## **Original Article**

# Cytogenetic biomonitoring in children submitting to a complete set of radiographs for orthodontic planning

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#### ABSTRACT

**Objectives:** To evaluate the DNA damage (micronucleus) and cellular death (pyknosis, karyolysis, and karyorrhexis) in exfoliated buccal mucosa cells from children undergoing orthodontic radiographs. **Materials and Methods:** A total of 25 healthy children undergoing orthodontic therapy partook in a complete set of orthodontic radiographs (lateral cephalographic, posteroanterior cephalographic, panoramic, full periapical exam, and bitewing). The micronucleus test in the buccal exfoliated cells was applied. The paired-samples *t*-test and the Wilcoxon test were used to compare the frequencies of alterations before and after X-ray exposure.

**Results:** We found no statistically significant differences (P > .05) between micronucleated buccal mucosa cells before and after exposure to radiation. However, radiation did cause other nuclear alterations closely related to cytotoxicity (P = .007).

**Conclusion:** According to the micronucleus test, the complete set of radiographs requested in the orthodontic planning may not be a factor that induces chromosomal damage, but it is able to promote cytotoxicity. (*Angle Orthod.* 2012;82:585–590.)

KEY WORDS: Micronucleus test; Buccal mucosa cells; Dental radiography

## INTRODUCTION

Several cellular biological effects can result from exposure to ionizing radiation, but the nucleus, including its genetic material, is more radiosensitive than the cytoplasmic structures of a cell.<sup>1</sup> These genetic effects may include changes in the number and structure of chromosomes and mutations<sup>2</sup> and are closely related to cancer development. However, a research article involving the risk of cancer from

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diagnostic X-rays was published in an important periodical, but the dental diagnostic radiation was largely ignored,<sup>3</sup> and this type of radiation is possibly the most common form of head and neck radiation in children and adolescents, especially in orthodontics.

A typical orthodontic patient in one university setting had three cephalometric radiographs, three panoramic radiographs, and one full-mouth set of intraoral radiographs.<sup>4</sup> Thus, the orthodontic patient may receive an effective radiation dose of up to 477  $\mu$ Sv, which corresponds to 58 days of natural background radiation, according to the radiation doses of dental radiographs described in the literature,<sup>5</sup> and a previous study<sup>6</sup> has demonstrated that the 22- $\mu$ Sv dose is sufficient for a positive response in oral mucosa cells. Therefore, it is important to emphasize the diagnostic radiation associated with orthodontic care and the need to include it in etiologic studies on head and neck cancers,<sup>4</sup> especially as a result of the high and increasing prevalence of orthodontic treatment.<sup>7</sup>

Biomonitoring tests have been used to elucidate alterations produced by genotoxic agents, such as Xrays, through the use of biomarkers. Biomarkers allow us to gather information about environmental exposure to carcinogenic agents, its biological effects, and individual's susceptibility to the agents.<sup>8</sup> For this purpose, a simple, minimally invasive, low-cost, and enthusiastic

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method for monitoring genetic damage in humans is the micronucleus (MN) test in epithelial exfoliated cells.<sup>9</sup> Micronuclei arise from acentric fragments or whole chromosomes that are not included in the main nuclei of the daughter cells and can be induced by agents that cause chromosome breakage or affect the spindle apparatus<sup>10</sup> and can be used as a biomarker in buccal cells to evaluate the mutagenic effect of many agents, including ionizing radiation.<sup>6,11–21</sup>

Previous studies conducted by our research group have demonstrated that the panoramic X-ray is able to induce damage in the oral mucosa cells of children.13 In this regard, it would be interesting to know if, and to what extent, children submitted to orthodontic radiographic protocol are a more sensitive group as a result of the use of X-ray in oral mucosa cells, particularly because there are no previous reports. Moreover, no previous biomonitoring study has evaluated the effects of full-mouth X-rays (FMX). Therefore, the aim of this study was to evaluate the frequency of micronucleated cells (MNC)-or the mutagenic effect-in the buccal mucosa cells of children who had undergone orthodontic radiographs, including FMX. To monitor the cytotoxic effects, pyknosis, karyolysis, and karyorrhexis were also scored in this setting.

## MATERIALS AND METHODS

The subjects of this study comprised 25 healthy children (n = 25; 15 boys and 10 girls, mean age 11.2 $\pm$  1.4 years). None were alcohol or tobacco consumers. They did not utilize mouth rinses or medicine and were not submitted to ionizing radiation in the 16 days prior to the study. Patients were subjected to the following radiographs: lateral cephalographic (LAT), posteroanterior cephalographic (PA), and panoramic (PAN), FMX (periapical-six of anterior teeth and eight of posterior teeth; two bitewings). Radiographs were taken using Rotograph Plus equipment (Dabi Atlante, Ribeirão Preto-SP, Brazil: LAT: 80 kV, 10 mA, 1.3 seconds, 0.003 mSv; PA: 85 kV, 10 mA, 1.6 seconds, 0.03 mSv; and PAN: 70 kV, 10 mA, 17 seconds, 0.03 mSv) and Spectro 70X Seletronic equipment (Dabi Atlante: anterior periapical: 70 kV, 8 mA, 0.4 seconds, 0.008 mSv, round collimation; posterior periapical and bitewing: 70 kV, 8 mA, 0.45 seconds, 0.008 mSv, round collimation). All exams were requested for orthodontic planning and treatment. The study was approved by the Institutional Human Ethics Committee, and informed consent was obtained from the parents of the included individuals.

## **MN Test in Oral Mucosa Cells**

Buccal exfoliated cells (BEC) were collected immediately before the X-ray exposure and after 10 days. After rinsing the mouth with tap water, cells were obtained by scraping the right/left cheek mucosa with a moist wooden spatula. Cells were transferred to a tube containing saline solution, fixed in 3:1 methanol/acetic acid, and dropped onto pre-cleaned slides. Later, the air-dried slides were stained using the Feulgen/Fast Green method and examined under a light microscope at  $400 \times$  magnification to determine the frequency of MNC. A total of 1000 cells were scored from each patient for each sampling time (before and after X-ray exposure).

## **Data Analysis**

All slides were analyzed by an experienced and blinded cytopathologist. The MNC (measure of DNA damage) were scored according to the criteria described by Sarto et al.22 (MN was identified taking into consideration the following conditions-intact nucleus and cytoplasm; diameter of one-third of the main nucleus; same staining and texture as the main nucleus, and MN was in the same focal plane as the main nucleus). For cytotoxicity, the following nuclear alterations were considered, as described by Tolbert et al.<sup>23</sup>: pyknosis, karyolysis, and karyorrhexis (Figure 1a-d). A total of 1000 cells were assessed per person in this study for the MN frequency and other parameters of cytotoxity. On average, a total of 50 cells were assessed per field. The results were calculated by assessing percent of altered cells only. Similar analyses were conducted in previous studies.13,15,19

## **Statistical Methods**

The paired-samples *t*-test and the Wilcoxon test were used to compare the frequencies of cell death and MNC, respectively, before and after X-ray exposure. The level of statistical significance was set at 5%.

The reliability of the evaluation was verified by digital pictures of 600 BEC from this research. These cells were numbered and classified according to their nuclear characteristics: normal, pyknosis, karyolysis, karyor-rhexis, and MNC. After 30 days these cells were reclassified, and the Kappa test was applied to investigate the concordance between the two evaluations.

## RESULTS

According to the Kappa test, the concordance between the two evaluations was good (Kappa value = 0.752). Table 1 shows the frequency of MNC and other nuclear alterations in children undergoing radiographs necessary for orthodontic treatment. Before the X-ray exposure, the mean frequency of MNC was 0.008% for the radiograph group. No statistically significant differences (P > .05) were noted after



**Figure 1.** Nuclear alterations observed (400× magnification, Feulgen/Fast Green stain): (a) micronucleated (arrow) and normal cells; (b) karyorrhexis (arrow); (c) karyolysis (arrow); and (d) pyknosis (arrow).

ionizing radiation exposure. However, a significant increase in other nuclear alterations was observed after these exams, specifically karyorrhexis, pyknosis, and karyolysis (P = .007). These data are summarized in Table 1. None of the children evaluated were exposed to other known genotoxic agents.

### DISCUSSION

MN assay in buccal exfoliates is an in vivo exam that permit elucidation of the effects of toxic agents directly in a target tissue, the buccal epithelium. The limited cost, the ease of scoring, the human time required, and the precision obtained from scoring large numbers of cells<sup>6</sup> enhance the popularity of this noninvasive method. Accordingly, utilization of the MN test in buccal exfoliates has attracted much attention from research groups, such as the Human  $MN_{XL}$  Project.<sup>9</sup>

The damage that led to the formation of micronuclei occurs in the basal layer of the epithelial tissue, where cells undergo mitosis. Rapid turnover of epithelial tissues brings the cells to the surface, where they

 Table 1.
 Frequency (%) of Micronucleated Cells and Other Nuclear Alterations (Karyorrhexis, Pyknosis, and Karyolysis) in Children Undergoing

 Radiographs.
 Values Are Means ± Standard Deviation (SD)

Group	No. of Children	Frequency, %	
		Micronucleated Cells	Other Nuclear Alterations
Prior to X-ray exposure	25	$0.008 \pm 0.03$	12.2 ± 5.3
After X-ray exposure	25	$0.024 \pm 0.05$	14.4 ± 5.1*

\* P = .007 (vs children prior to exam exposure).

exfoliate.<sup>24</sup> In general, cells take 7–16 days to emerge to the surface and exfoliate.<sup>25</sup> Results<sup>22</sup> described in patients suffering from cancer of the oral cavity and undergoing mouth radiotherapy showed that the frequency of MNC decreased to the initial background level 7 to 12 days after the end of radiotherapy. For this reason, exfoliated oral mucosa cells were collected immediately before ionizing radiation exposure and after 10 days, in accordance with the methods of similar studies,<sup>12,13,18</sup> a period that allowed time for the basal layer exposed to radiation to mature and to be collected when exfoliated.

Human biomonitoring studies in buccal cells demand attention, given the confounding factors, such as age, lifestyle, oral hygiene (eg, mouth rinse utilization), dental health, smoking, and use of alcohol.9 These factors were controlled in our study. The sample comprised only children with suitable oral hygiene and dental health (no periodontal disease or caries). Some studies have pointed toward a relationship between age and MN occurrence,<sup>26,27</sup> whereas others have not.<sup>12,19</sup> As a result of the homogeneity in the sample, it was not possible to correlate the frequency of MNC with age in this setting. Children are affected to a lower extent by confounders such as cigarette smoking and drinking habits, occupational exposure, and lifestyle (mainly dietary factors), which are factors of great concern in adults.<sup>26</sup> Moreover, because each patient was considered to be his own control, any effect of other genotoxic agents must have been present in the first cell count. Therefore, potential differences between the first and the second counts can be attributed to radiation.17

The MN assay is a measure of DNA damage.<sup>9,16,28</sup> The MNC frequencies were not significantly different before and after X-ray exposure in our sample. These results contrast with those of other authors,<sup>2,12,16,29,30</sup> who reported higher rates of chromosomal aberrations subsequent to X-ray exposure. However, despite higher radiation doses in our investigation, research with similar methodology with dental radiographs showed similar results compared to our study (ie, no mutagenic characteristic was evidenced by the MN test).<sup>6,11,13,14,17–21</sup>

Differences in radiation dose, frequency of exposition, type of cells evaluated, and site of collected cells may influence the results of the MN test. Some authors investigated patients undergoing radiotherapy five to six times per week over the course of 5–7-weeks<sup>16,29</sup> and others observed the effects of frequent occupational exposition to low doses of X-ray in radiation workers,<sup>2,30</sup> whereas some pointed out the results of only a single dental radiograph exposition.<sup>6,13,17–21</sup> The literature shows that MN-MNC<sup>31</sup> and cellular death<sup>17</sup> increase with radiation dose. With regard to the

Additionally, in the cytogenetic studies of dental radiograph effects, different sites were elected from which to collect buccal cells: buccal cheek mucosa,<sup>11,13,14,21</sup> the lateral border of the tongue,<sup>17,21</sup> and keratinized mucosa of the upper dental arch.<sup>12</sup> One of these studies<sup>21</sup> showed that the lateral border of the tongue is more sensitive to cytotoxic insult than is the cheek buccal mucosa. The research<sup>12</sup> that evidenced the genetic damage capacity of PAN was unique in that it utilized keratinized mucosa of the upper dental arch. These facts emphasize the need for more comparisons between different buccal sites and help us to explain the divergence found in the biological effects in radiation studies. Based on our findings, we assumed a lack of mutagenic effects related to the radiographic protocol utilized in children in orthodontic planning, a piece of information that has not yet been reported.

Researchers<sup>11,23</sup> have called attention to nuclear changes other than MN that characterize cellular death and may increase the sensitivity of tests to detect genotoxicity. Thus, cytotoxic effects were investigated through the frequencies of karyorrhexis, karyolysis, and pyknosis. In contrast, with respect to genetic damage, radiographs caused cellular death, as indicated by a statistically significant difference (P = .007) between values before and after X-ray exposure, in agreement with the findings of other studies.<sup>11,13,17,19</sup> This result endorsed the notion that X-rays are cytotoxic, and based on the knowledge that cytotoxicity interferes with MN induction because some MNC are inevitably lost after a cytotoxic insult,<sup>13</sup> the lack of a mutagenic effect on this set of X-rays is confirmed.

Nevertheless, repeated exposure to cytotoxic agents can result in chronic cell injury, compensatory cell proliferation, hyperplasia, and, ultimately, tumor development. These cytotoxic/non-genotoxic agents act by interfering with the molecules intimately involved in cell growth and cell death. Increased cell proliferation appears to be a unifying feature of epigenetic carcinogens. Proliferation may increase the risk of mutations within target cells and may also be important in the selective clonal expansion of initiated cells.<sup>32</sup>

Furthermore, pyknosis, karyorrhexis, and karyolysis are evident in cells undergoing necrosis, a form of cell death that occurs following injury by cytotoxic agents, and pyknosis and karyorrhexis (but not karyolysis) accompany the early stages of another type of cell death, apoptosis, believed to be the major mode of death in living tissues under physiological control. Because it is stimulated by ionizing radiation and by chemicals that bind to DNA, apoptosis may also serve in a surveillance role, eliminating cells with genetic damage.<sup>23</sup> The most worrisome aspect of irradiation is the genetic insult that may lead to neoplastic transformation of oral epithelial cells. For cells with DNA damage, self-repair or apoptosis is the best-case scenario. If the cells are unable to undergo repair or apoptosis, a neoplastic process may be initiated. Although DNA damage is able to induce apoptosis, it is important to stress that the MN assay detects DNA damage as a result of chromosome breakage or loss, that is, a genetic damage unable to repair. The assay is a putative indicator of mutagenicity as far as cancer risk (initiation phase of carcinogenesis process) is concerned. Other nuclear alterations, such as pyknosis, karyorrhexis, and/or karyolysis, reflect cellular death, either to necrosis or apoptosis. Therefore, we did not consider it relevant biologically to analyze MNC and cytotoxicity together because the biological mechanisms involved in these processes are different.

### CONCLUSIONS

- According to the results of this investigation, children's exposure to X-rays during the orthodontic radiographic protocol caused some DNA damage, which is lower than the threshold value required for carcinogenesis. Despite the increase after irradiation, the number of micronucleated cells was not statistically different among the observed periods. On the other hand, the orthodontic radiographic protocol was cytotoxic to buccal mucosa exfoliated cells.
- Thus, despite the importance of radiographs to orthodontic treatment, we cannot consider this set of radiographs to be a risk-free procedure, and other radiographs needed during or at the end of treatment should be requested only when necessary, always considering the risk/benefit relationship for the patients. This precaution is enhanced by the emerging trend of using another diagnostic exam (based on ionizing radiation) in dentistry, cone beam computerized tomography, and clearly points to the need for further studies in this field, even to confirm our findings.

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