Evaluation of Possible DNA Damage in Oral Mucosa Cells Using the Micronucleus Test in Pediatric Patients with Removable Appliances *

Evaluación de posibles daños en el ADN de células de mucosa bucal mediante la prueba de micronúcleos en pacientes pediátricos con aparatología removible

Avaliação de possíveis danos ao DNA em células da mucosa oral pelo teste do micronúcleo em pacientes pediátricos portadores de aparelhos removíveis

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DOI : https://doi.org/10.11144/Javeriana.uo42.epdd
Submission Date: 5 July 2023
Acceptance Date: 13 December 2023
Publication Date: 30 December 2023

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ABSTRACT

Background: Orthodontic and orthopedic materials are made of stainless steel, with chemical elements such as nickel and chromium, generally associated with allergenic effects and hypersensitivity, expressed locally**. Purpose:** To examine, using the micronucleus test in oral mucosa cells, DNA damage in patients undergoing removable metallic orthopedics. **Methods:** Samples were collected before the appliance adaptation and 30 days later, the buccal cells of each individual were collected by gently brushing the internal part of the cheeks with a cytological brush. The plates were immersed in a Coplin jar with 0.2 % Light Green solution for 1 minute, and then were rinsed with deionized water or Milli Q to remove excess dye. Later, these were placed on a drying plate between 50ºC and 80ºC for 10 to 15 minutes. **Results:** Compared with the initial samples, after 1 month of orthopedic treatment, a significant increase in the frequency of cells with micronuclei, nuclear budding and karyorrhexis was observed. No changes or evidence of an increase were observed in cells with condensed chromatin.

Conclusions: The stages of nuclear alteration that took place during the development of this study were MN frequency, nuclear budding and karyorrhexis, registering a higher frequency in the second sampling and attributed to the presence of removable appliances in the patients. Pyknosis, karyolysis, and karyorrhexis do not constitute stages of nuclear alteration, the latter being the one with the greatest trend observed in the first and second sampling. For this stage, a significant increase in number was found, attributed to the presence of removable appliances in the patients.
Keywords: cytotoxicity; dental orthopedics; dentistry; genotoxicity; micronucleus test; oral mucosa; oral pathology; orthodontic appliance; saliva; toxicity

RESUMEN

Antecedentes: Los materiales de ortodoncia y ortopedia están hechos de acero inoxidable, con elementos químicos como níquel y cromo, generalmente asociados a efectos alergénicos e hipersensibilidad, expresadas a nivel local. **Objetivos:** Examinar por la prueba de micronúcleos en células de mucosa bucal, el daño al ADN en pacientes sometidos a ortopedia metálica removible. **Métodos:** Las muestras recolectadas antes de la adaptación del aparato y después de 30 días obtenidas las células bucales de las mejillas con un cepillo citológico, las placas sumergidas en un coplin con solución Light Green al 0.2 % durante 1 minuto, enjuagadas con agua desionizada o Milli Q para retirar el exceso de colorante y posteriormente dispuestas en una plancha para secado entre 50ºC y 80ºC de 10 a 15 minutos. **Resultados:** después de 1 mes de tratamiento ortopédico se observó un aumento significativo en la frecuencia de células con micronúcleos, brotes nucleares y cariorrexis. No se observaron cambios ni evidencia de un aumento en células con cromatina condensada. **Conclusiones:** Las etapas de alteración nuclear que tuvieron lugar en el desarrollo de este estudio fueron frecuencia de MN, brotes nucleares y cariorrexis registrándose mayor frecuencia en la segunda toma de muestra, atribuido a la presencia de aparatología removible en los pacientes. La picnosis, cariolisis y cariorrexis no constituyen etapas de la alteración nuclear, esta ultima la de la mayor tendencia observada en la primera y la segunda toma, encontrándose un aumento significativo en número, atribuido a la presencia de aparatología removible.
Palabras clave: aparatos ortodónticos; citotoxicidad; genotoxicidad; mucosa oral; odontologia; ortopedia dentofacial; patología oral; prueba de micronúcleos; saliva; toxicidad

RESUMO

Antecedentes: Os materiais ortodônticos e ortopédicos são confeccionados em aço inoxidável, com elementos químicos como níquel e cromo, geralmente associados a efeitos alergênicos e hipersensibilidade, expressos localmente. **Objetivo:** Examinar danos ao DNA em pacientes submetidos à ortopedia metálica removível por meio do teste do micronúcleo em células da mucosa oral. **Métodos**: Amostras coletadas antes da adaptação do aparelho e após 30 dias obtiveram células bucais das bochechas com pincel citológico, as placas foram imersas em coplin com solução Light Green 0,2 % por 1 minuto, enxaguadas com água deionizada ou Milli Q para retirada do excesso. corante e posteriormente colocado em ferro para secar entre 50ºC e 80ºC por 10 a 15 minutos. **Resultados:** após 1 mês de tratamento ortopédico foi observado aumento significativo na frequência de células com micronúcleos, brotos nucleares e cariorrexe. Nenhuma alteração ou evidência de aumento foi observada em células com cromatina condensada. **Conclusões:** Os estágios de alteração nuclear ocorridos no desenvolvimento deste estudo foram frequência de MN, brotos nucleares e cariorrexe, com maior frequência registrada na segunda amostragem, atribuída à presença de aparelhos removíveis nos pacientes. Picnose, cariólise e cariorrexe não constituem estágios de alteração nuclear, sendo esta última a que apresenta maior tendência observada na primeira e segunda ingestão, com aumento significativo do número, atribuído à presença de aparelhos removíveis.
Palavras-chave: aparelhos ortodônticos; citotoxicidade; genotoxicidade; mucosa bucal; odontologia; ortopedia dentofacial; patologia oral; saliva; teste de micronúcleo; toxicidade

INTRODUCTION

A person's health can be observed in their oral cavity since the mucosa that lines the mouth functions as a protective barrier for the body against the environment and toxic substances. When metallic accessories are exposed to the oral environment, they undergo degradation such as corrosion, fatigue fracture, increased friction coefficient, or microbiological degradation (1).

In most cases in orthodontics and orthopedics, hypersensitivity reactions present local manifestations, which may eventually cause systemic disorders. Most of the apparatus and devices used in the specialty are composed of chemical elements such as metals (nickel, iron, chromium) and alloys (stainless steel, nickel titanium or nickel-cobalt) and usually include supports, bands, arches, wires and

springs. related to changes in the immune system (2, 3, 4,5).

The characteristics and properties of the mouth, its warm and humid condition, offer an ideal environment for the degradation of these attachments, facilitating the release of metal ions related to adverse health effects, such as cellular toxicity, cancer and other malignant oral pathologies. Nickel is the element most associated with cytotoxicity and genotoxicity processes. These elements have clastogenic and/or aneugenic capacity; they are responsible for the induction of chromosomal and chromatid alterations that result in the production of micronuclei (MN) and other cellular alterations (6, 7, 8).

The use of exfoliated cells from the oral mucosa for the evaluation of cellular changes associated with genomic instability has grown exponentially in recent years. presenting the first physical barrier to contact with metals and alloys in orthodontic appliances. It is an important target in the evaluation of the long-term effects of this type of treatment (6).

The correlation between a significant increase in the frequency of MN in oral mucosa cells of individuals and the presence of malignant disorders and oral carcinoma suggests a link between this biomarker and the process of neoplastic progression. In this way, the determination of the frequency of MN and other mucosal cellular abnormalities can be used as biomarkers to identify different preneoplastic conditions before any clinical manifestation occurs, constituting an important and powerful biomarker to evaluate populations with high cancer risk (9-11)

The oral mucosa cells being the first tissue where a localized corrosion effect occurs.

Is the use of removable metal orthopedic treatments capable of inducing cellular lesions associated with DNA damage?

MATERIALS AND METHODS

A quasi-experimental study was developed to determine DNA damage in patients undergoing removable orthopedic treatment, with appliances that aim to prevent, correct and treat growth problems of the jaws and rehabilitate the masticatory function in patients in deciduous and mixed dentition. .

The initial sample consisted of 46 individuals aged between 6 and 12 years, it was reduced to 25 who needed treatment with removable orthopedic appliances, the appliances were made of acrylic and stainless metal with silver solder between the metal and the bands, thus which will allow adaptation in the patient's mouth, and controlled after a month of treatment. In the sociodemographic survey, data such as age, gender, level of education and socioeconomic stratum were recorded.

The samples were obtained before the adaptation of the device and the buccal cells of each individual were collected 30 days later by gently brushing the inner part of the cheeks with a cytological brush, after washing the mouth several times with distilled water to eliminate the exfoliated dead cells. The brushes are shaken in 50 ml plastic tubes containing 20 ml of phosphate-buffered saline (PBS). Cells were washed twice, centrifuged at 1500 rpm for 10 minutes at room temperature, and suspended in PBS.

Genotoxicity testing is designed to detect compounds that induce genetic damage, including DNA lesions, genetic mutation, chromosome breakage, altered DNA repair capacity, and cellular transformation (12).

For the micronucleus test in oral mucosa, cell suspensions were dripped onto previously clean and preheated (37 °C) slides. After dropping, cells were air-dried and fixed in methanol (80 %, v/v) at 0ºC for 20 minutes. Staining with fulgen and counter staining with light Green, which identifies the different stages of cellular damage, the MN were identified according to the following characteristics at, less than 1/3 of the diameter of the main nucleus, the same plane of focus, the same color, texture and refraction as the main nucleus, smooth oval or round shape and clearly separated from the main nucleus, according to Thomas protocol (13)

To stain the cells, the already prepared slides went through a washing process that consisted of

immersing them for 1 minute in a Coplin box containing a 50 % ethanol solution and then repeating this procedure in another Coplin with 20 % ethanol solution for 1 minute and then with the final wash in a Coplin with deionized water or Milli Q for 2 minutes.

After washing, the hydrolysis process follows, which consists of placing the plates in a Coplin with 5M HCl for 30 minutes and then rinsing with tap water for 3 minutes. A negative control was established by subjecting a fixed plate to immersion in a Coplin containing deionized water or Milli Q to measure the effectiveness of the hydrolysis treatment for 30 minutes, then immersing it in a Coplin with Schiff's reagent for 60 minutes at room temperature in darkness. After 60 minutes, the plates were rinsed with tap water for 5 minutes.

Once the previous step was completed, the plates were immersed in a Coplin with 0.2 % Light Green solution for 1 minute, then rinsed with deionized water or Milli Q to remove excess dye and subsequently placed on an iron for drying between 50 ºC and 80 ºC for 10 to 15 minutes. Subsequently, the plates were observed under an optical microscope with a magnification of 10x or 40x. For the registration of cytological events and at 100x for the registration of MN.

The research did not represent a risk to the population studied and complied with the requirements and ethical standards of health research in accordance with the regulations of the Colombian Ministry of Health. 8430 resolution No. 008430 of 1993 (October 4, 1993) and international (Helsinki declaration) (14,15)

For the statistical analysis, a descriptive analysis was conducted, and the variability of the variance was evaluated using an Excel database to calculate the indicators and their analysis, the variables were calculated, analysis of these in tables and graphs to represent the results, trend measures such as proportions and percentages were calculated.

After the evaluation of homogeneity and homoscedasticity of the data with the Shapiro Wilk and Levi test respectively, the Wilcoxon test was performed to determine if there was a significant difference before and after orthopedic treatment.

RESULTS

Samples were taken from 46 patients between 5-12 years old who attended the Juan Manuel Méndez Bechara Dental Clinic practice center in the period 2018, subtracting 8 patients to whom treatment with orthopedic appliances was not applied, defining a total sample of 25 patients in the second sample collection.

When observing the plaques before and after orthopedic treatment, significant changes were observed in the sampling after 1 month of treatment and a greater number of micronuclei, cells with nuclear sprouting, and cells with karyorrhexis. No changes or evidence of cells with condensed chromatin were observed. It can be seen in figure 1.

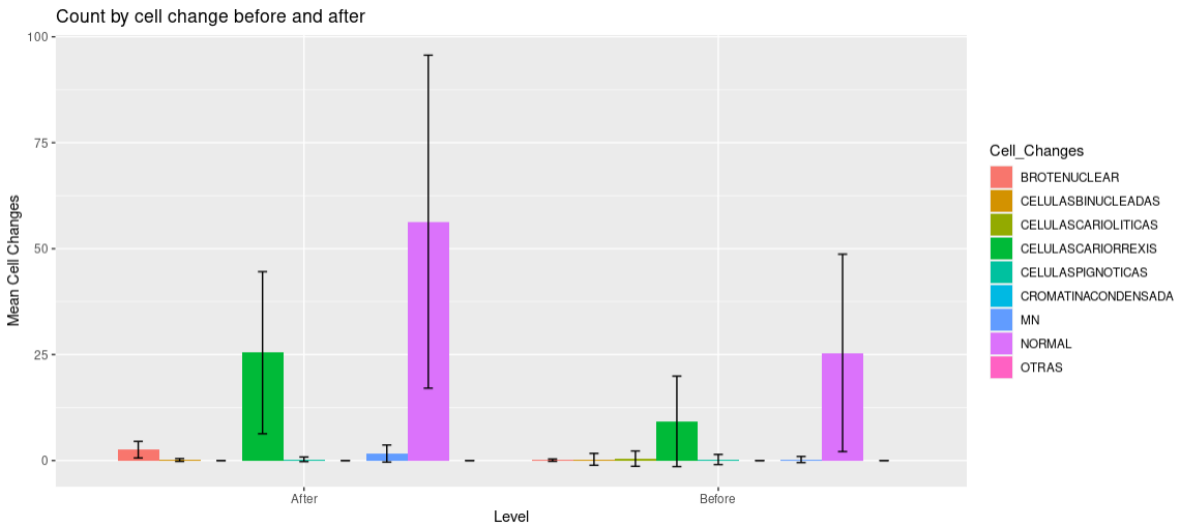

FIGURE 1
Comparison of cellular changes before and after orthopedic treatment

Particularly, the presence of micronuclei increased after one month of orthopedic treatment (values here) with a p value of 0.088875, observed in Figure 2.

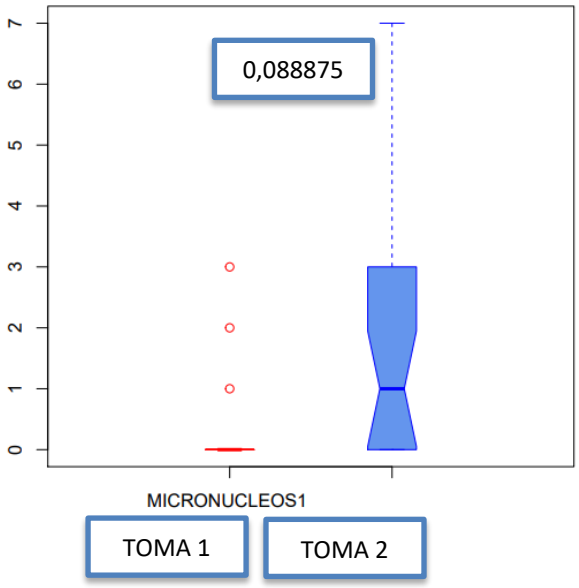

FIGURE 2
Number of micronuclei in the plates before and after orthopedic treatment

The presence of nuclear micronuclei sprouts increased after one month of orthopedic treatment with a p value of 0.05432, observed in Figure 3.

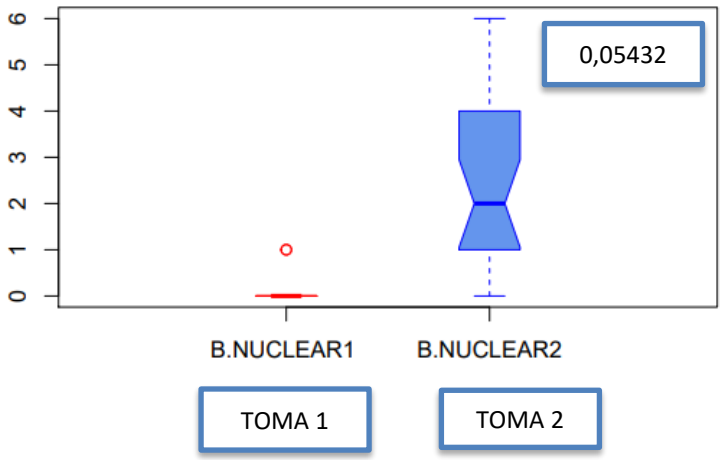

FIGURE 3
Number of nuclear sprouts in the plates before and after orthopedic treatment.

Before and after orthopedic treatment, no significant changes were observed in the sampling after one month of treatment in relation to pyknotic cells, as seen in Figure 4.

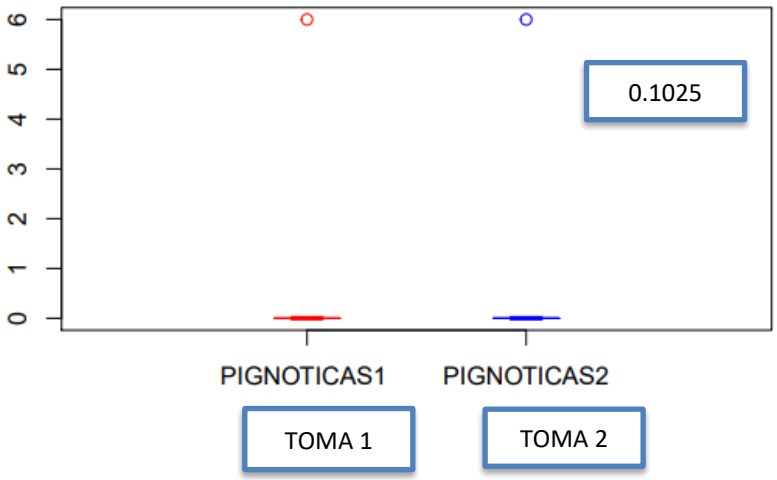

FIGURE 4
Number of pyknotic cells in the plates before and after orthopedic treatment

Destructive fragmentation of the nucleus of a cell, or karyorrhexis, increased after one month of treatment in patients, shown in Figure 5.

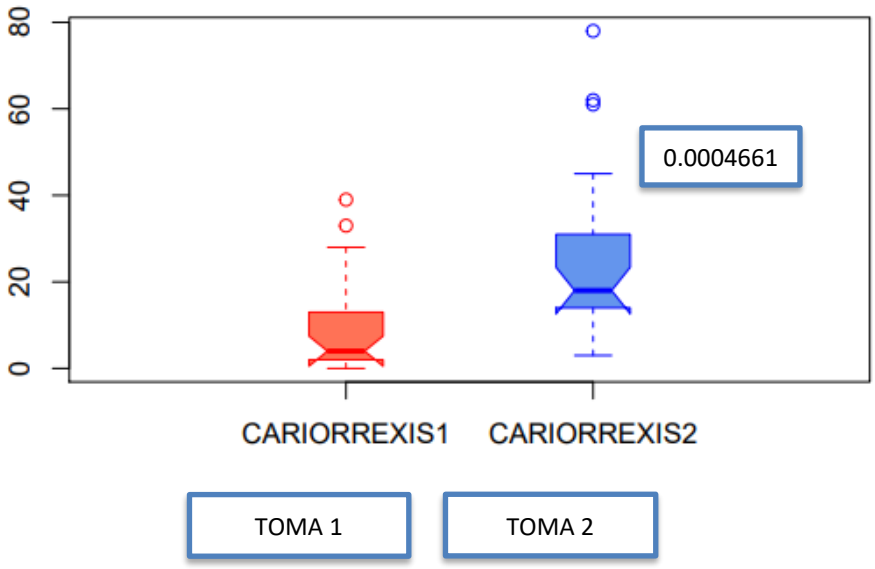

FIGURE 5
Number of cells that present karyorrhexis in the plates before and after orthopedic treatment.

DISCUSSION

MNs have been used as a measure and biomonitoring of the genotoxicity of several carcinogens, poisoning by heavy metals, antineoplastic drugs and contaminants, for this reason MNs are projected as a biomarker of chromosomal damage (16).

This technique has been widely used to assess the presence and extent of chromosomal damage in human populations exposed to genotoxic agents and has been shown to have a sensitivity of 94 %, a specificity of 100 %, and an accuracy of 95 % (16).

Most orthodontic and orthopedic appliances are made of stainless steel and Nickel Titanium (NiTi) alloys which can release metal ions into the oral cavity, with corrosion of the appliances and subsequent release of metal ions into the oral environment causing It is governed by the manufacturing process, which includes the type of alloy and the characteristics of the metals used, in addition to environmental factors such as mechanical stress, diet, time of day, salivary flow rate, and health and psychosomatic status of the patient. individual (17)

In studies conducted by Martín-Cameán A *et al.* In 2015, they observed significant differences regarding cytotoxic and genotoxic damage after a short treatment with orthodontic materials (1-3 months) and a longer term (24-48 months). Some studies that evaluated post-treatment effects (2 of 3 months) did not find significant differences with respect to controls after eliminating orthodontic applications and in comparison, with the current investigation, no damage was found at the genotoxic level, since karyorrhexis occurred in the second sample with a P value of 0.0004661, statistically significant, but karyorrhexis do not constitute stages of nuclear alteration (12)

Other authors studied the release of Ni and Cr ions from orthodontic appliances, extraoral anchorages, bands, brackets and arches. The result was that the largest amount of Ni released after 14 days came from the extraoral anchors, which contained a high quantity of silver solder(12)

Various investigations have failed to demonstrate a significant increase in Ni and Cr ions in saliva of treated patients one month after insertion of the application compared to levels before insertion (Gjerdet *et al,* 1991; Kerosuo *et al,* 1997). While other studies report an increase in the concentration of Ni and Cr in saliva after the insertion of the applications (Kocadereli *et al,* 2000; Eliades *et al.*, 2003b; Fors and Persson, 2006).

In short, there are various in vivo studies carried out that have evaluated the amount of metals released from orthodontic applications under different physical and chemical conditions, with a disparity in the criteria for experimentation and obtaining results (Gjerdet *et al.*, 1991; Bishara *et al,* 1993; Kerosuo *et al*, 1997; Kocadereli *et al,* 2000; Agaoglu *et al.* 2001; Eliades *et al*, Introduction / Introduction 47 2003b; Fors and Persson, 2006; Amini *et al*, 2008; Petoumeno *et al*, 2008;Matos de Souza *et al,* 2008).

Furthermore, the concentration required to produce a local adverse effect may be much lower than the concentrations to cause systemic effects via the oral route. In fact, it has been proven that various metal cations released from them can cause important biological alterations (DNA synthesis, alkaline phosphatase activity, etc.) at non-cytotoxic concentrations (Geurtsen, 2002). Occasionally, the patient's response to this release of metallic elements will differ depending on the nature and amount of said release.

CONCLUSIONS

The oral mucosa provides a barrier to carcinogens and its constant evaluation serves to monitor the first genotoxic events caused by carcinogens that enter the body. However, the presence of orthopedic appliances in the oral cavity represents a predisposing factor for corrosion, which constitutes the final phase of the metal alloys used (Ni-Ti/Ni-Cr).

Pyknosis, karyolysis and karyorrhexis do not constitute stages of nuclear alteration, the latter having the greatest tendency observed in the first and second sampling, with a significant increase in number, attributed to the presence of removable appliances in the patients. On the other hand, no changes were found in the cells associated with pyknosis and karyolysis in the initial sampling and the following sampling one month after treatment.

In the same sense, the changes that were most relevant were associated with micronuclei and nuclear sprouting, contemplated only in the second sample taken from the patients one month after treatment; however, the number of cells found does not represent a risk that generates genotoxicity and cytotoxicity in the oral cavity.

Through the technique for detecting micronuclei in oral mucosa cells, it was possible to determine that there is no DNA damage in those healthy patients undergoing removable metal orthopedic treatment.

RECOMMENDATIONS

Consider in future and long-term studies the technique for detecting micronuclei in oral mucosa cells with different materials, in order to have new findings, increase evidence and be able to determine the possible association of environmental and sociocultural factors.

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*Original research.

How to cite this article: Vergara Mercado ML, Salcedo Arteaga S, Espitia Pérez L, Hoyos Estrada LD, Gómez Montiel VA. Evaluation of possible DNA damage in oral mucosa cells using the micronucleus test in pediatric patients with removable appliances. Univ Odontol. 2023; 42. https://doi.org/10.11144/Javeriana.uo42.epdd