



# Genotoxic and Cytotoxic Effects of Dental Radiographic Modalities on Buccal Mucosal Cells in Children

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## Abstract

Dental radiography is an important diagnostic tool for the detection and assessment of the extent of dental caries and accurate treatment planning. There is no safe limit for X-ray exposure. The associated risks of X-ray exposure are higher in children due to a higher rate of cell proliferation in them, compared with adults. This study aimed to assess the genotoxic and cytotoxic effects of dental radiographic modalities on buccal mucosal cells in children. This interventional study evaluated 80 children between 3 and 12 years who required periapical, panoramic, bitewing, or bitewing plus panoramic radiography for treatment planning. Twenty eligible patients were assigned to each of the aforementioned four groups. Buccal mucosal cells were scraped bilaterally by a plastic spatula after complete rinsing of the oral cavity. The collected specimens were directly mounted on microscopic slides and after air-drying, they were fixed with 80% methanol and Giemsa stain. The cells were then inspected under a light microscope at 400x magnification for cytogenetic changes. Data were tabulated and analyzed by SPSS version 20 at a  $p < 0.001$  level of significance. The results showed a significant increase in the frequency of karyolysis, karyorrhexis, and pyknosis in all four groups after dental radiography ( $p < 0.001$ ). Also, the number of micronuclei significantly increased after panoramic plus bitewing radiography ( $p < 0.05$ ). X-ray exposure in panoramic, periapical, bitewing, and bitewing plus panoramic radiographies can be cytotoxic, while bitewing plus panoramic radiography can be genotoxic in children as well.

## Keywords

- ▶ cytotoxicity
- ▶ genotoxicity
- ▶ X-ray
- ▶ dental radiography
- ▶ buccal mucosal cells

## Introduction

Radiography is an important diagnostic tool commonly used in dentistry. It can be of great help in all steps of diagnosis, treatment planning, checkups, and follow-up.<sup>1</sup> The three most commonly prescribed dental radiographic modalities include the bitewing, periapical, and extraoral dental radiographies.<sup>2</sup> Children often require several dental radiographs

in their dental treatment process. However, considering their developmental stage, they are more susceptible to the adverse effects of X-ray radiation than adults. Thus, the effects of X-ray exposure in dental radiography on children should be more precisely evaluated.<sup>3</sup> A study on X-ray exposure in the United States demonstrated that about 90% of X-ray exposures are related to medical and dental radiography. At present, almost all dental offices are equipped with a

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dental X-ray unit to confirm or complement the suspected diagnoses and use it for more accurate treatment planning.<sup>4</sup> Bitewing radiography is the most efficient dental radiographic modality for the detection of interproximal caries in teeth with closed contacts such that 75% of interproximal caries cannot be detected without bitewing radiography.<sup>1</sup> Panoramic radiography is an extraoral radiographic modality used as a diagnostic tool for developmental disorders such as missing teeth, supernumeraries, ectopic tooth eruption, delayed primary root resorption, and detection of cysts, tumors, and some genetic disorders.<sup>3</sup> It should be noted that despite the widespread use of dental radiographic modalities, there is no definite safety level for X-ray exposure.<sup>5</sup>

Sarto et al categorized a parameter for cytotoxicity that includes karyolysis, karyorrhexis, and pyknosis.<sup>6</sup> Li et al<sup>7</sup> also considered the increase in the number of micronuclei as a criterion for the study of genotoxicity which according to the article by Thomas et al<sup>8</sup> is based on several criteria: (1) dimensions less than 1.3 of the diameter of the nucleus, (2) color similar to the nucleus, (3) round or oval shape, and (4) completely separated from the main nucleus.

Cytotoxic changes are the presence of cellular biomarkers such as karyorrhexis (nucleus decomposition), karyolysis (destruction of the nucleus completely), and pyknosis (shrinkage of the cell nucleus). Genotoxic changes are genetic changes in the cell that are characterized by an increase in the number of micronuclei.

Collection of the exfoliated buccal mucosal cells directly exposed to X-ray radiation is a suitable method to assess the cytotoxicity and genotoxicity of X-ray radiation. These cells can be easily collected by noninvasive techniques such as scraping. This method is more accurate than the assessment of blood lymphocytes since a high number of cells (around 2,000) can be collected and evaluated as such.<sup>9</sup> These cells have a fast turnover of around 7 to 21 days, and after this period, changes in the cell nucleus (karyorrhexis, karyolysis, and pyknosis) can be evaluated by cytogenetic biomonitoring.<sup>8</sup> This method has long been used for the assessment and detection of the risk of the development of diseases or for determining the stage of disease.<sup>10</sup> Also, it is widely used for the assessment of the genotoxic effects of tobacco, alcohol, and many other potentially carcinogenic substances.<sup>11,12</sup> Popova et al in 2007 reported no change in the number of micronuclei after X-ray exposure in panoramic radiography.<sup>9</sup> However, Li et al in 2018 demonstrated that cone-beam computed tomography had genotoxic effects on buccal mucosal cells.<sup>7</sup> Considering the controversial results of previous studies on this topic and lack of studies regarding the effects of X-ray exposure in periapical, panoramic, and bitewing radiographies on children (particularly in Zanjan, Iran), who might need repeated radiographs owing to factors such as high dental caries incidence, little cooperation while making radiographs, and occurrence of errors such as motion artefact—all of which may lead to more exposure, this study aimed to assess the genotoxic and cytotoxic effects of dental radiographic modalities on buccal mucosal cells in children.

## Materials and Methods

### Study Design

This study was conducted in accordance with the World Medical Association Declaration of Helsinki (of 1975 as revised in 2000) and was approved by the ethics committee of Zanjan University of Medical Sciences. (Institutional Review Board: IR.ZUMS.REC.1396.345).

The sample size was calculated to be 80 assuming 95% confidence interval,  $\alpha = 0.05$ , and study power of 80%.<sup>13</sup> Eighty children between 3 and 12 years residing in the city of Zanjan, Iran who required periapical ( $n = 20$ ), panoramic ( $n = 20$ ), bitewing ( $n = 20$ ), or bitewing plus panoramic ( $n = 20$ ) radiography were enrolled in this before-after interventional study.

All of the collected data from the population of the study, including first and second sampling, were performed in a short period of time in spring. Demographic information of patients was recorded, and systemically healthy children with no history of radiotherapy or radioiodine therapy of the head and neck region were selected. The most recent radiograph of patients had to be taken at least 6 months earlier. The patients did not require pulpotomy or pulpectomy, had no oral ulcer or trauma, and were not using antibacterial mouthwashes. According to the factors affecting the outcome of studies, such as diet, smoking and exposure to cigarette smoke, chemicals such as insecticides, and occupation of parents, therefore considering the control group for this study may confound the results of the study.<sup>14-17</sup>

The mucosa cytology samples were collected as follows.

### Sample Collection

Before sampling, the children were requested to rinse their mouths with water. After obtaining written informed consent from the parents, a plastic spatula was used to scrape the exfoliated buccal mucosal cells bilaterally at the line of occlusion from the mesial of the first molar to distal of canine.

One slide was prepared for each of the right and left sides, and two specimens were mounted on each slide. Thus, two slides were prepared for each patient. The collected specimens were immediately mounted on the microscopic slides and allowed 30 minutes to dry at room temperature. Next, the specimens were fixed with 80% cold methanol and stained with 10% Giemsa stain. A dentist and a student evaluated the slides under the supervision of an oral and maxillofacial pathologist under a light microscope (Olympus, Japan) at  $\times 400$  magnification. The children then underwent the prescribed radiographic modalities. It should be mentioned that all radiographic modalities had been requested by the attending dental clinicians of children for purposes not related to this study. Ten days after radiography, the patients were recalled, and the parents were questioned about any recent history of air travel, not having a cell phone or tablet, and not taking any other radiograph during this period. If the parents responded negatively to the above-mentioned questions, postintervention samples were collected as explained for baseline sampling. Since the cells of the buccal mucosa have a fast turnover of around 7 to

21 days, and after this period, changes in the cell nucleus (karyorrhexis, karyolysis, and pyknosis) can be evaluated by cytogenetic biomonitoring.<sup>8</sup> Therefore, like many of the present articles, we can review the result after 10 days.

Dental radiographs were obtained with the following exposure settings: panoramic radiography (Instrument Iom, Soredex): 63 to 75 kVp, 9 to 14 mA, 14 to 16 seconds time, and bitewing and periapical radiographies (Minray, Soredex): 70 kV, 7 mA, and 0.2 to 0.25 seconds time using photostimulable phosphor plate sensors.<sup>18</sup>

**Statistical Analysis**

Data were tabulated and analyzed by SPSS version 20 at a  $p < 0.001$  level of significance. The Kolmogorov-Smirnov's test analyzed the normality of data distribution. Analysis of variance (ANOVA) and paired *t*-test were applied for normally distributed data. In the case of the significance of ANOVA, pairwise comparisons were performed by Tukey's post hoc test. The Kruskal-Wallis' and Wilcoxon's tests were applied to analyze the data with nonnormal distribution.

**Results**

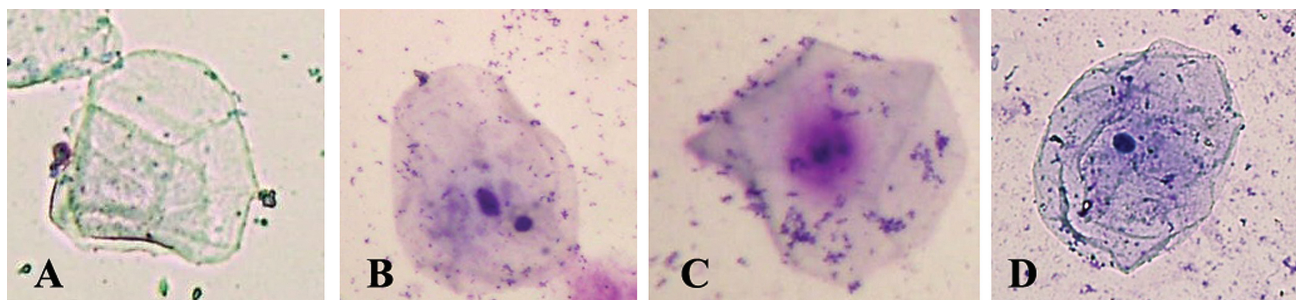
A total of 80 children were evaluated in this study; out of which, 41 (51.2%) were male and 39 (48.8%) were female. Of all, 42.5, 32.5, and 25% of patients were between 6 to 9, 3 to 6, and 9 to 12 years, respectively. The mean body mass index (BMI) of children was  $17.96 \pm 1.96$ .

The results of the assessment of children between 3 and 12 years at  $10 \pm 2$  days after radiography showed a significantly higher rate of pyknosis, karyorrhexis, and karyolysis (**► Fig. 1**) in all four groups compared with baseline ( $p < 0.001$ ). Also, a significant increase was noted in the number of micronuclei (**► Fig. 1**) after bitewing plus panoramic radiography compared with baseline ( $p < 0.001$ ; **► Fig. 2**).

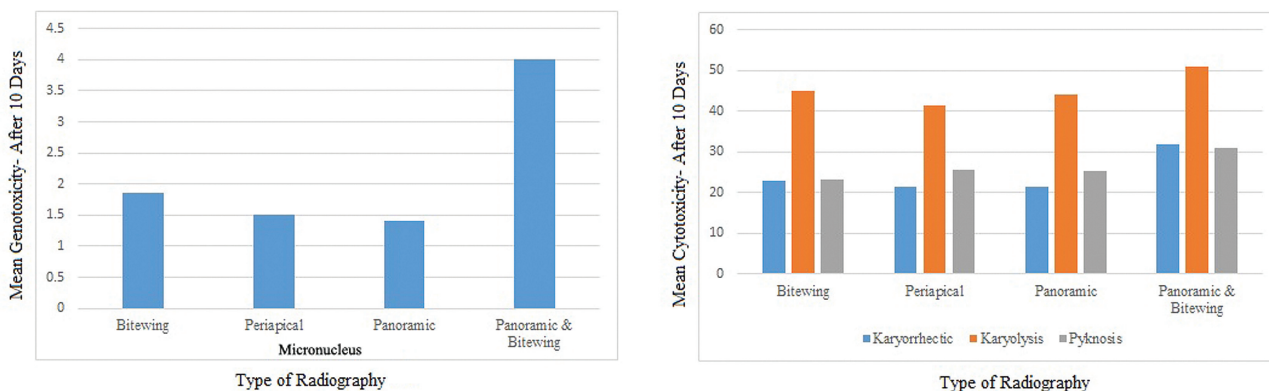
In this study, we evaluate the changes after bilateral bitewing and posterior periapical radiographs. Certainly, one-sided posterior radiographs are needed to study the effects of X-rays on exposed and unexposed mucosa, as well as a different study design.

**Discussion**

There is no safe limit for X-ray exposure in dental radiography.<sup>5</sup> Although the cytotoxicity and genotoxicity rates of dental radiography are low, they are not zero.<sup>4</sup> Children are more susceptible to the adverse effects of X-ray exposure than adults due to their higher cell proliferation rate and higher sensitivity.<sup>3,18</sup> Another reason that makes them more sensitive to radiation is their anatomical characteristics and longer lifespan.<sup>19,20</sup> Moreover, the X-ray effects are cumulative and can lead to X-ray-induced tumorigenesis in the future.<sup>21</sup> The current results showed that all types of dental radiographic modalities can be cytotoxic, and a combination of bitewing and panoramic radiography can also have a genotoxic effect in addition to cytotoxic effects on the buccal



**Fig. 1** Micrograph of cellular changes after X-ray exposure: (A) karyolysis; (B) micronucleus; (C) karyorrhexis; and (D) pyknosis (Giemsa stain,  $\times 400$ ).



**Fig. 2** Number of micronuclei, pyknosis, karyorrhexis, and karyolysis after panoramic, periapical, bitewing, and bitewing plus panoramic radiographies.

mucosal cells of children. Regarding genotoxicity, Preethi et al<sup>3</sup> evaluated the effects of X-ray exposure in bitewing and panoramic radiography on children between 6 and 12 years and reported a significant increase in the number of micronuclei as an index of genotoxicity in both groups ( $p < 0.001$ ). Also, they added that the effects of X-ray in bitewing radiographs were 1.5 times greater than those in panoramic radiography. They offered two explanations for this finding: The first reason was explained to be the X-ray scattering in panoramic radiography since the scanner rotates around the jaw for image acquisition, while X-ray is concentrated at one point in bitewing radiographs; thus, the X-ray scattering is lower in the latter. The second reason was explained to be the lower voltage of panoramic radiography (64 vs. 70 kVp).<sup>3</sup> Similar to the results of this study about the cytotoxic effects of X-ray, studies have been conducted by Angelieri et al,<sup>22</sup> Antonio et al,<sup>23</sup> and Agarwal et al<sup>24</sup> between the cases in which panoramic radiographs were taken. They demonstrated no genotoxic effects can be due to the lack of bitewing images alongside the panoramic radiographs.<sup>22–24</sup> In contrast, Li et al evaluated the effects of X-ray exposure in dental radiographic modalities requested for orthognathic surgery and orthodontic treatment on patients with age 8 to 42 years. They reported a significant increase in the number of micronuclei only when multiple radiographic series such as panoramic radiography + lateral + frontal + craniofacial images were requested. No genotoxic effect was noted following low-dose radiographic modalities such as panoramic radiography.<sup>7</sup> Such a controversy in the results of studies can be due to the interference of cytotoxicity with the presence of micronuclei because cells with micronuclei may undergo cell death (i.e., cytotoxicity).<sup>25</sup> On the other hand, Angelieri et al in 2010 assessed the effects of lateral and frontal cephalometric X-rays in combination with panoramic radiography among patients who were referred for orthodontic therapy. The outcome of the study revealed no chromosomal damage while cytotoxicity occurred. The difference between the genotoxicity results can be because of the small number of samples (18 cases) in the Angelieri et al's study as well as the higher average age (14 years) or due to the absence of bitewing radiographs.<sup>26</sup> The effects of confounding factors such as diet, exposure to cigarette smoke, place of residence, age, and occupation of the father and mother are among other parameters that may be responsible for the existing controversy in the literature.<sup>7,14–17,21,27</sup> In this study, demographic information of patients was collected to control for the confounding factors as much as possible. Some previous studies assessed the effect of confounders in this respect. For instance, Chen et al evaluated the oral buccal mucosal cells of rats and reported that alcohol can cause carcinogenicity.<sup>27</sup> Also, Fagundes et al evaluated the effect of beta-carotene and showed that carotenoids have protective effects and can repair the damaged DNA.<sup>16</sup> In such studies, since each patient serves as his/her own control, the changes after 10 days are attributed to X-ray exposure.<sup>28</sup>

The cytotoxicity and genotoxicity of other radiographic modalities have also been evaluated. For instance, Palla et al assessed the cytotoxic and genotoxic effects of computed

tomography on buccal mucosal cells. They collected the buccal mucosal cell samples 10 and 20 days after exposure and evaluated them by using light microscopy.<sup>29</sup> They concluded that genotoxicity significantly increased after 20 days; however, the level of cytotoxicity observed after 10 days significantly decreased in the second cell cycle at 20 days.

One of the strong points of this study was the exclusion of patients who required pulpotomy and pulpectomy since the materials used for such treatments could serve as a confounder. Also, the patients were standardized in terms of BMI, which was another strength. After calculation of BMI and by using one-way ANOVA, we found no significant difference in BMI among the four groups. Thus, the effect of this confounder was also controlled for. The current results showed a significant increase in cytotoxicity in all four groups after X-ray exposure and a significant increase in genotoxicity in the bitewing + panoramic radiography group. However, considering the controversy in the results of studies on this topic, future studies with a larger sample size are required while controlling for the effect of some other confounders such as cigarette smoke. It is noteworthy that future studies with a larger sample size would be of importance.

## Conclusion

In conclusion of this study, the assessment of buccal mucosal cells before and 10 days after different dental radiographic modalities revealed that X-ray exposure in the process of panoramic, periapical, bitewing, and panoramic + bitewing radiographies can cause cytotoxic changes that might induce tumorigenesis. Also, a significant increase was noted in the number of micronuclei following X-ray exposure in panoramic plus bitewing radiography indicating their genotoxic effects, which are indicators of carcinogenic activity.

## Conflict of Interest

None declared.

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