” Brazilian dental journal vol. 31,3 (2020): 281-289. doi:10.1590/0103-6440202002898

Apatzidou, Danae Anastasia. “The role of cigarette smoking in periodontal disease and treatment outcomes of dental implant therapy.” Periodontology 2000 vol. 90,1 (2022): 45-61. doi:10.1111/prd.12449

Bergström, Jan et al. “Tobacco smoking and dental periapical condition.” European journal of oral sciences vol. 112,2 (2004): 115-20. doi:10.1111/j.1600-0722.2004.00112.x

Borges JS, Costa VC, Irie MS, de Rezende Barbosa GL, Spin-Neto R, Soares PBF. Definition of the Region of Interest for the Assessment of Alveolar Bone Repair Using Micro-computed Tomography. J Digit Imaging. 2023 Feb;36(1):356-364. doi: 10.1007/s10278-022-00693-w.

César-Neto, João B et al. “A comparative study on the effect of nicotine administration and cigarette smoke inhalation on bone healing around titanium implants.” Journal of periodontology vol. 74,10 (2003): 1454-9. doi:10.1902/jop.2003.74.10.1454

Chaffee, Benjamin W et al. “Oral and periodontal implications of tobacco and nicotine products.” Periodontology 2000 vol. 87,1 (2021): 241-253. doi:10.1111/prd.12395

da Silva ACR, Vasques AMV, Bueno CRE, Ervolino E, Cintra LTÂ, Biguetti CC, Matsumoto MA, Dezan-Junior E. Effects of cigarette smoke inhalation on the immune-inflammatory profile of experimental apical periodontitis in rats. Int Endod J. 2023 Dec;56(12):1559-1570. doi: 10.1111/iej.13981.

Gomes, Maximiliano Schünke et al. “Can apical periodontitis modify systemic levels of inflammatory markers? A systematic review and meta-analysis.” Journal of endodontics vol. 39,10 (2013): 1205-17. doi:10.1016/j.joen.2013.06.014

Gupta, Ruchika et al. “Risk of Coronary Heart Disease Among Smokeless Tobacco Users: Results of Systematic Review and Meta-Analysis of Global Data.” Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco vol. 21,1 (2019): 25-31. doi:10.1093/ntr/nty002

Javed F, Kellesarian SV, Abduljabbar T, Abduljabbar AT, Akram Z, Vohra F, Rahman I, Romanos GE. Influence of involuntary cigarette smoke inhalation on osseointegration: a systematic review and meta-analysis of preclinical studies. Int J Oral Maxillofac Surg. 2018 Jun;47(6):764-772. doi: 10.1016/j.ijom.2017.11.009.

Kalatzis-Sousa NG, Spin-Neto R, Wenzel A, Tanomaru-Filho M, Faria G. Use of micro-computed tomography for the assessment of periapical lesions in small rodents: a systematic review. Int Endod J. 2017 Apr;50(4):352-366. doi: 10.1111/iej.12633.

Kang B, Cheong S, Chaichanasakul T, Bezouglaia O, Atti E, Dry SM, Pirih FQ, Aghaloo TL, Tetradis S. Periapical disease and bisphosphonates induce osteonecrosis of the jaws in mice. J Bone Miner Res. 2013 Jul;28(7):1631-40. doi: 10.1002/jbmr.1894.

Kirkevang, Lise-Lotte, and Ann Wenzel. "Risk indicators for apical periodontitis." Community dentistry and oral epidemiology vol. 31,1 (2003): 59-67. doi:10.1034/j.1600-0528.2003.00032.x

Krall, E A et al. "Cigarette smoking increases the risk of root canal treatment." Journal of dental research vol. 85,4 (2006): 313-7. doi:10.1177/154405910608500406

Lazenby RA, Skinner MM, Kivell TL, Hublin JJ. Scaling VOI size in 3D μCT studies of trabecular bone: a test of the over-sampling hypothesis. Am J Phys Anthropol. 2011 Feb;144(2):196-203. doi: 10.1002/ajpa.21385.

Leite, Fábio R M et al. "Effect of Smoking on Periodontitis: A Systematic Review and Meta-regression." American journal of preventive medicine vol. 54,6 (2018): 831-841. doi:10.1016/j.amepre.2018.02.014

Leonardi Dutra K, Haas L, Porporatti AL, Flores-Mir C, Nascimento Santos J, Mezzomo LA, Corrêa M, De Luca Canto G. Diagnostic Accuracy of Cone-beam Computed Tomography and Conventional Radiography on Apical Periodontitis: A Systematic Review and Meta-analysis. J Endod. 2016 Mar;42(3):356-64. doi: 10.1016/j.joen.2015.12.015.

Li, Xiangjun et al. "Effect of environmental tobacco smoke on COX-2 and SHP-2 expression in a periodontitis rat model." Oral diseases vol. 27,2 (2021): 338-347. doi:10.1111/odi.13538

López-López, José et al. "Tobacco smoking and radiographic periapical status: a retrospective case-control study." Journal of endodontics vol. 38,5 (2012): 584-8. doi:10.1016/j.joen.2012.02.011

Moon JH, Kong MH, Kim HJ. Effect of Secondhand Smoking, Determined by Urinary Cotinine Level on Bone Health. Int J Prev Med. 2018 Feb 8;9:14. doi: 10.4103/ijpvm.IJPVM_280_16.

Oliveira LM, Oliveira MDM, Ardenghi TM, Zanatta FB. Is secondhand smoke exposure associated with poor periodontal status in children and adolescents? A systematic review

and meta-analysis. Eur Arch Paediatr Dent. 2022 Aug;23(4):513-525. doi: 10.1007/s40368-022-00709-7.

Paljevic, Ema et al. “Impact of Smoking on the Healing of Apical Periodontitis after Nonsurgical Endodontic Treatment.” European journal of dentistry, 10.1055/s-0043-1761451. 28 Mar. 2023, doi:10.1055/s-0043-1761451

Pesce, Paolo et al. “Evaluation of periodontal indices among non-smokers, tobacco, and e-cigarette smokers: a systematic review and network meta-analysis.” Clinical oral investigations vol. 26,7 (2022): 4701-4714. doi:10.1007/s00784-022-04531-9

Pinto, K P et al. a “Does tobacco smoking predispose to apical periodontitis and endodontic treatment need? A systematic review and meta-analysis.” International endodontic journal vol. 53,8 (2020): 1068-1083. doi:10.1111/iej.13316

Pinto, K P et al. b “Effects of alcohol and nicotine consumption on the development of apical periodontitis in rats: a correlative micro-computed tomographic, histological and immunohistochemical study.” International endodontic journal vol. 53,9 (2020): 1238-1252. doi:10.1111/iej.13344

Sahni V, Gupta S. The association between second-hand smoke and the periodontal status of children and adolescents. Evid Based Dent. 2022 Sep;23(3):106-107. doi: 10.1038/s41432-022-0812-3.

Santiago HA, Zamarioli A, Sousa Neto MD, Volpon JB. Exposure to Secondhand Smoke Impairs Fracture Healing in Rats. Clin Orthop Relat Res. 2017 Mar;475(3):894-902. doi: 10.1007/s11999-016-5184-6.

Segura-Egea, J J et al. “Endodontic medicine: connections between apical periodontitis and systemic diseases.” International endodontic journal vol. 48,10 (2015): 933-51. doi:10.1111/iej.12507

Segura-Egea, J J et al. “High prevalence of apical periodontitis amongst smokers in a sample of Spanish adults.” International endodontic journal vol. 41,4 (2008): 310-6. doi:10.1111/j.1365-2591.2007.01365.x

Sun Z, Wang L, Peng B. Kinetics of glycogen synthase kinase (GSK)3β and phosphorylated GSK3β (Ser 9) expression in experimentally induced periapical lesions. Int Endod J. 2014 Dec;47(12):1107-16. doi: 10.1111/iej.12258.

Tang Y, Zhou X, Gao B, Xu X, Sun J, Cheng L, Zhou X, Zheng L. Modulation of Wnt/β-catenin signaling attenuates periapical bone lesions. J Dent Res. 2014 Feb;93(2):175-82. doi: 10.1177/0022034513512507.

Vasques, Ana Maria Veiga et al. “Inflammatory profile of apical periodontitis exacerbated by cigarette smoke inhalation: Histological and immunohistochemical analysis in rats.” International endodontic journal vol. 56,4 (2023): 465-474. doi:10.1111/iej.13883

von Stechow D, Balto K, Stashenko P, Müller R. Three-dimensional quantitation of periradicular bone destruction by micro-computed tomography. J Endod. 2003 Apr;29(4):252-6. doi: 10.1097/00004770-200304000-00005.

Yang S, Zhu L, Xiao L, Shen Y, Wang L, Peng B, Haapasalo M. Imbalance of interleukin-17+ T-cell and Foxp3+ regulatory T-cell dynamics in rat periapical lesions. J Endod. 2014 Jan;40(1):56-62. doi: 10.1016/j.joen.2013.09.033

Table 1: Mean and standard deviation of BV/TV% values for the first molar.

First Molar	Control	AP	ETS	ETS+AP
Cervical	94,1±6,7^{Aa}	66,2±12,9^{BCb}	83,8±7,5^{Aa}	55,4±11,4^{Ca}
Distant Apical	96,6±0,7^{Aa}	84,7±10,5^{Ba}	92,8±7,2^{Aa}	50,7±11,5^{Ca}
Furcation	92,1±4,7^{Aa}	52,0±13,1^{Bb}	76,5±13,1^{Ab}	22,2±13,5^{Cb}
Near Apical	79,7±3,5^{Ab}	52,6±9,0^{Bb}	57,0±5,7^{BDb}	22,9±12,0^{Cb}

Different letters indicate statistically significant differences determined by Tukey's test ($p < 0.05$). Uppercase letters are used to compare intergroup regions of interest (cervical, distant apical, furcation, and near apical) (line); lowercase letters are used to compare intragroup regions of interest (column).

Table 2: Mean and standard deviation of BV/TV% values for the second molar.

Second Molar	Control	AP	ETS	ETS+AP
Cervical	88,3±13,3^{Aa}	77,1±4,4^{ABa}	83,3±4,7^{ABb}	57,1±14,2^{Bc}
Distant Apical	92,7±4,7^{Aa}	85,2±12,3^{Aa}	88,5±4,9^{Aab}	94,6±6,0^{Aa}
Furcation	89,8±5,4^{Aa}	96,6±3,5^{Aa}	90,8±6,8^{Aab}	86,9±6,8^{Aa}
Near Apical	92,2±6,5^{Aa}	58,6±12,1^{Bb}	93,0±4,4^{Aa}	60, 3± 12,5^{Bb}

Different letters indicate statistically significant differences determined by Tukey's test ($p < 0.05$). Uppercase letters are used to compare intergroup regions of interest (cervical, distant apical, furcation, and near apical) (line); lowercase letters are used to compare intragroup regions of interest (column).

Table 3: Mean and standard deviation of the periodontal ligament space (mm) values for the first molar

First Molar	Control	AP	ETS	ETS+AP
M1	0,08±0,03^{AB}	0,08±0,05^{B}	0,07±0,03^{B}	0,10±0,04^{A}
M2	0,06±0,02^{B}	0,11±0,14^{AC}	0,08±0,03^{BC}	0,11±0,05^{A}
M3	0,08±0,04^{C}	0,14±0,06^{AB}	0,10±0,04^{BC}	0,20±0,12^{A}
M4	0,15±0,05^{B}	0,30±0,08^{A}	0,15±0,07^{B}	0,34±0,15^{A}
M5	0,10±0,04^{BC}	0,15±0,10^{AC}	0,09±0,04^{B}	0,23±0,13^{A}
M6	0,06±0,02^{B}	0,09±0,04^{A}	0,05±0,02^{B}	0,13±0,07^{A}
M7	0,05±0,02^{B}	0,11±0,05^{A}	0,05±0,02^{B}	0,10±0,05^{A}

Different letters indicate statistically significant differences as determined by Tukey's test ($p < 0.05$). Uppercase letters are used to compare intergroup regions of interest (line).

Table 4: Mean and standard deviation of the periodontal ligament space (mm) values for the second molar

Second Molar	Control	AP	ETS	ETS+AP
M1	0,09±0,03^{B}	0,08±0,03^{B}	0,08±0,03^{B}	0,12±0,04^{A}
M2	0,06±0,03^{B}	0,08±0,10^{A}	0,05±0,02^{B}	0,09±0,03^{A}
M3	0,05±0,03^{B}	0,07±0,02^{A}	0,05±0,02^{B}	0,10±0,06^{A}
M4	0,11±0,04^{B}	0,17±0,08^{A}	0,11±0,06^{B}	0,20±0,10^{A}
M5	0,06±0,02^{B}	0,07±0,02^{B}	0,06±0,03^{B}	0,10±0,04^{A}
M6	0,06±0,02^{B}	0,06±0,02^{B}	0,05±0,02^{B}	0,09±0,03^{A}
M7	0,06±0,02^{B}	0,06±0,03^{AB}	0,05±0,02^{B}	0,09±0,05^{A}

Different letters indicate statistically significant differences as determined by Tukey's test ($p < 0.05$). Uppercase letters are used to compare intergroup regions of interest (line).