Genetic Damage in Exfoliated Cells from Oral Mucosa of Individuals Exposed to X-rays after Panoramic Radiograph: A Cross-sectional Study

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ABSTRACT

Objectives: In the past decades, X-rays have been used widely for diagnosis in dentistry. However, it is well known that ionizing radiation causes damage (including single- and double-strand breaks) to deoxyribonucleic acid (DNA) and DNA—protein crosslinks, and induces cellular death. Therefore, outlining the cytogenetic effects induced by X-ray is necessary to identify the degree of cancer risk and minimize potential risks to patients and clinicians.

Materials and methods: Cytogenetic biomonitoring studies focusing on oral mucosa cells in individuals exposed to dental X-ray were reviewed.

Results: Dental X-ray can induce DNA damage and cytotoxicity in oral mucosa cells.

Conclusion: These results will contribute to a better understanding of X-ray-induced effects upon the cellular system in individuals continually exposed to known genotoxic/cytotoxic agents.

Keywords: Micronuclei, Apoptosis, Oral mucosal changes, Nuclear alterations.


INTRODUCTION

X-rays are a potent mutagenic agent capable of inducing both gene mutations and chromosomal aberrations. They act directly on the DNA molecule or indirectly through the formation of reactive compounds that interact with this molecule. In spite of their mutagenic potential, this kind of radiation is an important tool for diagnosis. Panoramic dental radiography is been widely used to compliment clinical examinations and is considered less harmful than performing several periapical radiographs. As a result and because of inadequate in vivo evidence; the present study is aimed to investigate genetic damage from the exfoliated epithelial cells of oral mucosa in patients exposed to panoramic dental radiograph. To evaluate the magnitude of DNA damage and genetic effect from panoramic dental radiography, the micronucleus test is used.

According to Tobert et al (1991)\(^1\)\(^2\) the sensitivity of micronucleus test is increased by recording degenerative nuclear alterations indicative of apoptosis and necrosis, such as karyorrhexis, karyolysis, pyknosis, nuclear bud and condensed chromatin, in addition to micronucleus. In order to monitor cytotoxic effects, micronucleus, pyknosis, karyolysis, karyorrhexis, nuclear bud and condensed chromatin were evaluated in this study.

AIMS AND OBJECTIVES

To evaluate the possible genotoxic effect of radiation exposure after dental panoramic radiographs and to evaluate and score the apparent genetic damage to cells by identifying and scoring micronuclei, nuclear projections (buds and broken eggs) and degenerative nuclear alterations (condensed chromatin, karyolysis and karyorrhexis) immediately before and 10 days after exposure to OPG.

OBTAINING APPROVAL FROM THE AUTHORITIES

Permission from the ethical committee of the Ragas Dental College and Hospital was obtained before the starting of the study for examining and interpretation of patients. Also an informed consent was obtained from the patients forming the study sample, to participate in the study.

MATERIALS AND METHODS

The study population consisted of healthy individuals subjected to panoramic dental radiograph attending the Extra Oral Radiology Department in Ragas Dental College and Hospital. The study group comprised of 35 healthy individuals of both sexes who were submitted to panoramic dental radiographic examination in the age group of 18 to 65 years. Normal healthy individuals who were subjected to panoramic dental radiograph for various diagnostic purposes other than pathological conditions were included in the study. Satelec dental panoramic radiographic unit with specifications of 70 kV, 10 mA was used for obtaining panoramic radiograph.

Prior to the radiographic procedure buccal smear was obtained from both sides of the buccal mucosa through gentle scraping with a brush and it was smeared over clean
slides and were fixed in a methanol—acetic acid (3:1) solution for 15 to 30 minutes. Slides were air-dried for 10 minutes prior to staining. Slides were then treated in 5 M hydrochloric acid for 30 minutes and then washed in running tap water for 3 minutes. Slides were drained but not allowed to dry out before being treated in Schiff’s reagent in the dark for 60 minutes. Slides were washed in running tap water for 5 minutes and rinsed well in distilled water for 1 minute. Slides were stained for 30 seconds in 0.2% light green and rinsed well in distilled water for 2 minutes. Slides were allowed to air dry. Nuclei and micronuclei are stained magenta, while the cytoplasm appears green. Slides were scored using a light microscope.

CYTOLOGICAL ANALYSIS

Analysis was performed in a blind fashion in 2,000 cells. The scoring was done according to the criteria established by Tolbert et al (1991).

APPEARANCE OF CELLS

Normal differentiated cells: These cells have a uniformly stained nucleus that is usually oval or round in shape. They are distinguished from basal cells by their larger size and by a smaller nuclear to cytoplasmic ratio. No other DNA containing structures apart from the nucleus are observed in these cells.

Cells with micronuclei: Presence of both a main nucleus and one or more smaller nuclei. Micronuclei are round or oval in shape and their diameter may range between 1/3 and 1/16 the diameter of the main nucleus. Cells with micronuclei usually contain only one micronucleus.

MN SCORING CRITERIA

Fenech et al (2003):

- The diameter of the micronucleus should be less than one-third of the main nucleus.
- Micronucleus should be separated from or marginally overlap with main nucleus as long as there is clear identification of the nuclear boundary.
- Micronucleus should have similar staining as the main nucleus.

Condensed chromatin: Cells have nuclei with regions of condensed or aggregated chromatin exhibiting a speckled or striated nuclear pattern. In these cells, it is apparent that chromatin is aggregating in some regions of the nucleus while being lost in other areas.

Karyorrhectic cell: Cells are characterized by the more extensive appearance of nuclear chromatin aggregation (relative to condensed chromatin cells) leading to fragmentation and eventual disintegration of the nucleus.

Pyknotic cell: Cells are characterized by a small shrunken nucleus, with a high density of nuclear material that is uniformly but intensely stained. The nuclear diameter is usually one- to two-thirds of a nucleus in normal differentiated cells.

Karyolytic cell: Nucleus is completely depleted of DNA and apparent as a ghost-like image that has no Feulgen staining. These cells thus appear to have no nucleus.

Cells with nuclear bud: Nuclei has an apparent sharp constriction at one end of the nucleus suggestive of a budding process, i.e. elimination of nuclear material by budding. The nuclear bud and the nucleus are usually in very close proximity and are apparently attached to each other.

Statistical analysis were obtained using paired/matched/ dependent t-test.

RESULTS

In our study the total number of micronucleus before exposure was 39 (0.06%) and postexposure was 41 (0.06%), number of karyorrhexis before exposure were 2,125 (3.04%) and post exposure were 2,915 (4.16%), the number of condensed chromatin found before exposure were 1,650 (2.36%) and postexposure were 2,915 (3.48%), the number of pyknosis found before exposure were 429 (0.61%) and postexposure were 516 (0.74 %), the number of karyolysis found before exposure were 37 (0.04%) and postexposure were 46 (0.07%), the number of broken eggs found before exposure were 37 (0.05%) and postexposure were 46 (0.06%), the number of nuclear buds found before exposure were 170 (0.24%) and postexposure were 193 (0.28%) and the number of nuclear projections found before exposure were 207 (0.24%) and postexposure were 260 (0.37%).

On comparison between the pre-exposure and post-exposure values among females the p-value for karyorrhexis was 0.001, condensed chromatin p = 0.001 pyknosis p = 0.01, and broken eggs p = 0.006 these values are statistically significant before and after exposure indicative of cytotoxicity. The p-value for micronuclei was 0.33, karyolysis p = 0.16, nuclear buds p = 0.57 and nuclear projections p = 0.59. These values are statistically not significant before and after exposure. In contrast to males, in females the process of karyolysis is insignificant but this cannot be confirmed due to less number of sample size.
On comparison of the pre-exposure values between males and females, the p-value for micronuclei was 0.64, karyorrhexis p = 0.51, condensed chromatin p = 0.81, pyknosis p = 0.16, karyolysis p = 0.16, broken eggs p = 0.58, nuclear buds p = 0.71 and nuclear projections p = 0.81. These values are statistically not significant between males and females which indicates that sex does not influence the formation of nuclear anomalies.

On comparison of the postexposure values between males and females the p-value for micronuclei was 0.71, karyorrhexis p = 0.25, condensed chromatin p = 0.38, pyknosis p = 0.27, karyolysis p = 0.40, broken eggs p = 0.30, nuclear buds p = 0.40 and nuclear projections p = 0.40. These values are statistically not significant between males and females which indicates that sexually there is no difference between males and females in formation of nuclear anomalies after low dose radiation exposure.

**DISCUSSION**

Biomonitoring studies of populations exposed to X-rays are quite difficult and rather specific because each population is exposed to different doses of radiation. This could explain why some studies find an increase of genetic damage in populations exposed to X-rays. Despite the lack of micronuclei formation, the results demonstrated that panoramic dental radiography was able to induce cellular death and cytotoxicity as depicted by statistically significant differences between values before and after X-ray exposure. Also in the postexposure period, a significant higher number of nuclear alterations characterized by disruption of nuclear contour and chromatin shrinkage was seen, which may result from cytotoxicity.

Different laboratories have reported variable normal background micronucleus frequency in human oral epithelial cells: 0.16% (Tolbert et al 1991), 0.04% (Karhalil et al 1999), 0.1 to 0.3% (Fenech et al 1999) and 0.08% (Burgaz et al 1999). In our study, the occurrence of micronucleus frequencies were not altered before and after exposure with p-value of 0.54 and is statistically insignificant which states that panoramic dental radiography does not produce chromosomal alterations.

The presence of karyorrhexis, condensed chromatin and pyknosis was increased after the exposure with a p-value of 0.01 which is statistically significant, which is indicative of apoptosis. The presence of karyolysis were increased after the exposure with a p-value of 0.009 which is statistically significant suggesting that the cellular response to X-rays produce a cytotoxic effect which may lead to necrosis. The presence of broken eggs was increased after the exposure with a p-value of 0.001 which is statistically significant and should be considered as genotoxicity biomarker. The presence of nuclear buds was increased after the exposure with a p-value of 0.31 which is statistically not significant and is indicative of normal epithelial differentiation. The presence of nuclear projections was increased after the exposure with a p-value of 0.15 though not statistically significant it is indicative of mild cellular damage due to radiation. This is similar to the results obtained by Cerqueira et al (2004), Angeliieri et al (2007), Da silva et al (2007), Cerqueira et al (2008), Ribeiro, Angeliieri (2008), Ribeiro et al (2008), Popova et al (2007) in a similar study.

On comparison between pre-exposure and postexposure values among males and among females karyorrhexis, condensed chromatin, pyknosis karyolysis and broken eggs values are statistically significant before and after exposure. On comparison of the pre-exposure and postexposure values between males and females, the values are statistically not significant between males and females which indicates that sex does not influence the formation of nuclear anomalies. A similar study was done by Ribeiro (2012) and Carlin (2010) in cone beam computed tomography which also produced similar results.

Taking into consideration that activated histone 2AX (γ-H2AX) and activated checkpoint kinase 2 (pChk2) are DNA damage response molecules produced in irradiated cells, these proteins are signature molecules of radiation exposure. A recent study conducted by Yoon et al (2009) revealed high expression levels of pChk2 and γ-H2AX in oral cells after radiation exposure. This suggests that pChk2 and γ-H2AX may serve as sensitive indicators of low-dose radiation exposure.

**CONCLUSION**

According to the results from this investigation, exposure to X-rays during panoramic radiography induces genotoxic effects in oral mucosal buccal epithelial cells that increase chromosomal damage and induce apoptosis. Thus, panoramic dental radiography should be requested only when necessary because it cannot be considered a risk-free procedure. In some cases the cells with nuclear anomalies were greater before X-rays suggesting that they may be associated with normal process of cell differentiation. Repeated exposure to cytotoxicants can result in chronic cell injury, compensatory cell proliferation, hyperplasia and, ultimately, tumor development. The risks associated with dental radiographs are small but should not be overlooked. Since, cellular death is considered to be a prime mechanism in nongenotoxic mechanisms of carcinogenesis, dental X-rays should be used only when necessary. Panoramic radiography should be carefully performed in order to avoid retakes and increase in radiation doses. More frequent, both as substitute for and as a complement to intraoral...
radiographs, their indication should always follow the concept of maximum benefit with minimum risk.

REFERENCES


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