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Editorial

Genotoxicity of Fixed Orthodontic Treatment

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Abstract

Introduction: Fixed orthodontic treatment involves the use of materials, including metals that can cause cell damage such as genetic alterations during cell division, some of which include the presence of micronuclei (MN), oxidation of deoxyribonucleic acid (DNA) and its degradation. **Objective:** To evaluate the genotoxic damage caused by fixed orthodontic therapy with metals. **Material and methods:** Oral epithelial cells of 51 patients were evaluated before and one year after placement of fixed orthodontic treatment with Equilibrium® 2 stainless steel brackets. MN frequency was analysed by Feulgen staining, DNA extraction was performed with the DNeasy® Blood & Tissue Kit and DNA degradation was observed by electrophoresis. In addition, DNA oxidation was assessed by quantifying the 8-hydroxydeoxyguanosine (8-OHdG) molecule

using enzyme-linked immunosorbent assay (ELISA). **Results:** An increase in the frequency of MN was detected in oral epithelial cells when comparing before (0%) and after treatment (18%) ($p<$ 0.004). DNA degradation also increased, detecting 45% of samples degraded before treatment to 88% after treatment (p=0.002). The concentration of 8-OHdG increased approximately two-fold after treatment (13.6 ± 1.2 ng/mL) compared to the before-treatment condition (5.8 ± 0.49 ng/mL) (p=0.04). **Conclusions:** The results presented here demonstrate DNA damage after one year of metal-based fixed orthodontic therapy.

Keywords: fixed orthodontics, 8-hydroxydeoxyguanosine, DNA, micronuclei.

INTRODUCTION

Fixed orthodontic treatment using materials made from metals involves the generation of corrosion of these materials in the oral cavity, which can cause effects on oral epithelial cells that include damage to deoxyribonucleic acid (DNA), which represents a significant impact since it can initiate malignant lesions[1].

Metal damage to the DNA molecule can be caused directly by hydrolysis of the phosphodiester bond, generating fragmentation of the molecule[2-4], or indirectly by inhibiting DNA repair mechanisms[5] or by oxidation processes that generate reactive species that oxidize the DNA[6-7]. Oxidative damage causes lesions in DNA, the most frequent being oxidation of its nitrogenous bases, especially guanine, which is more easily oxidized to form 8-hydroxydeoxyguanosine (8-OHdG). This molecule is considered one of the most significant biomarkers for oxidative DNA damage[8]. Buljan *et al.*[9] demonstrated an increase in 8-OHdG in mouse cell lines exposed for 48 h to materials used in orthodontic treatments. *In vivo* studies in humans investigating oxidative DNA damage induced by fixed orthodontic treatment and 8-OHdG quantification are limited, controversial, and only evaluate shorter periods of time and up to 6 months after treatment[10-12]. Guler *et al.*[10] demonstrated an increase in 8-OHdG in saliva of children after 3 months of fixed orthodontic treatment with metals and different adhesive materials. In contrast, Atuğ Özcan *et al.*[11] did not detect changes in 8-OHdG levels in saliva and gingival crevicular fluid after 6 months of orthodontic treatment. Similarly, Esenlik *et al.*[12] evaluated 7 days after orthodontic treatment with metals and did not detect changes in 8-OHdG levels.

At the cellular level, small nuclei or micronuclei (MN) are formed from chromosomes or fragments of these when they are not incorporated into one of the nuclei of the daughter cells during cell division; the presence of these MN is one of the most used tests to evaluate DNA damage[13]. A recent review explored the average increase in MN due to the use of fixed orthodontic treatment and found that the increase in MN was 3 times greater as compared to oral epithelial cells without orthodontic treatment[14]. Regarding the time of exposure to the procedure, MN have been analysed from 7 days to 6 years after treatment, observing that the damage begins from the first week of placement, but suggests that it may decrease with exposure time[15,16].

At the institutional level, in the Mexican Secretariat of National Defence, the orthodontic treatment used in the last 15 years in the Dental Specialties Unit (UEO for its acronym in Spanish) is Fixed Orthodontic Appliances based on the MBT technique. The MBT philosophy belongs to the third generation of pre-adjusted appliances, created from the knowledge and clinical experience of doctors Richard McLaughlin and John Bennet[17]. The present study evaluated DNA

damage by MN frequency analysis, visualization of DNA degradation by electrophoresis, and quantification of the 8-OHdG molecule before and after one year of fixed orthodontic placement.

MATERIAL AND METHODS

Patients who attended the Orthodontic Service of the UEO in the Mexican Secretariat of National Defence were included. Patients selected as candidates for orthodontic treatment were 51, aged between 12 and 36 years, without caries, without previous orthodontic treatments, without periodontal disease and without metal restorations. The orthodontic supplies provided by the UEO based on 0.022" MBT were used and included: stainless steel brackets (Equilibrium® 2, Dentaurum GmbH & Co. KG, Ispringen, Germany), buccal tube bands (3M™ Unitek™, Victory Series™ Bands, 3M™, Minnesota, USA) with anatomical stainless steel contour, stainless steel bonding mini tubes (3M™ Victory Series™ Mini Tubes, 3M™, Minnesota, USA), nitinol heat-activated archwires (3M™ Unitek™ Nitinol Heat-Activated Archwire, 3M™, Minnesota, USA) sizes: 0.014", 0.016", 0.016x0.022" and 0.019x0.025", 0.019x0.025" stainless steel archwire, 0.019x0.025" multifilament stainless steel braided archwires (Dentaflex®, Dentaurum GmbH & Co. KG, Ispringen, Germany), 0.010" stainless steel wire ligature, 0.021" brass wire, and nitinol opening spring.

Patients and legal guardians of minor patients voluntarily agreed to participate in the study and signed the informed consent letter, and minors signed an informed assent letter. The UEO institutional committee at the Mexican Secretariat of National Defence approved the protocol and informed consent (approval number 19CI09016025). Two samples of oral epithelial cells were taken from each patient using a cytological brush. One sample was used for MN analysis and another for DNA extraction. Sample collection was performed before treatment placement and after one year after the procedure.

Oral epithelial cell samples were obtained after rinsing the oral cavity with water, gently scraping the right and left cheek with the cytological brush. For MN analysis, the sample was spread on a clean slide and fixed with ethanol for 10 minutes. Finally, Feulgen staining was performed. After staining, the cells were examined under a light microscope (VE-B2 Binocular Microscope, VELAB Co., Texas, USA). Slides were prepared in triplicate for each patient, and in the same way, 2000 epithelial cells were counted for each patient to evaluate MN frequency. For DNA extraction, the commercial kit (DNeasy® Blood & Tissue Kit, QIAGEN, Hilden, Germany) was used; the extraction protocol was followed considering the manufacturer's instructions. The DNA concentration was measured in a spectrophotometer (NanoDrop 1000, Thermo Fisher Scientific Inc., Delaware, USA) and adjusted to 50 ng/µL, and electrophoretic runs of the DNA were then performed in 1.5% agarose gels to value its integrity. DNA oxidation was measured in the DNA samples by quantifying the 8-OHdG molecule using enzyme-linked immunosorbent assay (ELISA) with a commercial kit (DNA damage ELISA kit, Enzo® Life Sciences Inc., New York, USA), following the protocol and all the manufacturer's instructions. The detection range was 0.94 to 60 ng/mL.

Statistical analysis was performed using IBM® SPSS® Statistics 23 software, version 23.0. The McNemar's test was used to evaluate differences in MN frequencies and DNA degradation before and after one year of treatment. A comparison of the means of the concentration levels of the 8-OHdG molecule before and after one year of treatment was performed with the Student's *t*-test for related samples to determine the difference. Values of $p<0.05$ indicated statistical significance.

RESULTS

The study included 30 women and 21 men with an average age of 15.8 ± 4.8 years, 86% of the patients presented Class I Malocclusion and 14% presented Class II Malocclusion. Oral epithelial cells were sampled and evaluated for the presence of MN (Figure 1). No MN-containing cells were detected in the 2000 oral cells examined from any patient prior to orthodontic therapy. 0 to 1 cell with MN was detected out of 2000 buccal cells examined in 9 patients after treatment. The percentage of patients who presented MN before (0%, control) and after one year of treatment (18%) showed a significant difference (p=0.004) (Table 1).

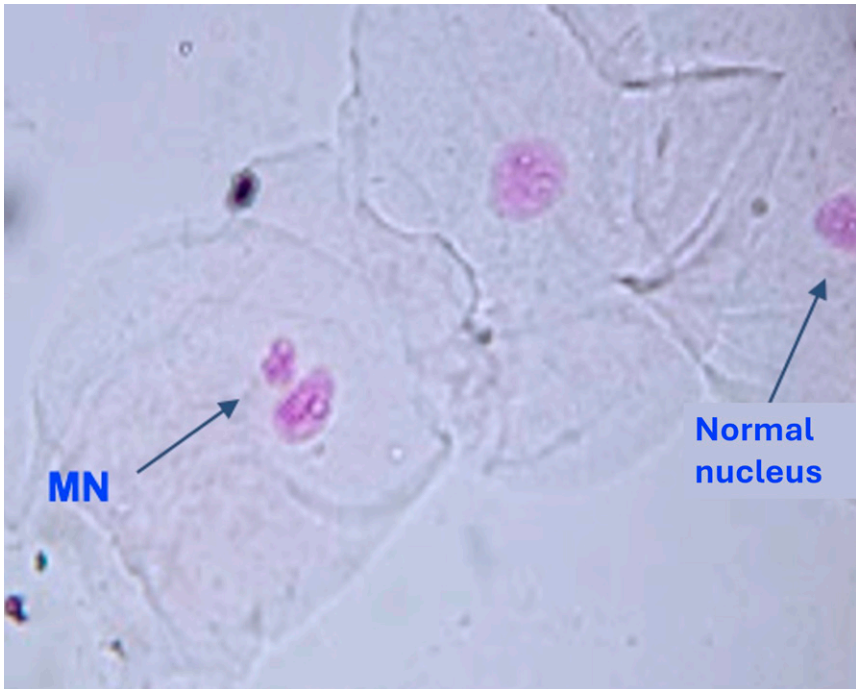

Figure 1. Oral epithelial cells stained with Feulgen stain. The nucleus is stained pink-purple, the cytoplasm remains colourless or with a slight pinkish hue. The arrows indicate a cell with a normal nucleus and a cell with a micronucleus (MN). Observed under a light microscope with a 40x objective.

Table 1. Frequency of patients who presented micronuclei (MN) in oral epithelial cells before and after one year of fixed orthodontic treatment

Variable	Before treatment	After treatment	*p* value
Presence of MN	0 / 51 (0%)	9/ 51 (18%)	0.004*
Absence of MN	51 / 51 (100%)	42/51 (82%)	

* McNemar's test, $p<0.05$ shows statistical significance

After DNA extraction, the concentration was adjusted and electrophoresis was performed. The most representative electrophoretic scans of some samples studied before and after one year of orthodontic treatment are shown (Figure 2). DNA degradation was detected in 45% of samples

before orthodontic treatment placement. This degradation was visualised only as a slight scan along the entire lane, without loss of high molecular size DNA. However, after treatment, the degradation was more evident and around 88% of the samples presented fragments of smaller sizes. In addition, a decrease in high molecular size DNA was observed in some samples. Statistical analysis shows significant difference in DNA degradation before and after orthodontic treatment studied by electrophoresis (Table 2).

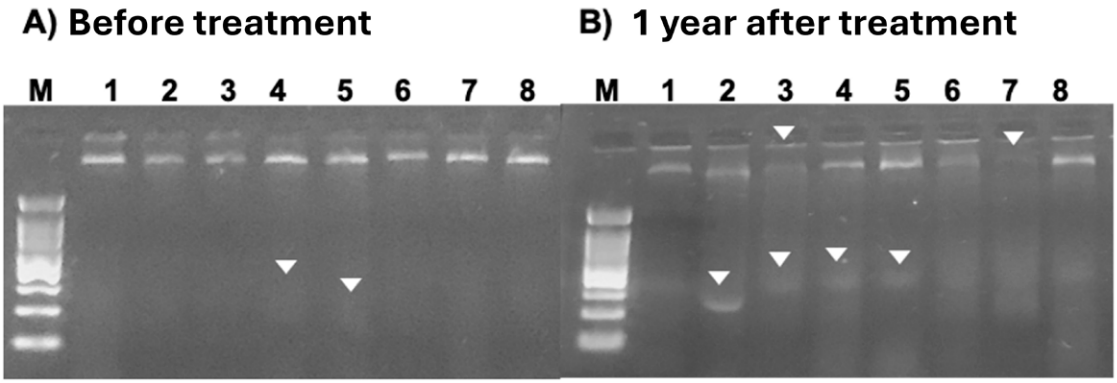

Figure 2. Representative samples of DNA degradation derived from oral epithelial cells evaluated in this study. Representative DNA samples 1-8 are shown. Agarose gel at 1% with molecular size marker (M). a) Before and b) After one year of fixed orthodontic therapy. The arrows indicate the presence of DNA fragments, marked with the arrowhead. They are observed in greater numbers after therapy.

Table 2. DNA degradation assessed by electrophoresis in buccal cells before and after fixed orthodontic treatment

Variable	Before treatment	After treatment	*p* value
	n=51	n=51	
Presence of degradation	23/51 (45%)	45/51 (88%)	0.002*
Absence of degradation	28/51(55%)	6/51 (12%)	

* McNemar's test, $p<0.05$ shows statistical significance

Analysis of oxidative DNA damage was assessed by quantifying the 8-OHdG molecule in DNA samples extracted from oral epithelial cells. The data, which indicate the average of the values before and after one year of treatment, show a statistically significant increase in DNA oxidation after one year of treatment when comparing the averages (Table 3).

Table 3. Levels of 8-OHdG in DNA extracted from buccal epithelial cells before and after one year of fixed orthodontic treatment

8-OHdG level ng/mL	Before treatment	After treatment	p-value
Mean ± standard deviation	5.8 ± 0.49	13.6 ± 1.2	0.04*

T-test for related samples $p<0.05$ shows statistical significance

DISCUSSION

In our study we evaluated oxidative damage to DNA before and after the application of fixed orthodontic treatment. The patients mostly presented Class I Malocclusion, coinciding with prevalence reports in the world and in America that place this type of Malocclusion in first place[18]. Concerning the frequency of MN, the data are similar to those by Flores-Bracho *et al.*[19] in which they demonstrate increases in the frequency of MN after 4 years of treatment, while they differ from the report by Goncalves *et al.*[20] in which no damage is detected after 1 year of treatment. In both studies, they used stainless steel brackets from the Morelli Sorocaba Sp® Brazil brand, and explored long periods after treatment placement. But the differences observed in the results between them may be mainly due to the wide difference in the times at which the damage was evaluated. Also, differences could be due to specific requirements of the mechanical force exerted during treatment on patients, which participate in both environmental alteration and DNA damage[21].

In relation to DNA degradation analysed by electrophoresis, a two-fold increase was detected after one year of treatment placement. The electrophoresis assay has low sensitivity, compared to studies in the literature that examine degradation mainly by gel electrophoresis analysis in single cells or comet assay, a technique described as one of the most sensitive[22-26]. Fernández-Miñano *et al.*[23] compared the before and after one month of application of the therapy, and demonstrated DNA damage with stainless steel, titanium and nickel materials. Faccioni *et al.*[24] reported damage to oral epithelial cells in patients with fixed orthodontic therapy with different metals for a period of 2-4 years when compared to the population without orthodontics, but did not evaluate the before and after in the same population. In contrast, other authors using the same comet assay to evaluate damage do not describe changes after performing evaluations after 10 and 30 days, and up to 6 months of application of orthodontic treatment with the same type of metals[25, 26]. Although the discrepancy in the results can be explained by the variation in the time evaluated, it could also be due to slight differences in metal concentration resulting from the variation in the brands of the materials. This is supported by reports such as that of Buljan *et al.*[9], which have shown different degrees of damage in mouse gingival fibroblast cultures when exposed to different orthodontic materials like ceramic and metal. Similarly, Loyola-Rodríguez *et al.*[27] demonstrated different damage in human gingival fibroblasts due to exposure to brackets of different brands and materials.

Our study detected an increase in the level of 8-OHdG molecule of approximately 2 times more after one year of treatment placement. The changes detected in 8-OHdG values are similar to those described by other authors who studied mouse fibroblast cells after 48 hours of exposure to different materials used in orthodontics including stainless steel, ceramic and polyurethane, when compared with unexposed fibroblasts[9]. In addition, *in vivo* studies detected increases in 8-OHdG of approximately 1.5 times more in the saliva of children after 1 and 3 months of orthodontic treatment with metals[10]. However, there are authors who, using metal treatment, did not detect changes in the levels of 8-OHdG in samples of saliva and gingival crevicular fluid before and after 6 months of treatment[11]. Similarly, Esenlik *et al.*[12], using metals in the treatment, did not show changes in 8-OHdG levels in short periods of 4-5 h and 7 days post-treatment. The variation in results between studies can be justified both by the different types of samples considered, as well as by the different examination times after orthodontic therapy. We consider that variation in sampling is essential since saliva and gingival crevicular fluid may have a lower concentration of metabolites than the direct analysis of oral epithelial cells, because the fluids reflect products derived from the cellular epithelium.

With respect to the time of exposure to metals, recent studies from a meta-analysis show that the maximum release of metals occurs after one month of starting treatment and decreases after two and three months of treatment[28], and accordingly the levels of DNA damage are higher in the first months of treatment[29]. Our research group reported previous work evaluating DNA damage through capillary electrophoresis analysis of fragments obtained by polymerase chain reaction of short tandem repeat (STRs) sequences at shorter times (3 months after treatment). DNA degradation and loss of stability were detected, although the data were not statistically significant[30]. Therefore, we consider that DNA damage is due to many factors including the release of metals, damage resulting from inflammation and stress caused by the mechanical force exerted in orthodontics[21], or by the change in the oral environment caused by the bacterial microbiota that commonly increases during orthodontic treatment due to the difficulty of hygiene and the irritating effects of the materials[31].

The results presented here are for consideration by orthodontists, since the continuous improvement of the materials used in the treatment and the duration of the treatment can contribute to reducing the DNA damage that occurs during orthodontic treatment. The initial malocclusion problem and the particular conditions of each patient will always be a priority for the orthodontic specialist who will determine the techniques, times, materials, among other factors that will define the patient's treatment.

CONCLUSIONS

The results presented here demonstrate DNA damage. For now, there are studies that are evaluating levels of antioxidant enzymes that could indicate whether oxidative damage is being counteracted and detected as a process of biological adaptation.

BIBLIOGRAPHIC REFERENCES

1. Downarowicz P, Mikulewicz M. Trace metal ions release from fixed orthodontic appliances and DNA damage in oral mucosa cells by in vivo studies: A literature review. *Adv Clin Exp Med*. 2017; 26(7): 1155-1162. DOI: 10.17219/acem/65726
2. Sreedhara A, Cowan JA. Catalytic hydrolysis of DNA by metal ions and complexes. *J Biol Inorg Chem*. 2001; 6(4): 337-347. DOI: 10.1007/s007750100209
3. Katada H, Komiyama M. Artificial restriction DNA cutters as new tools for gene manipulation. *Chembiochem*. 2009; 10(8): 1279-1288. DOI: 10.1002/cbic.200900040
4. Camargo MA, Neves A, Bortoluzzi AJ, Szpoganicz B, Martendal A, Murgu M, *et al*. New gadolinium complex with efficient hydrolase-like activity: a 100-million-fold rate enhancement in diester hydrolysis. *Inorg Chem*. 2008; 47(8): 2919-2921. DOI: 10.1021/ic702167p
5. Iarmarcovai G, Sari-Minodier I, Chaspoul F, Botta C, De Meo M, Orsiere T, *et al*. Risk assessment of welders using analysis of eight metals by ICP-MS in blood and urine and DNA damage evaluation by the comet and micronucleus assays; influence of XRCC1 and XRCC3 polymorphisms. *Mutagenesis*. 2005; 20(6): 425-432. DOI: 10.1093/mutage/gei058
6. Jeżowska-Bojczuk M, Kaczmarek P, Bal W, Kasprzak KS. Coordination mode and oxidation susceptibility of nickel (II) complexes with 2´-deoxyguanosine 5´-monophosphate and l-histidine. *J Inorg Biochem*. 2004; 98(11): 1770-1777. DOI: 10.1016/j.jinorgbio.2004.08.002

7. Kaczmarek P, Jeżowska-Bojczuk M, Bal W, Kasprzak, KS. Determination of the stability constants and oxidation susceptibility of nickel (II) complexes with 2´-deoxyguanosine 5´-triphosphate and l-histidine. *J Inorg Biochem*. 2005; 99(3): 737-746. DOI: 10.1016/j.jinorgbio.2004.12.006
8. Chiorcea-Paquim AM. 8-oxoguanine and 8-oxodeoxyguanosine biomarkers of oxidative DNA damage: A review on HPLC–ECD determination. *Molecules*. 2022; 27(5): 1620. DOI: 10.3390/molecules27051620
9. Buljan ZI, Ribaric SP, Abram M, Ivankovic A, Spalj S. In vitro oxidative stress induced by conventional and self-ligating brackets. *Angle Orthod*. 2012; 82(2): 340-345. DOI: 10.2319/061811-395.1
10. Guler C, Toy E, Ozturk F, Gunes D, Karabulut AB, Otlu O. Evaluation of salivary total oxidant-antioxidant status and DNA damage of children undergoing fixed orthodontic therapy. *Angle Orthod*. 2015; 85(2): 239-244. DOI: 10.2319/110113-798.1
11. Atuğ Özcan SS, Ceylan I, Özcan E, Kurt N, Dağsuyu IM, Çanakçi CF. Evaluation of oxidative stress biomarkers in patients with fixed orthodontic appliances. *Dis Markers*. 2014; 2014: 59792. DOI: 10.1155/2014/597892
12. Esenlik E, Gümüş EB, Albayrak GE, Doğuç DK. Does puberty affect oxidative stress levels and antioxidant activity of saliva in patients with fixed orthodontic appliances? *J Orofac Orthop*. 2023; 84: 54-64. DOI: 10.1007/s00056-021-00346-7
13. Fenech M, Knasmueller S, Bolognesi C, Bonassi S, Holland N, Migliore L, *et al*. Molecular mechanisms by which in vivo exposure to exogenous chemical genotoxic agents can lead to micronucleus formation in lymphocytes in vivo and ex vivo in humans. *Mutat Res Rev Mutat Res*. 2016; 770(Pt A): 12-25. DOI: 10.1016/j.mrrev.2016.04.008
14. Ángeles-Estrada L, Santos-Quintanilla AR, Sánchez-Monroy V. Evaluación de efectos citotóxicos y genotóxicos con ensayo de micronúcleos en la mucosa bucal de pacientes con aparatología ortodóncica. Revisión de la literatura. *Odontol Sanmarquina*. 2021; 24(2); 165-172. DOI: 10.15381/os.v24i2.19903
15. Francis PG, Thomas M, Antony V, Shaloob M, Hassan KJ, Roshan G. Cytomorphometric analysis on the effects of components of orthodontic appliances on the epithelial cells of the buccal mucosa. *J Int Soc Prev Community Dent*. 2017; 7(3): 142-6. DOI: 10.4103/jispcd.JISPCD_58_17
16. Mariachelliah SJL, Sathiasekar AC, Mathew DG, Ramachandran KL, Kesavaram P, Kangasabapathy UG. Localized toxicity in patients with fixed orthodontic appliance: A case control study. *Indian J Public Health Res Dev*. 2019; 10(12): 989-994. DOI: 10.37506/v10%2Fi12%2F2019%2Fijphrd%2F192253
17. McLaughlin RP, Bennett JC. Bracket placement with the preadjusted appliance. *J Clin Orthod*. 1995; 29(5): 302-311. PMID: 8617853
18. Lombardo G, Vena F, Negri P, Pagano S, Barilotti, Paglia L, *et al*. Worldwide prevalence of malocclusion in the different stages of dentition: A systematic review and meta-analysis. *Eur J Paediatr Dent*. 2020; 21(2): 115-122. DOI: 10.23804/ejpd.2020.21.02.05
19. Flores-Bracho MG, Takahashi CS, Castillo WO, Saraiva MCP, Küchler EC, Matsumoto MAN, *et al*. Genotoxic effects in oral mucosal cells caused by the use of orthodontic fixed appliances in patients after short and long periods of treatment. *Clin Oral Investig*. 2019; 23(7): 2913-2919. DOI: 10.1007/s00784-018-02795-8
20. Goncalves TS, De Menezes LM, Trindade C, Thomas P, Fenechc M, Henriques JAP. In vivo evaluation of the genotoxic effects of Hyrax auxiliary orthodontic appliances containing silver-soldered joints. *Mutat Res Genet Toxicol Environ Mutagen*. 2015; 791: 25-29. DOI: 10.1016/j.mrgentox.2015.07.007
21. Morii A, Miyamura Y, Sago MI, Mizuhara M, Shikayama T, Naniwa M, *et al*. Orthodontic force-induced oxidative stress in the periodontal tissue and dental pulp elicits nociception via activation/sensitization of TRPA1 on nociceptive fibers. *Free Radic Biol Med*. 2020; 147: 175–186. DOI: 10.1016/j.freeradbiomed.2019.12.016

22. Singh NP. Microgels for estimation of DNA strand breaks, DNA protein crosslinks and apoptosis. *Mutat Res – Fund Mol Mechan Mutagen*. 2000; 455(1-2): 111-127. DOI: 10.1016/s0027-5107(00)00075-0
23. Fernández-Miñano E, Ortiz C, Vicente A, Calvo JL, Ortiz AJ. Metallic ion content and damage to the DNA in oral mucosa cells of children with fixed orthodontic appliances. *Biometals*. 2011; 24(5): 935-941. DOI: 10.1007/s10534-011-9448-z
24. Faccioni F, Franceschetti P, Cerpelloni M, Fracasso ME. In vivo study on metal release from fixed orthodontic appliances and DNA damage in oral mucosa cells. *Am J Orthod Dentofacial Orthop*. 2003; 124(6): 687-693. DOI: 10.1016/j.ajodo.2003.09.010
25. Westphalen GH, Menezes LM, Prá D, Garcia GG, Schmitt VM, Henriques JAP, *et al*. In vivo determination of genotoxicity induced by metals from orthodontic appliances using micronucleus and comet assays. *Genet Mol Res*. 2008; 7(4): 1259-1266. DOI: 10.4238/vol7-4gmr508
26. Hafez HS, Selim EMN, Eid FHK, Tawfik WA, Al-Ashkar EA, Mostafa YA. Cytotoxicity, genotoxicity, and metal release in patients with fixed orthodontic appliances: a longitudinal in-vivo study. *Am J Orthod Dentofacial Orthop*. 2011; 140(3): 298-308. DOI: 10.1016/j.ajodo.2010.05.025
27. Loyola-Rodríguez JP, Lastra-Corso I, García-Cortés JO, Loyola-Leyva A, Domínguez-Pérez RA, Ávila-Arizmendi D, *et al*. In vitro determination of genotoxicity induced by brackets alloys in cultures of human gingival fibroblasts. *J Toxicol*. 2020; 1467456. DOI: 10.1155/2020/1467456
28. Imani MM, Mozaffari HR, Ramezani M, Sadeghi M. Effect of fixed orthodontic treatment on salivary nickel and chromium levels: a systematic review and meta-analysis of observational studies. *Dent J*. 2019; 7(1): 21. DOI: 10.3390/dj7010021
29. Pineda-Zayas A, Menéndez López-Mateos L, Palma-Fernández JC, Iglesias-Linares A. Assessment of metal ion accumulation in oral mucosa cells of patients with fixed orthodontic treatment and cellular DNA damage: a systematic review. *Crit Rev Toxicol*. 2021; 51(7): 622-633. DOI: 10.1080/10408444.2021.1960271
30. Díaz-Rojas AR, Ángeles-Estrada L, Pérez-Vielma NM, Sánchez-Monroy V. Short tandem repeat (STR) instability in the oral mucosa of patients submitted to fixed orthodontic therapy: a limitation of STR profile quality for human identification. *Forensic Sci Med Pathol*. 2022; 18(1): 57–63. DOI: 10.1007/s12024-021-00451-4
31. Reichardt E, Geraci J, Sachse S, Rödel J, Pfister W, Löffler B, *et al*. Qualitative and quantitative changes in the oral bacterial flora occur shortly after implementation of fixed orthodontic appliances. *Am J Orthod Dentofacial Orthop*. 2019; 156(6): 735-744. DOI: 10.1016/j.ajodo.2018.12.018