Genotoxic and cytotoxic effects of X-ray on buccal epithelial cells following panoramic radiography: A pediatric study

ABSTRACT

Background: Ionizing radiation is a potent mutagenic agent capable of inducing both mutation and chromosomal aberrations. Non-lethal doses of ionizing radiation may induce genomic instability favoring carcinogenesis. In spite of their mutagenic potential, this kind of radiation is an important tool for diagnosis of the disease and is used in medical and dental practice. It has been believed that the number of micronucleus and increased frequency of other nuclear alterations, including karyorrhexis, condensed chromatin and pyknosis, are related to the increasing effects of carcinogens. Many approaches and techniques have been developed for the monitoring of human populations exposed to various mutagens, but the analysis of micronuclei (MN) has become a standard approach for the assessment of chromosomal damage in human populations.

Aim: To assess the effects of radiation exposure from panoramic radiography on the buccal epithelial cells (BECs) of pediatric patients.

Materials and Methods: The study included 20 pediatric patients who had to undergo panoramic radiography for further dental treatment. Exfoliated BECs were obtained and examined immediately before and 10 days after radiation exposure. The cells were stained using rapid Papanicolaou (PAP) kit. Evaluation for MN and nuclear alterations was carried out by an oral pathologist and data were statistically analyzed using the “t” test.

Results: The mean number of MN in the BECs before exposure of pediatric patients to panoramic radiography was 4.25 and after exposure was 4.40. This difference was not found to be statistically significant (P<0.0001). However, the mean nuclear alterations of 8.70 and 15.75 before and after exposure were statistically significant (P<0.0001).

Conclusion: Panoramic radiographs can induce cytotoxicity but not genotoxic effects in buccal mucosal cells. Hence, dental radiographs should be prescribed only when deemed indispensable.

Key words: Cytotoxicity; ionizing radiation; micronuclei; panoramic radiography

Introduction

Radiation is indispensable in modern medicine. Radiographic examination is one of the principal diagnostic methods used in all fields of medical and dental services. The risk associated with low-level diagnostic exposures could be expected to be low but greater than zero. Panoramic radiography is widely used to complement clinical examination and is considered less harmful than performing several periapical radiographs. Because the estimated risk from diagnostic radiation exposure for the child patient is twice that of the adult, pediatric dental radiography was a particular focus of concern.

Genomic damage is one of the most important fundamental causes of developmental and degenerative disease. It is thought to be produced by environmental exposure...
to genotoxins, medical procedures (e.g., radiation and chemicals), micronutrient deficiency (e.g., folate), lifestyle factors (e.g., alcohol, smoking, drugs and stress) and genetic factors.\[4\] It is essential to have reliable and relevant minimally invasive biomarkers to improve the implementation of biomonitoring, diagnostics and treatment of diseases caused by, or associated with, genetic damage. The micronucleus assay in exfoliated buccal cells is potentially an excellent candidate to serve as such a biomarker.\[5\]

During panoramic radiography, buccal epithelial cells (BECs) are a primary target for radiation and can therefore be used for monitoring human exposure.\[6,7\] The basal epithelial layer cells, which may or may not contain micronucleus, eventually differentiate into the prickle cell layer and the keratinized superficial layer and then exfoliate into the buccal cavity. Some of these cells may degenerate into cells with condensed chromatin, fragmented nuclei (karyorrhectic cells) or pyknotic nuclei, or completely lose their nuclear material (karyolytic). In rare cases, some cells may be blocked in a binucleated stage or may exhibit nuclear buds (broken eggs), a biomarker of gene amplification. These biomarkers of genome damage and cell death can be observed in buccal cell systems and thus provide a more comprehensive assessment of genome damage than only micronucleus in the context of cytotoxicity and cytostatic effects.\[4\] It is important to stress that cytotoxicity interferes with micronucleus induction as some micronuclei (MN) are inevitably lost after cytotoxic insult therefore confirming the lack of a mutagenic effect induced by X-rays. Nevertheless, it has been postulated that repeated exposure to cytotoxicants can result in chronic cell injury, compensatory cell proliferation, hyperplasia and, ultimately, tumor development.\[9\]

Increase in the frequency of MN does not necessarily indicate the formation of pre-neoplastic lesion or carcinoma but reveals the genotoxicity of carcinogens and may indicate an elevated probability for the formation of particular chromosome changes, which in turn can lead to transformation into lesions.\[5\] Therefore, the micronucleus test was used in the present study to assess the genotoxicity in exfoliated buccal cells of children exposed to panoramic radiography.

Materials and Methods

The study group consisted of 20 pediatric patients who had to undergo panoramic radiography for further dental treatment. Panoramic dental radiographs were obtained using the Kodak 8000 C system with the following parameters: 65-90 kV, 15 mA, 13 s, 110 mGy cm and effective dose 21.4 mSv. The parents of the participants answered a questionnaire before their X-ray examination. The main characteristics gathered were age, gender, regular use of oral antiseptic solutions, use of prostheses, previous exposure to dental radiography and use of antibiotics or any other chemical substance. Exclusion criteria comprised children with damages in the oral mucosa that preclude the collection of cells, regular use of oral antiseptics, radiographic examination in the past 6 months, recent use of antibiotics and whose parents had not signed the consent form or did not want to participate in the study.

Children were asked to rinse their mouth thoroughly with normal water. Exfoliated buccal mucosa cells were obtained by scraping the right/left buccal mucosa with a wooden spatula immediately before the X-ray exposure and 10 days after exposure. Cells were smeared over a clean glass slide and spread over a large area, preventing clumping of cells. The slides were immediately preserved with Biofix™ spray fixative and stained using Rapid PAP™ (Biolab Diagnostic Private Limited, Tarapur, Maharashtra). All the slides were observed under an optical microscope and the micronucleus count [Figure 1] was carried out by an oral pathologist, scoring 1000 cells on each slide using the criteria described by Sarto et al.\[9\] Nuclear alterations were considered: Pyknosis, karyolysis [Figure 2] and karyorrhexis [Figure 3], according to the criteria used by Tolbert et al.\[10\]

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences version 17 (SPSS). The paired “t” test was used to determine the significance of difference between micronucleated cell frequencies and micronucleus before

Figure 1: Photomicrograph showing micronuclei (Rapid Pap, ×400)
and after exposure to radiation. Descriptive statistics was performed and statistical inference was made by the t test.

**Results**

The mean number of MN before exposure was 4.25, which was found to increase to 4.40 after exposure. Statistical analysis showed no significant difference in the number of MN in the BECs ($P > 0.001$). However, a statistically significant increase in the number of other nuclear alterations after X-ray exposure was observed, as shown by the overall combined frequency of karyorrhexis, pyknosis and karyolysis ($P < 0.001$), with a mean of 8.70 and 15.75 before and after exposure, respectively. The standard deviation for MN was found to be 1.51 and 1.76 and for nuclear alterations was 2.45 and 3.95 before and after exposure, respectively [Table 1].

**Discussion**

Human biomonitoring has become a central tool in environmental and occupational medicine and also in research for the identification, control and prevention of population exposure to potentially harmful compounds.[11] So far, a variety of assays have been proposed in biomonitoring studies, including those that assess metaphase chromosomal aberrations, sister chromatid exchanges, DNA damage and host cell reactivation.[12] However, these methods are typically laborious and time-consuming or require highly trained technicians to accurately read and interpret slides. For these reasons, the application of the micronucleus test to uncultured exfoliated cells has been greeted enthusiastically.[13] The key advantage of the micronucleus assay is the relative ease of scoring, the limited costs, time efficiency and the precision obtained from scoring larger numbers of cells.[14]

Currently, two methods are used to perform the assay. In the original method proposed by Countryman and Heddle,[15] micronucleus test underestimates micronucleus frequency when nuclear division is inhibited or when cells are allowed sufficient time to divide more than once. To improve the in vitro micronucleus assay, Fenech[16] proposed that micronucleus should only be scored in cells that had completed one nuclear division, both to obtain an accurate estimate of spontaneous micronucleus frequency as well as a reliable estimate of micronucleus induced by radiation or chemicals. Scoring of micronucleus is usually performed in peripheral blood lymphocytes, but the micronucleus can also be relatively easily scored in other cell types relevant to human biomonitoring such as fibroblasts, exfoliated BECs and in erythrocytes.[2]

Buccal cells are the first barrier for the inhalation or ingestion route and are capable of metabolizing proximate carcinogens to reactive products. Approximately 90% of human cancers originate from epithelial cells. Therefore, it could be argued that oral epithelial cells represent a preferred target site for early genotoxic events induced by carcinogenic agents entering the body via inhalation and ingestion.[17] The advantages with BEC are that they can be easily and rapidly sampled, do not have to be cultivated and do not require stimulation or metaphase preparations and have limited DNA repair capacity relative to peripheral blood lymphocytes; therefore, they may more accurately reflect genomic instability events in epithelial tissues.[2]
In the present study, a 10-day interval was taken because chromosomal damage leading to micronucleus formation occurs in dividing cells from the basal layer of the oral epithelium but is only observed later in exfoliated cells after the differentiation. Rapid turnover of epithelial tissues brings the cells to the surface, where they exfoliate. As a result, the maximal rate of MN formation in exfoliated cells is seen between 1 and 3 weeks after exposure to the genotoxic agent.\[7,18\] Analysis of micronucleus was carried out according to the criteria given by Tolbert et al.,[11] and Sarto et al.,[9] Cytotoxic effects like pyknosis, karyolysis and karyorrhexis were also studied as their inclusion in assessment increases the sensitivity of biomonitoring studies.\[10,19\]

Micronucleated cell indices may reflect genomic instability.\[20\] The mean prevalence of cells with micronucleus in the general population is 0.0-0.9%. Any different range of MN can be the result of chromosomal alternations. The micronucleus is the result of genomic damage to the cells. It has been believed that the number of MN is related to increasing the effects of carcinogens.\[21\]

The results of this study showed that there was no statistically significant increase in MN following radiation exposure from panoramic radiography [Table 1]. However, radiation did lead to other nuclear alterations closely related to cytotoxicity, including karyorrhexis, pyknosis and karyolysis: There were statistically significant differences ($P < 0.001$) before and after radiation exposure [Table 1]. Nevertheless, it has been postulated that repeated exposure to cytotoxicants can result in chronic cell injury, compensatory cell proliferation, hyperplasia and, ultimately, tumor development.\[9\]

The variations between studies with respect to MN are difficult to interpret clinically due to complex interactions between the environment and the genotype within the matrix of growth dynamics, development and adaptation. In such studies, it is important to consider some confounding factors like viruses, alterations in the immune system, failures in the DNA repair system, etc. Cell proliferation may vary by age and in different cell types.\[22\]

This information is important to the timing of the cell collection to assess exposure of genotoxicity events. Furthermore, increased DNA damage and decreased DNA repair capacity have been found in children suffering from malnutrition, a common problem in developing countries.\[23\] The population characteristics and methodological aspects like differences in sites, collection of cells, fixing techniques, various staining procedures, number of cells counted and scoring criteria for micronucleus, etc. may affect the results.\[9\]

Biomonitoring studies of populations exposed to some radiographic methods are quite difficult and rather specific because each population is exposed to different doses of radiation.\[24\]

This could explain why some studies find an increase in genetic damage in populations exposed to radiographic procedures.

**Conclusion**

In conclusion, the results of the present study suggest that panoramic radiographs can induce cytotoxic but not mutagenic effects in the oral mucosal cells. Hence, dental X-rays should be prescribed only when deemed indispensable. Furthermore, greater emphasis should be given to methods that detect the genotoxic human activity. The biomarkers may be used in the future for the prevention of serious diseases and detection of high-risk patients.

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