

## CYTOLOGICAL CHANGES OF ORAL MUCOSA FOLLOWING LATERAL CEPHALOMETRY AND PANORAMIC RADIOGRAPH

Roza Haghgoo, Reza Sedaghat, Motahare Ahmadvand, Zahra Ogaghi

## ABSTRACT

**Background:** Despite the wide use of radiographic examination as a diagnostic tool in dental practice radiography can induce cytotoxic effect. **Aims and objectives:** To assess DNA damages of buccal mucosa cells in children and adults who have been undergone panoramic radiography and cephalometry during orthodontic treatment. **Materials and Method:** The study sample consists of thirty randomly selected patients who were under going panoramic radiography and cephalometry examination for their orthodontic therapy. The patients were allotted to two groups of 15 adults and 15 children. Exfoliated buccal mucosa cells were collected immediately before and 10 days after X-ray exposure and the cellular changes were evaluated. Statistical analysis of the data was done by SPSS v16. **Results:** The results of this study showed that there was no significant difference in micronuclei of oral mucosa cells before and after oral radiography in children ( $P < 0.05$ ). Both group post exposure samples had increased cytotoxicity indicators such as karyolysis, karyolexy and pyknosis. There was no statistically significant difference in micronuclei numbers between children and adults ( $P < 0.05$ ). **Conclusion:** This study results concludes that absorbed dose from dental X-ray cannot make genotoxicity changes.

**Keywords:** Buccal mucosa cells; Micronucleus Test; X-ray

## Introduction

Nowadays, radiographs have been used widely for diagnosis in dentistry. However, it is well known that ionizing radiation causes cell damage including single and double strand breaks to deoxyribonucleic acid (DNA) and DNA protein crosslinks leading to cellular death.<sup>1</sup> Radiation can cause molecular changes in cells and its effect can remain for some hours, months or generations leading to genotoxicity or cytotoxicity.<sup>2,5</sup> The risk of cytogenetic damage in children exposed to x-rays during a dental treatment is one of the main concerns for the parents and dentists, because radiograph is an essential diagnostic tool for the successful treatment.<sup>3</sup> It is reported that, in general, younger tissues and organs are more sensitive to X-rays than adult tissues.<sup>6</sup> This study was conducted to compare and evaluate the possible cytotoxic effect of radiation exposure for dental diagnostic purposes in children and adults after panoramic and lateral cephalometric radiographs.

## Materials and Method

This study was conducted in Dental School, Shahed University, Tehran, Iran. The study population consists of thirty randomly selected patients who were advised for panoramic radiograph and lateral cephalometric radiograph as a diagnostic tool for their orthodontic treatment. The study protocol conforms to the guidelines of the Helsinki Declaration on human experimentation. The ethics committee of Dental School, Shahed University, accepted the study design. Written informed consent was obtained from all adult participants and guardians for minors. The thirty healthy volunteers were divided into two groups i.e., Group A -15 adults and Group B-15 children. Inclusion criteria were good oral hygiene, absence of tooth decay and restorations. Patients with more than four lost teeth, repeated aphthous stomatitis and skin reactions were excluded from the study. Volunteers with a history of alcoholism and smoking and who were using any oral antiseptic solutions at the time of the study were too excluded. Buccal cell-exfoliated epithelium samples were collected at two separate time points from each patient. Exfoliated buccal epithelial cells were

scraped from the middle part of the inner cheeks with sterile cement spatulas before the radiographs were taken and were regarded as the baseline control. The second sample was collected at 10 days after the radiographs were taken. All patients were instructed to rinse their mouths twice with tap water before the investigator collected exfoliated epithelial cells from the buccal mucosa. All samples were scraped from the middle part of the inner cheeks with sterile cement spatulas by the same investigator. Spatula was moved in same direction, i.e., from inside to outside of mouth parallel to a horizontal line that begins near posterior teeth towards the corner of lip.

Samples obtained were transferred to test tubes and were dissolved in 2ml normal saline. The test tubes were placed in a centrifuge machine and were centrifuged 800-rpm speed for 5minutes. To increase the accuracy of deposited cells, conical end tubes were used. After removing the tubes from the centrifuge device, normal saline solution was removed using a sampler. The cells were smeared onto clean microscope glass slides. Sample were prepared, air-dried and immediately fixed in a methanol:glacial acetic acid (3:1) mixture and were transferred to pathology laboratory of Mustafa Khomeini Hospital to be painted with Papanicola. Samples were stained in laboratory by following the steps shown in table 1. Following the staining cells nuclei became blue; cytoplasm pink and the space between the cells green-blue. These painted samples were investigated with optic dual eyepiece microscope with a magnitude of 400x. Frequency of micronucleus cells were considered as an index for DNA damage (genotoxicity), because micronucleus formation can be caused by Chromosome breakage and factors affecting Duk system.<sup>7</sup> Cells that undergo artifact changes were deleted to increase the accuracy of cell count and just clearly spread cells were counted. Finally an average of 600 cells per slide was investigated.

**Statistical Analysis:** Mean, standard deviation, minimum and maximum values of degenerative nuclear abnormalities, as appropriate, were calculated as the descriptive measurements

## Steps for Slide Staining

Washing in 50% - 70 %- 80% alcohol
Washing in Distilled water
Putting in Hematoxylin Haris solution for 5 to 30 seconds
Washing in Distilled water
Putting in acid – alcohol solution for one minutes
Washing in Distilled water
Washing in 50% - 70 %- 80% alcohol
Putting in OG6 Solution for 90 seconds
Washing in two absolute alcohol dishes
Putting in EA50 Solution for 90 seconds
Washing in three absolute alcohol dishes
Washing in Xylene four times
Share coverslip on the slides

Table 1 : Slide Staining Procedure

of this study. Repeated measure ANOVA tests were used to calculate statistical differences in degenerative nuclear abnormalities during pre and post exposure periods within these groups. The level of significance was set at  $p < 0.05$ . The data were computationally tested using SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA).

### Results

Micronucleus distribution analysis between two age groups had  $P=0.841$  ( $p < 0.05$ ) and was not showing any statistically significant differences. There was no significant difference in the frequency of micronuclei between two groups i.e., before and after radiography was  $P=0.548$  ( $p < 0.05$ ). Both panoramic and cephalometric radiographies had no genotoxicity effect in each age group. Evaluation of necrotic cell frequency between two age groups showed no significant difference in the frequency of necrotic cells between two groups i.e.,  $P=0.151$  ( $p < 0.05$ ). Evaluation of necrotic cells frequency before and after radiography in each age group was showing significant difference in necrotic cells frequency in each age group before and after radiography, i.e.,  $P=0.00$  ( $p < 0.05$ ). This result indicates that x- ray can induce necrosis and cause cytotoxic changes in buccal mucosal cells of adults and children.

### Discussion

The previous studies in literature investigated frequency of cell necrosis and DNA damage in buccal mucosal cells sampled from patients exposed to digital lateral radiography.<sup>1,2,8</sup> The present study results were similar to those observations that even though there were no significant difference in micronucleus frequency before and after radiation exposure exposure to dental x-ray increased other cytotoxicity associated ratio. In general digital lateral radiography does not cause chromosomal damages but can initiate cytotoxic changes in cells.<sup>1,2,8</sup> Similar study on DNA damage and cell death in buccal cells of children and adults reported that panoramic radiography does not cause chromosomal damage but can produce genotoxicity effects.<sup>3,9-11</sup> This study compared the pre and post

exposure effect of cephalometric and panoramic radiography between children and adults and similar study was not done in Iran. Cells are constantly exposed to internal and external harmful agents such as viruses and chemicals which can lead to changes in cells function and structure. These factors can cause cell necrosis or changes in nucleus genetic information.<sup>10</sup> X-ray is one of the electromagnetic forms that can cause changes in organisms.<sup>11,12</sup> Today, X-ray is widely used for diagnosis and treatment in medicine and dentistry. Children are also exposed to the radiation. Because the younger organs and tissues are more sensitive to radiation, this has raised concerns that whether radiation can cause genetic damage in children.

In this study we used cytology method to evaluate the x-ray effects on cells after panoramic and lateral cephalometric radiography. Results of this study showed X ray from panoramic and lateral cephalometric radiography in both age groups had no genotoxicity effect on cells but had cytotoxicity effects and could induce cell death. Present study results agree with Ribeiro<sup>2,13</sup> studies. Ribeiro in one study investigated cytological changes of panoramic radiography in children and adults and in other study he also investigated cytological changes of cephalometric radiography in adults. We investigated these changes after both panoramic and lateral cephalometric radiographies in both children and adults.

Popova<sup>9</sup> study results agree with the present study. In popova research the age range of the sample is extensive and this problem can affect results because increasing age is a risk factor for cellular changes. In our study all adults were in same age range. On the other hand oral mucosal epithelium by a renewal system keeps in homeostasis condition, which automatically compensates cell loss caused by abrasion. Control mechanism of this automatic system is complex and different factors like hormones affect it, so collected cells in one day are more reliable than cells were collected in three days.

Angelieri study results agree with present study. Of course in the Angelieri study radiation effects after panoramic radiographies in children were investigated and these effects were not compared with adults. Riberio investigated DNA damage and cell death after panoramic radiography in smoking and non-smoking healthy cases.<sup>14</sup> Riberio study results agree with our study. In present study confounding factors such as smoking excluded because only the radiation role be compared in two age groups. Angelieri investigated cytogenic effects of panoramic radiography in buccal mucosa and marginal surface of tongue in smoking and non- smoking adults.<sup>15</sup> Cerqueira<sup>16</sup> investigated genotoxicity effects of panoramic radiography in gingival epithelial cells and showed X ray increased genotoxicity in these cells that caused chromosomal damages. Micronucleus index reflects genomic instability. Diagnosis of micronucleus increased frequency in a population shows increased risk for cancer.<sup>17</sup> The damage because micronuclei formation happens in epithelial basal cells where mitosis is happens.<sup>18</sup> Epithelial cell turn over brings them to the surface thus most rates of micronuclei formation happens in mucosal separated

cells one to three weeks after genotoxic factors exposure. That is why, in this study cells were collected 10 days after x-ray exposure.<sup>19</sup> Necrotic cells (karyolysis, karyorexy, and pyknosis) were evaluated as an index for cell death (cytotoxicity).<sup>14,15,20</sup> Results of this study showed amounts of absorbed dose in dental radiation cannot cause genotoxicity changes but since repeating use of cytotoxic factors can result to chronic cell damages and degenerative changes and finally made appropriate field to neoplastic changes.<sup>20</sup>

### Conclusion

In conclusion, dental radiographs should be used only when absolutely necessary and every effort should be made to keep the dose to all individuals as low as possible.

### Authors Affiliations

1. Roza Haghgoo, DDS, Professor, Department of Pediatric Dentistry, Dental School, Shahed University, Tehran, Iran, 2.Reza Sedaghat, DDS, Assistant Professor, Department of Pathology, Medical School, Shahed University, Tehran, Iran, 3.Motahare Ahmadvand, DDS, Post Graduate Student, Department of Pediatric Dentistry, Dental School, Shahed University, Tehran, Iran, 4.Zahra Ogaghi, DDS, Dentist, Private Office, Tehran, Iran.

### References

- Ribeiro DA. Cytogenetic biomonitoring in oral mucosa cells following dental X-ray. *Dentomaxillofac Radiol.* 2012;41(3):181-4.
- Ribeiro DA, Sannomiya EK, Pozzi R, Miranda SR, Angelieri F. Cellular death but not genetic damage in oral mucosa cells after exposure to digital lateral radiography. *Clinical oral investigations.* 2011;15(3):357-60.
- Angelier F, de Oliveira GR, Sannomiya EK, Ribeiro DA. DNA damage and cellular death in oral mucosa cells of children who have undergone panoramic dental radiography. *Pediatric radiology.* 2007;37(6):561-5.
- Suk WA, Murray K, Avakian MD. Environmental hazards to children's health in the modern world. *Mutation Research/Reviews in Mutation Research.* 2003;544(2):235-42.
- Robbins SL, Kumar V, Abbas AK, Aster JC. *Robbins basic pathology: Elsevier Health Sciences;* 2012.
- McDonald RE, Avery DR, Dean JA. *Dentistry for the Child and Adolescent: CV Mosby;* 1969.
- Wang C-C. *Radiation therapy for head and neck neoplasms. Chicago, IL (USA): Year Book Medical Publishers;* 1990.
- Carlin V, Artioli AJ, Matsumoto MA, Borgo E, Oshima CTF, Ribeiro DA. Biomonitoring of DNA damage and cytotoxicity in individuals exposed to cone beam computed tomography. 2014.
- Popova L, Kishkilova D, Hadjidekova VB, Hristova RP, Atanasova P, Hadjidekova VV, et al. Micronucleus test in buccal epithelium cells from patients subjected to panoramic radiography. *Dentomaxillofacial Radiology* 2007;36:168-71.
- Sari-Minodier I, Orsiere T, Bellon L, Pompili J, Sapin C, Botta A. Cytogenetic monitoring of industrial radiographers using the micronucleus assay. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis.* 2002;521(1):37-46.
- White SC, Pharoah MJ. *Oral radiology: principles and interpretation: Elsevier Health Sciences;* 2013.
- Xu G-L, Bestor TH, Bourc'his D, Hsieh C-L, Tommerup N, Bugge M, et al. Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. *Nature.* 1999;402(6758):187-91.
- Ribeiro DA, De Oliveira G, De Castro GM, Angelieri F. Cytogenetic biomonitoring in patients exposed to dental X-rays: comparison between adults and children. *Dentomaxillofacial Radiology.* 2008;37(7):404-7.
- Ribeiro DA, Angelieri F. Cytogenetic biomonitoring of oral mucosa cells from adults exposed to dental X-rays. *Radiation medicine.* 2008;26(6):325-30.
- Angelier F, Moleirinho TdCG, Carlin V, Oshima CTF, Ribeiro DA. Biomonitoring of oral epithelial cells in smokers and non-smokers submitted to panoramic X-ray: comparison between buccal mucosa and lateral border of the tongue. *Clinical oral investigations.* 2010;14(6):669-74.
- Cerqueira EMM, Meireles JRC, Lopes MA, Junqueira VC, Gomes-Filho IS, Trindade S, et al. Genotoxic effects of X-rays on keratinized mucosa cells during panoramic dental radiography. *Dentomaxillofacial Radiology.* 2008;37(7):398-403.
- Baciuchka-Palmaro M, Orsiere T, Duffaud F, Sari-Minodier I, Pompili J, Bellon L, et al. Acentromeric micronuclei are increased in peripheral blood lymphocytes of untreated cancer patients. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis.* 2002;520(1):189-98.
- Stick HF, Rosin MP. Quantitating the synergistic effect of smoking and alcohol consumption with the micronucleus test on human buccal mucosa cells. *International Journal of Cancer.* 1983;31(3):305-8.
- King RJB, Robins MW. *Cancer biology.* 3rd ed. London: Pearson Education; 2006.
- Madhavan R, Kumaraswamy M, Kailasam S, Kumar SM. Genetic Damage in Exfoliated Cells from Oral Mucosa of Individuals Exposed to X-rays after Panoramic Radiograph: A Cross-sectional Study. *Journal of Indian Academy of Oral Medicine and Radiology.* 2012;24(2):102-5.

### How cite this article

Haghgoo R, Sedaghat R, Ahmadvand M, Ogaghi Z. Cytological Changes of oral mucosa following lateral cephalometry and panoramic radiograph. *International Journal of Dental Clinics.* 2014;6(3):1-3.

### Address for Correspondence

Motahare Ahmadvand, DDS,  
Post Graduate Student,  
Department of Pediatric Dentistry,  
Dental School, Shahed University,  
Tehran, Iran.  
Email: motahare.ahmadvand@yahoo.com

Source of Support: Nil

Conflict of Interest: None Declared