

Cellular death but not genetic damage in oral mucosa cells after exposure to digital lateral radiography

Daniel A. Ribeiro · Eduardo K. Sannomiya ·
Renan Pozzi · Sandra R. Miranda · Fernanda Angelieri

Received: 31 December 2009 / Accepted: 4 March 2010 / Published online: 25 March 2010
© Springer-Verlag 2010

Abstract The aim of the present study was to evaluate DNA damage (micronucleus) and cellular death (pyknosis, karyolysis, and karyorrhexis) in exfoliated buccal mucosa cells from individuals following digital lateral radiography. A total of 30 healthy patients (15 men and 15 women) indicated to the orthodontic therapy were submitted to digital lateral X-ray. Exfoliated oral mucosa cells were collected immediately before the X-ray exposure and after 10 days. The results pointed out no significant statistically differences ($p > 0.05$) of micronucleated oral mucosa cells. On the other hand, X-ray was able to increase other nuclear alterations closely related to cytotoxicity such as karyorrhexis, pyknosis, and karyolysis. In summary, these data indicate that exposure to digital lateral radiography may not

be a factor that induced chromosomal damage, but it is able to promote cytotoxicity.

Keywords Buccal mucosa cells ·
Digital lateral radiograph · Micronucleus test

Introduction

Radiology is a fundamental method for diagnosis and planning in dentistry. Investigations on this issue have primarily addressed the reduction in the radiation dose to the patient and improvement in definition of the radiographic image [1]. In this regard, there are many motivations for radiologists to introduce digital radiography systems. One major concern of radiologists is of course obtaining better image quality. A second interesting feature is the separation of the image acquisition from the image processing step [2]. From an organizational point of view, the capability to store images electronically in a picture archiving and communications system is most interesting to both radiologists and patients. Furthermore, digital radiography systems allow acceleration of patient throughput by different means, i.e., less data typing, shorter time to image, and no cassette manipulation for some systems [2].

Radiation cytogenetics, which is defined as the study of radiation-induced chromosome alterations, goes back more than 70 years and has continued to contribute to our understanding of the fundamental genetic and molecular bases of the biological effects of radiation, such as mutation, cell killing, and carcinogenesis [3]. Biophysical arguments and mathematical modeling of aberration induction have stimulated the development of radiobiological theories, including the nature of the effects in response to the quality and quantity of radiation [3]. To date, a variety

D. A. Ribeiro · S. R. Miranda
Departamento de Patologia, Universidade Federal de Sao Paulo,
São Paulo, Brazil

D. A. Ribeiro (✉) · R. Pozzi
Departamento de Biociências,
Universidade Federal de São Paulo—UNIFESP,
Av. Ana Costa 95,
11060-001 Santos, São Paulo, Brazil
e-mail: daribeiro@pesquisador.cnpq.br
e-mail: daribeiro@unifesp.br

E. K. Sannomiya
Department of Dentomaxillofacial Radiology,
São Paulo Methodist University, UMESP,
Sao Bernardo do Campo,
São Paulo, Brazil

E. K. Sannomiya · F. Angelieri
Department of Orthodontics, São Paulo Methodist University,
UMESP, Sao Bernardo do Campo,
São Paulo, Brazil

of assays has been idealized, including those that assess metaphase chromosomal aberrations, sister chromatid exchanges, and host cell reactivation. However, these methods are typically laborious and time consuming or require highly trained technicians to accurately read and interpret slides. For this purpose, a great deal of enthusiasm was raised by the application of the micronucleus test to uncultured exfoliated cells [4]. Micronucleus arises from acentric fragments or whole chromosomes which are not included into the main nuclei of the daughter cells. The formation of micronuclei can be induced by substances that cause chromosome breakage (clastogens) as well as by agents that affect the spindle apparatus (aneugens) [5]. As a result, the present study was undertaken to investigate the frequencies of micronucleated cells in oral mucosa from individuals submitted to digital lateral radiography. To monitor cytotoxic effects, pyknosis, karyolysis, and karyorrhexis were also evaluated in this setting. Certainly, such data will contribute to a better understanding on the outcomes induced by digital radiographic documentation upon cellular system.

Material and methods

Subjects

The sample was composed of 30 digital lateral radiographies of 15 females and 15 males aged 20–23 years. These individuals were patients before the onset of orthodontic treatment at the post graduate Orthodontics Clinic of São Paulo Metodista Dental School. The digital lateral radiographies were obtained on a panoramic radiographic machine, Rotography Plus (Dabi Atlante, Ribeirão Preto, Brazil) with exposure time of 0.7–0.8 s, set at 70–80 KVp and 10 mA, with 24×30-cm cassette, on phosphor plate (Fuji Medical, Tokyo, Japan). The entrance dose was 0.046 R. Immediately after achievement of the latent image, reading was performed in a phosphor plate reader, FCR XG1 (Fuji Medical, Tokyo, Japan) and processed in a Dell Precision 490 workstation with a Xeon Dell Core processor (Dell computer, Taiwan, China).

Informed consent was obtained from the individuals included in the study or from their parents. All individuals were nonsmokers. All procedures adopted in this study were approved by the Institutional Ethics Committee of the Methodist University, UMESP, São Bernardo do Campo, São Paulo, Brazil.

Micronucleus test in oral mucosa cells

Exfoliated oral mucosa cells 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, centrifuged (800 rpm) during 5 min, fixed in 3:1 methanol/acetic acid, and dropped onto precleaned slides. Later, the air-dried slides were stained using the Feulgen/Fast Green method and examined under a light microscope at 400× magnification to determine the frequency of micronucleated cells as described elsewhere [5]. Two thousand cells were scored from each patient for each sampling time (before and after X-ray exposure).

Data analysis

Micronuclei were scored according to the criteria described by Sarto et al. [6] as a parameter of DNA damage (mutagenicity). Micronucleus was identified under the following conditions: intact main nucleus and cytoplasm; diameter 1/3 of the main nucleus; same staining and texture as the main nucleus; and micronuclei were in the same focal plane as the main nucleus [6]. For cytotoxicity, the following nuclear alterations were considered: pyknosis, karyolysis, and karyorrhexis. Results were expressed in percentage. Such analysis was established in a previous study conducted by our research group [7].

Statistical methods

The Wilcoxon's test for dependent samples was used to compare the frequencies of micronuclei and other cellular alterations among the samples before and after X-ray exposure using SigmaStat software, version 1.0 (Jandel Scientific, USA). The level of statistical significance was set at 5%.

Results

Table 1 shows the frequencies of micronucleated cells in patients undergoing digital lateral radiographies. Before X-ray exposure, the mean frequency of micronucleated cell was 0.3%. No significant statistical differences ($p>0.05$) were noticed after X-ray exposure. On the other hand, an increase of other nuclear alterations was observed after radiography exposure as depicted by the frequency of karyorrhexis, pyknosis, and karyolysis. These data are summarized in Table 1.

In order to compare all data with accuracy, all patients included in this study were nonsmokers. The daily alcohol consumption drunk was not considered in this study because recall bias phenomenon has occurred [8].

Table 1 Frequency of micronucleated cells (MNC) and other nuclear alterations (karyorrhexis, pyknosis, and karyolysis) in orthodontic patients undergoing digital lateral radiography

Groups	MNC (%)		Other nuclear alterations ^a (%)	
	No. of individuals	Mean±SD	No. of individuals	Mean±SD
Prior to X-ray exposure	30	0.3±0.1	30	6.5±4.2
After X-ray exposure	30	0.2±0.1	30	15.7±5.2 ^b

^a Karyorrhexis, pyknosis, and karyolysis

^b $p < 0.05$ when compared to individuals prior to X-ray exposure

Discussion

The aim of this study was to employ the micronucleus test to assess genetic damage and/or cellular death in individuals submitted in digital lateral radiography. To the best of our knowledge, the approach has not been addressed in the literature before.

Micronucleus assay in exfoliated buccal mucosa cells has been systematically used in genetic biomonitoring of populations exposed to several genotoxic chemicals, such as ethylene oxide, pesticides, and petroleum derivatives [8–10]. The key advantage of the micronucleus assay is the relative ease of scoring, the limited costs and person-time required, and the precision obtained from scoring larger numbers of cells. Damages that lead to the formation of micronuclei takes place 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 exfoliate. As a result, the maximal rate of micronuclei formation in exfoliated cells is seen between 1 and 3 weeks after exposure to the genotoxic agent [11]. For this reason, exfoliated oral mucosa cells were collected immediately before the X-ray exposure and after 10 days.

Micronucleated cell indexes may reflect genomic instability [12]. The detection of an elevated frequency of micronuclei in a given population indicates increased risk of cancer [13]. DNA double-strand breaks are a major threat to the genomic integrity of a cell. They can result in cell death if left unrepaired or if incorrectly repaired, can produce chromosomal aberrations, and are thought to induce cancer [14]. DNA double-strand breaks are induced by ionizing radiation, a range of chemotherapeutic drugs, and are formed endogenously during DNA replication or as initiators of programmed genetic rearrangement processes that occur during lymphocyte differentiation and meiosis [14]. It was surprising that the micronucleus frequencies were not significantly different before and after digital X-ray exposure in this trial. Such findings are fully in line with other authors [15–17]. Conversely, some authors have reported higher rates of cytogenetic damage induced by X-ray [18]. Biomonitoring studies of populations exposed

to X-ray 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-ray. Based on the results found, we postulated the lack of clastogenic and/or aneugenic effects related to digital lateral X-ray exposure in healthy individuals.

To monitor cytotoxic effects, the frequencies of karyorrhexis, karyolysis, and pyknosis were evaluated into this experimental design. Despite the lack of cytogenetic damage, our results demonstrated that lateral digital radiography were able to induce cellular death as depicted by statistically significant differences ($p < 0.05$) between values before versus after X-ray exposure. Analogous results were described by others [17, 18]. Exposure of eukaryotic cells to ionizing radiations is known to affect the normal progression through G1, S, and G2 phases of cell cycle [19]. In the recent years, the major molecular players taking part in pathways responsible for causing cell cycle delay have been identified. Taken as a whole, such results support the notion that X-ray was a cytotoxic agent. It is important to stress that cytotoxicity interferes with micronucleus induction since some micronucleated cells are inevitably lost after cytotoxic insult, confirming, therefore, to the lack of mutagenic effect induced by X-ray. Nevertheless, it has been postulated that repeated exposure to cytotoxicants can result in chronic cell injury, compensatory cell proliferation, hyperplasia, and ultimately tumor development [20]. In fact, a correlation between cell proliferation and induction of cancer is assumed [21]. Probably, proliferation may increase the risk of mutations within target cells and also be important in selective clonal expansion of (exogenously or endogenously) initiated cells from preneoplastic foci and eventually tumors [21].

In human cytogenetic studies, some confounding factors are important to be considered. Viruses, alterations in the immune system, failures in DNA repair system, and interindividual variations have already been associated with increased frequencies of chromosome aberrations [22]. Furthermore, an age-related increase of micronuclei has been postulated [22]. All participants of this had similar age

(ranging from 20 to 23 years old). Moreover, the influence of tobacco smoke has also been usually considered as relevant confounding factors [6]. Thus, all adults recruited to participate in this study were nonsmokers. Particularly, the mutagenic potential of alcohol is controversial and quite complicated to interpret using micronucleus assay in exfoliated cells. For example, in two reports, almost all participants consumed alcohol and tobacco; therefore, the influence of the individual factors could not be elucidated [23]. In another study, no genotoxic effect of alcohol was found [6]. In a study by Stich and Rosin [24], where the effects of alcohol consumption, cigarette smoking, and a combination of the two were examined, a synergistic effect of alcohol and nicotine was observed, while none of the two drugs alone caused an elevation of micronuclei frequencies.

In conclusion, the results of the present study suggest that digital lateral radiography is able to induce cytotoxicity but not genetic effects in oral mucosa cells. Therefore, radiographies should be used only when necessary. Further studies are necessary to confirm these findings.

Acknowledgments This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant number: 07/01228-4). Daniel A. Ribeiro is a recipient of the CNPq fellowship.

References

- Schmidt LD, Lima TC, Chinelatto LE, Bramante CM, Garcia RB, Moraes IG, Bernardinelli N (2008) Comparison of radiographic measurements obtained with conventional and indirect digital imaging during endodontic treatment. *J Appl Oral Sci* 16:167–170
- Kotter E, Langer M (2002) Digital radiography with large-area flat-panel detectors. *Eur Radiol* 12:2562–2570
- Sasaki MS (2009) Advances in the biophysical and molecular bases of radiation cytogenetics. *Int J Radiat Biol* 85:26–47
- Stich HF, Parida BB, Brunnemann KD (1992) Localized formation of micronuclei in the oral mucosa and tobacco-specific nitrosamines in the saliva of "reverse" smokers, Khaini-tobacco chewers and gudakhu users. *Int J Cancer* 21:172–176
- Belien JA, Copper MP, Braakhuis BJ, Snow GB, Baak JP (1995) Standardization of counting micronuclei: definition of a protocol to measure genotoxic damage in human exfoliated cells. *Carcinogenesis* 16:2395–2400
- Sarto F, Finotto S, Giacomelli L, Mazzotti D, Tomanin R, Levis AG (1987) The micronucleus assay in exfoliated cells of the human buccal. *Mutagenesis* 2:11–17
- Angelier F, de Oliveira GR, Sannomiya EK, Ribeiro DA (2007) DNA damage and cellular death in oral mucosa cells of children who have undergone panoramic dental radiography. *Pediatr Radiol* 37:561–565
- Martins RA, Gomes GA, Aguiar O Jr, Ribeiro DA (2009) Biomonitoring of oral epithelial cells in petrol station attendants: comparison between buccal mucosa and lateral border of the tongue. *Environ Int* 35:1062–1065
- Sarto FR, Tomanin L, Giacomelli G, Iannini G, Cupiraggi AR (1990) The micronucleus assay in human exfoliated cells of the nose and mouth: application to occupational exposures to chronic acid and ethylene oxide. *Mutat Res* 244:345–351
- Pastor S, Gutierrez S, Creus A, Cebulska-Wasilewska A, Marcos R (2001) Micronuclei in peripheral blood lymphocytes and buccal epithelial cells of Polish farmers exposed to pesticides. *Mutat Res* 495:147–156
- Majer BJ, Laky B, Knasmuller S, Kassie F (2001) Use of the micronucleus assay with exfoliated epithelial cells as a biomarker for monitoring individuals at elevated risk of genetic damage and in chemoprevention trials. *Mutat Res* 489:147–172
- Maluf SW, Erdtmann B (2001) Genomic instability in Down syndrome and Fanconi anemia assessed by micronucleus analysis and single cell-gel electrophoresis. *Cancer Genet Cytogenet* 124:71–75
- Ribeiro DA, Grilli DG, Salvadori DM (2008) Genomic instability in blood cells is able to predict the oral cancer risk: an experimental study in rats. *J Mol Histol* 39:481–486
- Jackson SP (2002) Sensing and repairing DNA double-strand breaks. *Carcinogenesis* 23:687–696
- Cerqueira EM, Gomes-Filho IS, Trindade S, Lopes MA, Passos JS, Machado-Santelli GM (2004) Genetic damage in exfoliated cells from oral mucosa of individuals exposed to X-rays during panoramic dental radiographies. *Mutat Res* 562:111–117
- Popova L, Kishkilova D, Hadjidekova V, Hristova R, Atanasova P, Hadjidekova V, Ziya D, Hadjidekov V (2007) Micronucleus test in buccal epithelium cells from patients subjected to panoramic radiography. *Dentomaxillofac Radiol* 36:168–171
- Ribeiro DA, de Oliveira G, de Castro G, Angelier F (2008) Cytogenetic biomonitoring in patients exposed to dental X-rays: comparison between adults and children. *Dentomaxillofac Radiol* 37:404–407
- He JL, Chen LF, Jin LF, Jin HY (2000) Comparative evaluation of the in vitro micronucleus test and the comet assay for the detection of genotoxic effects of X-ray radiation. *Mutat Res* 469:223–231
- Blakely EA, Kronenberg A (1998) Heavy-ion radiobiology: new approaches to delineate mechanisms underlying enhanced biological effectiveness. *Radiat Res* 150:S126–S145
- Swenberg JA (1993) Cell proliferation and chemical carcinogenesis: conferences summary and future directions. *Environ Health Perspect* 101:153–158
- Mally A, Jagetia JK (2002) Non-genotoxic carcinogens: early effects on gap junctions, cell proliferation and apoptosis in the rat. *Toxicology* 180:233–248
- Xu GL, Bestor HH, Bourc'his D, Hsieh CL, Tommerup N, Bugge M, Hulten M, Qu X, Russo JJ, Viegas-Pequignot E (1999) Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. *Nature* 402:187–191
- Burgaz S, Işcan A, Büyükbıngöl ZK, Bozkurt A, Karakaya AE (1995) Evaluation of micronuclei in exfoliated urothelial cells and urinary thioether excretion of smokers. *Mutat Res* 335:163–169
- Stich HF, Rosin MP (1983) Quantitating the synergistic effect of smoking and alcohol consumption with the micronucleus test on human buccal mucosa cells. *Int J Cancer* 31:305–308