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RESEARCH

Mutagenicity and cytotoxicity assessment in patients undergoing orthodontic radiographs

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Objectives: 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 radiography.
Methods: Lateral and frontal cephalometric X-ray and panoramic dental X-rays were taken of a total of 18 healthy patients (6 male and 12 female) referred for orthodontic therapy. Exfoliated oral mucosa cells were collected immediately before X-ray exposure and after 10 days.
Results: The results revealed no statistically significant difference ($P > 0.05$) in the frequency micronucleated oral mucosa cells after X-ray exposure. However, X-ray was able to increase other nuclear alterations closely related to cytotoxicity, such as karyorrhexis, pyknosis and karyolysis.
Conclusions: Data indicated that exposure to certain radiography may not be a factor in inducing chromosomal damage, but it does promote cytotoxicity.
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Keywords: buccal mucosa cells; radiographic documentation; micronucleus test

Introduction

Radiography is one of the most valuable diagnostic tools used in comprehensive dental care. Orthodontic radiographs carried out in radiological clinics are essential for diagnosis, planning and control of orthodontic treatment.[1] For example, a lateral cephalometric X-ray must be obtained for all patients, before the start of treatment, when the information obtained from this film is expected to benefit or enhance the formulation of the patient's diagnosis and treatment plan.[2] In the same way, a panoramic X-ray must be obtained for all patients, before the start of treatment, unless there are other suitable radiographs, such as full-mouth periapical radiography, that will enable the orthodontic practitioner to formulate an appropriate diagnosis and treatment plan.[2] Frontal cephalometric analysis is also a valuable complementary examination in establishing the correct diagnosis and orthodontic planning.[3] At present, lateral and frontal cephalograms are considered mandatory in orthodontic therapy.

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Although it is generally accepted that there is no safe level of radiation exposure, the possible risk associated with exposure to X-rays must be compared against the benefits of clinical interpretation.[4] It is well known that ionizing radiation damages DNA, including single and double strand breaks, and DNA protein cross-links.[5] The application of validated biomarkers helps to delineate the continuum of events between exposure and resulting disease; identify smaller exposures to specific agents; enhance group risk monitoring and assessment; and reveal toxicological mechanisms by which an exposure and a disease are related.[6] To date, a variety of assays have been proposed as potential biomarkers, 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 these reasons, there was a great deal of enthusiasm regarding the application of the micronucleus test to uncultured exfoliated cells.[7] A micronucleus arises from acentric fragments or whole chromosomes, which are not included in the main nuclei of the daughter cells. The formation of micronuclei can

be induced by substances that cause chromosome breakage (clastogens) as well as agents that affect the spindle apparatus (aneugens).[8] As a result, the present study was undertaken to investigate the frequency of micronucleated cells in oral mucosa from individuals following orthodontic radiography. To monitor cytotoxic effects, pyknosis, karyolysis and karyorrhexis were also evaluated. Such data will contribute to a better understanding of the outcomes on the cellular system following radiography.

Materials and methods

Participants

The participants of this study comprised 18 healthy volunteers (6 male and 12 female) with a mean age of 14.2 ± 1.4 years referred for orthodontic therapy at the Department of Orthodontics, Methodist University, Brazil. All the participants underwent lateral and frontal cephalometric X-rays and panoramic dental radiography prior to orthodontic therapy. A patient history was taken including gender, age, habits and exposure to genotoxic agents. All panoramic dental radiographs were requested by an orthodontist and were performed with Siemens Orthophos equipment (Erlangen, Germany), system 250–71 kV/15 mA/14 s/ 110 mGy/cm^2. Informed consent was obtained from the participants or from their parents or guardians. All individuals were non-smokers.

The research was approved by the Institutional Ethics Committee of the Methodist University UMESP, Brazil.

Micronucleus test in oral mucosa cells

Damage that leads 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 1–3 weeks after exposure to the genotoxic agent.[9,10] For this reason exfoliated oral mucosa cells were collected immediately before X-ray exposure and 10 days after. After rinsing the mouth with tap water, cells were obtained by scraping the right or left cheek mucosa with a moist wooden spatula. Cells were then transferred to a tube containing saline solution, centrifuged (800 rpm) for 5 min, fixed in 3:1 methanol/ acetic acid and dropped onto pre-cleaned slides. Air-dried slides were then 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.[8] 2000 cells were scored from each patient on both sampling occasions (before and after X-ray exposure).

Data analysis

Micronuclei were scored according to the criteria described by Sarto et al[11] as a parameter of DNA damage (mutagenicity). For cytotoxicity, the nuclear alterations pyknosis, karyolysis and karyorrhexis, were considered. Results were expressed as a percentage. This is the same analysis established in a previous study conducted by our research group.[12]

Statistical methods

The Wilcoxon test for dependent samples was used to compare the frequency of micronuclei and other cellular alterations among the before and after X-ray exposure samples using SigmaStat software, version 1.0 (Jadel Scientific, Rafael, CA). The level of statistical significance was 5%.

Results

The frequency of micronucleated cells in patients undergoing cephalometric and panoramic radiography can be seen in Table 1. Before X-ray exposure, the mean frequency of micronucleated cells was 0.02%. There was no significant statistical difference ($P > 0.05$) after X-ray exposure. However, an increase of other nuclear alterations after X-ray exposure was observed as shown by the frequency of karyorrhexis, pyknosis and karyolysis. The data are summarized in Table 1.

To compare the data with accuracy, all patients included in this study were non-smokers. In addition, 12 of the participants used oral antiseptic solutions regularly. Daily alcohol consumption was not recorded in this study because of possible recall bias phenomenon.

Discussion

The aim of this study was to use the micronucleus test to assess chromosome damage and/or cellular death in individuals who had undergone radiography by means of cephalometric and panoramic radiographs. To the

Table 1 Frequency of micronucleated cells (MNC) and other nuclear alterations (karyorrhexis, pyknosis and karyolysis) in orthodontic patients undergoing X-ray exposure

Groups	*MNC (%)*		*Other nuclear alterations* (%)*	
	No. of individuals	*Mean ± SD*	*No. of individuals*	*Mean ± SD*
Prior to X-ray exposure	18	0.2 ± 0.1	18	8.5 ± 3.2
After X-ray exposure	18	0.2 ± 0.1	18	14.4 ± 4.8[†]

[*]Karyorrhexis, pyknosis and karyolysis
[†]$P < 0.05$ when compared to individuals prior to X-ray exposure

best of our knowledge this approach has not been addressed in the literature before.

Micronucleus assay in exfoliated buccal mucosa cells has been used systematically in genetic biomonitoring of populations exposed to several genotoxic chemicals, such as tobacco products, pesticides and alcohol.[13–15] 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.

Micronucleated cell indexes are thought to reflect genomic instability.[16] Detection of an elevated frequency of micronuclei in a given population indicates an increased risk of cancer.[17] It was surprising that micronucleus frequency was not significantly different before and after X-ray exposure in this trial; however, such findings are fully in line with other authors.[18–20] Conversely, some articles have reported higher rates of cytogenetic damage induced by X-ray.[21] Biomonitoring studies of populations exposed to X-rays can be difficult and rather specific owing to the different doses of radiation each population is exposed to. This could explain why some studies have found increased genetic damage in populations exposed to X-rays. Based on our results, we postulate the lack of clastogenic and/or aneugenic effects are related to dental panoramic radiography and lateral or frontal cephalometric X-ray exposure in healthy individuals.

To monitor cytotoxic effects the frequency of karyorrhexis, karyolysis and pyknosis was evaluated in this study. Despite the lack of cytogenetic damage our results demonstrated that panoramic and cephalometric radiographs induced cellular death as demonstrated by the statistically significant differences ($P < 0.05$) between values before X-ray exposure compared with after. Analogous results have been reported by others.[18,20,21] Taken as a whole these results support the notion that X-ray is a cytotoxicant agent. It is important to stress that cytotoxicity does interfere with micronucleus induction, since some micronucleated cells are inevitably lost after cytotoxic insult, confirming, therefore, 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 tumour development.[22] In fact, a correlation between cell proliferation and induction of cancer is assumed.[23] It is likely that proliferation increases the risk of mutations within target cells, and is important in selective clonal expansion of (exogenously or endogenously) initiated cells from preneoplastic foci and eventually tumours.[22]

In human cytogenetic studies some confounding factors need to be considered. Viruses, alterations in the immune system, failures in DNA repair system and interindividual variations have already been associated with an increased frequency of chromosome aberration.[24] Furthermore, an age-related increase of micronuclei has been postulated in participants of a similar age.[24] The influence of tobacco smoke has also been considered as a relevant factor.[11] Thus, all adults recruited to participate in this study were non-smokers. The mutagenic potential of alcohol is controversial and quite complicated to interpret using the micronucleus assay in exfoliated cells. For example, in two reports almost all participants consumed alcohol and tobacco and, therefore, the influence of the individual factors could not be elucidated.[25] In the another study, no genotoxic effect of alcohol was found.[26] In a study by Stich and Rosin[27] the effects of alcohol consumption, cigarette smoking and a combination of the two were examined. A synergistic effect of alcohol and nicotine was observed, but the two drugs alone did not cause an elevation of micronuclei frequencies.

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

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References

1. Gonçalves FA, Schiavon L, Pereira Neto JS, Nouer DF. Comparison of cephalometric measurements from three radiological clinics. *Braz Oral Res* 2006; **20**: 162–166.
2. Jerrold L. Litigation, legislation, and ethics. Post treatment records. *Am J Orthod Dentofacial Orthop* 2008; **133**: 124–126.
3. Masuoka N, Muramatsu A, Ariji Y, Nawa H, Goto S, Ariji E. Discriminative thresholds of cephalometric indexes in the subjective evaluation of facial asymmetry. *Am J Orthod Dentofacial Orthop* 2007; **131**: 609–613.
4. Noffke CE, Snyman AM, Smit E, Chabikuli CE. Design of an X-ray room for a dental practice. *SADJ* 2006; **61**: 292–294.
5. Minicucci EM, Kowalski LP, Maia MA, Pereira A, Ribeiro LR, de Camargo JL, et al. Cytogenetic damage in circulating lymphocytes and buccal mucosa cells of head-and-neck cancer patients undergoing radiotherapy. *J Radiat Res* 2005; **46**: 135–142.
6. Hulka H. Epidemiological studies using biological markers: issues for epidemiologists. *Cancer Epidemiol Biomarkers Prev* 1991; **1**: 13–19.
7. Stich HF, Parida BB, Brunnemann KD. 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* 1992; **21**: 172–176.
8. Belien JA, Copper MP, Braakhuis BJ, Snow GB, Baak JP. Standardization of counting micronuclei: definition of a protocol to measure genotoxic damage in human exfoliated cells. *Carcinogenesis* 1995; **16**: 2395–2400.
9. Fenech M, Holland N, Chang WP, Zeiger E, Bonassi S. The HUman MicroNucleus Project—An international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. *Mutat Res* 1999; **428**: 271–283.

10. Majer BJ, Laky B, Knasmuller S, Kassie F. 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* 2001; **489**: 147–172.
11. Sarto F, Finotto S, Giacomelli L, Mazzotti D, Tomanin R, Levis AG. The micronucleus assay in exfoliated cells of the human buccal. *Mutagenesis* 1987; **2**: 11–17.
12. Angelieri 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. *Pediatr Radiol* 2007; **37**: 561–565.
13. Bloching M, Hofmann A, Lautenschlager C, Berghaus A, Grummt T. Exfoliative cytology of normal buccal mucosa to predict the relative risk of cancer in the upper aerodigestive tract using the MN-assay. *Oral Oncol* 2000; **36**: 550–555.
14. Sarto FR, Tomanin L, Giacomelli G, Iannini G, Cupiraggi AR. The micronucleus assay in human exfoliated cells of the nose and mouth: application to occupational exposures to chronic acid and ethylene oxide. *Mutat Res* 1990; **244**: 345–351.
15. Pastor S, Gutierrez S, Creus A, Cebulska-Wasilewska A, Marcos R. Micronuclei in peripheral blood lymphocytes and buccal epithelial cells of Polish farmers exposed to pesticides. *Mutat Res* 2001; **495**: 147–156.
16. Maluf SW, Erdtmann B. Genomic instability in Down syndrome and Fanconi anemia assessed by micronucleus analysis and single cell-gel electrophoresis. *Cancer Genet Cytogenet* 2001; **124**: 71–75.
17. Hagmar L, Bonassi S, Stomberg U, Brogger A, Knudsen LE, Norppa H, et al. Chromosomal aberrations in lymphocytes predict human cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). *Cancer Res* 1998; **58**: 4117–4121.
18. Cerqueira EM, Gomes-Filho IS, Trindade S, Lopes MA, Passos JS, Machado-Santelli GM. Genetic damage in exfoliated cells from oral mucosa of individuals exposed to X-rays during panoramic dental radiographies. *Mutat Res* 2004; **562**: 111–117.
19. 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. *Dentomaxillofac Radiol* 2007; **36**: 168–171.
20. Ribeiro DA, de Oliveira G, de Castro G, Angelieri F. Cytogenetic biomonitoring in patients exposed to dental X-rays: comparison between adults and children. *Dentomaxillofac Radiol* 2008; **37**: 404–407.
21. He JL, Chen LF, Jin LF, Jin HY. Comparative evaluation of the in vitro micronucleus test and the comet assay for the detection of genotoxic effects of X-ray radiation. *Mutat Res* 2000; **469**: 223–231.
22. Mally A, Jagetia JK. Non-genotoxic carcinogens: early effects on gap junctions, cell proliferation and apoptosis in the rat. *Toxicology* 2002; **180**: 233–248.
23. Swenberg JA. Cell proliferation and chemical carcinogenesis: conferences summary and future directions. *Environ Health Perspect* 1993; **101**: 153–158.
24. Xu GL, Bestor HH, Bourc'his D, Hsich CL, Tommerup N, Bugge M, et al. Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. *Nature* 1999; **402**: 187–191.
25. Ghose UR, Parida BB. Cytological study of exfoliated buccal mucosa cells of tribes in Orissa State (India) with high risk for oral cancer. *Indian J Cancer* 1995; **32**: 95–99.
26. Burgaz S, Işcan A, Büyükbingöl ZK, Bozkurt A, Karakaya AE. Evaluation of micronuclei in exfoliated urothelial cells and urinary thioether excretion of smokers. *Mutat Res* 1995; **335**: 163–169.
27. Stich HF, Rosin MP. Quantitating the synergistic effect of smoking and alcohol consumption with the micronucleus test on human buccal mucosa cells. *Int J Cancer* 1983; **31**: 305–308.