

## RESEARCH ARTICLE

# Cytogenetic biomonitoring in individuals exposed to cone beam CT: comparison among exfoliated buccal mucosa cells, cells of tongue and epithelial gingival cells

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**Objectives:** To evaluate chromosomal damage and cytotoxicity in exfoliated buccal mucosa cells, cells of the tongue and epithelial gingival cells from adults following CBCT scan and to compare the sensitivity of the different exfoliated cells to a same dosage of ionizing radiation.

**Methods:** The study included 46 healthy participants (median age 27 years; age range 23–42 years) who had a CBCT scan. Exfoliated mucosa cells were collected immediately before the CBCT scan and 10 days after. Cells were centrifuged, fixed in the fluid of methanol:glacial acetic acid (3:1) and stained using the method of Schiff's reagent and fast green. One observer analyzed all the slides. For interobserver variances, a second observer scored 16 slides chosen from all the subjects. The same set of 16 slides were analyzed once again a month later for intraobserver variances.

**Results:** There is no significant differences for micronucleated cells before and after a CBCT scan in exfoliated buccal mucosa cells ( $p = 0.476$ ), cells of the tongue ( $p = 0.884$ ) and epithelial gingival cells ( $p = 0.362$ ). The frequencies of pyknotic cell and karyolysis cell had significantly increased after CBCT scan in the three groups. No significant difference was found among the three kinds of mucosa cells ( $p = 0.557$ ). The interobserver ( $p = 0.624$ ) and intraobserver ( $p = 0.193$ ) variances were not significant.

**Conclusions:** A CBCT scan may induce cytotoxicity but not chromosomal damage in the oral mucosa cells, including buccal mucosa cells, cells of the tongue or epithelial gingival cells. The sensitivity of the different exfoliated cells to the same dosage of radiation had no statistically significant difference.

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**Keywords:** micronucleus; cone beam computed tomography; buccal mucosa; tongue; gingival

## Introduction

CBCT is a newly developed three-dimensional imaging system that is increasingly used by dental professionals for various clinical applications. Although the radiation dose from CBCT is generally lower than the dose from traditional CT,<sup>1</sup> it is higher than conventional dental radiography.<sup>2</sup>

Micronucleus cytome assay is a minimally invasive method for monitoring chromosomal instability, cell death and the regenerative potential of human oral mucosal tissues. Micronuclei are formed by whole chromosome or chromatid fragments that fail to attach to the mitotic spindle. It is not included in the nucleus of the daughter cells but remains in the cytoplasm as microstructures that are similar to the main nucleus.<sup>3</sup> Increase in the frequency of micronuclei does not necessarily indicate the formation of pre-neoplastic lesion or carcinoma but

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reveals the genotoxicity of carcinogens and might indicate an elevated probability of particular chromosome changes, which in turn leads to lesions.<sup>4</sup>

The chromosome damage could be caused by exposure to ionizing radiation or carcinogenic chemicals. For effective doses >50 mSv, the risk of cancer initiation proliferates linearly with dose.<sup>5</sup> This dose–response relation had not been demonstrated at doses <50 mSv. So many researchers work on the link between low dose of ionizing radiation and chromosome damage/cytotoxicity. In the study by Kanagaraj *et al.* who employed lymphocytes, the frequency of micronucleus has significantly increased after a CT scan.<sup>6</sup> However, the results from experiments of conventional dental radiography are inconsistent. In the studies by Popova *et al.*,<sup>7</sup> Angelieri *et al.*<sup>8</sup> and da Silva *et al.*,<sup>9</sup> there were no statistically significant difference in the frequency of micronucleated oral mucosa cells after panoramic dental radiography. Contrarily, a few studies revealed a significant increase in the micronucleated oral mucosa cells after a panoramic examination.<sup>10–12</sup>

Previous studies demonstrated that CBCT scan might not be a factor that induced chromosome change, but it was able to promote cytotoxicity in buccal mucosa cells.<sup>13,14</sup> With respect to exfoliated cells of the tongue and gingiva, no such study was found in the search of literature. Meanwhile, whether a difference exists between different oral exfoliated cells to the same ionizing radiation is unknown. Thus, the aim of the present study was (1) to evaluate chromosomal damage (micronucleus) and cytotoxicity (condensed chromatin, pyknosis, karyolysis and karyorrhexis) in exfoliated buccal mucosa cells, cells of tongue and epithelial gingival cells from adults following a CBCT scan and (2) to compare the sensitivity of the different exfoliated cells to the same dosage of ionizing radiation.

## Methods and materials

### *Subjects and CBCT scan*

The participant included 46 healthy adult patients who sought orthodontic treatment in the Peking University school and hospital of stomatology. The criteria for the inclusion of patients were as follows: (1) no smoking cigarette and/or drinking alcohol; (2) no exposure to radiographic examinations within the past 3 months; (3) no apparent oral mucosal diseases; (4) no local stimulation factors, such as fixed and removable metal prosthodontic appliances, betel nuts, calculus etc.

Informed consent forms were signed by all the participants. The experiment protocol was authorized by the local ethical committee (PKUSSIRB-201627029).

For each participant, one CBCT scan was taken with a CBCT unit NewTom VG (Quantitative Radiology, Verona, Italy). The exposure parameters were field of view (FOV) 15 × 15 cm, 110 kVp and 6.24–14.45 mA according to the patient size, with a subsequent dose of area product (DAP) 448.15–730.79 mGy cm<sup>2</sup>.

### *Micronucleus test in oral mucosa cells*

Exfoliated mucosa cells were collected immediately before the CBCT scan and 10 days after. To obtain oral exfoliated cells, the participants were firstly asked to rinse their mouth with tap water. After rinsing, a wooden spatula was used to collect samples over a great area of the right/left cheek mucosa (50 times respectively) with a circular expanding motion. Then cells of the tongue were collected from the one-third to two-third border of each side of the tongue (20 times, respectively), and gingival cells were obtained from the keratinized mucosa of the upper/lower dental arch by a cervical brush. The cells were immediately transferred into differently marked tubes each containing 5 ml of cell buffer. After the tubes were centrifuged (2000 rpm) for 3 min, the supernatant fluid was removed and replaced with 5 ml of fresh cell buffer. This procedure was repeated 3 times. With the help of cell count plate, the total number of cells in one tube was calculated, and cell viability was determined by using the trypan blue (0.4% weight per volume) exclusion assay. Then, the cells were fixed with methanol : glacial acetic acid (3 : 1) for 10 min. The fixed slides were dried and stained with Schiff's reagent in a dark area at room temperature for 60 min and further counterstained with 0.2% (weight per volume) fast green cytoplasmic stain for 2 min.

### *Cytological analysis*

The stained slides were analyzed under an Olympus BX51 fluorescence microscopy (Olympus Corp., Tokyo, Japan) in a fixed order. For each slide, 1000 cells were analyzed. Micronucleated cells, cells with nuclear buds, basal cells, binucleated cells, condensed chromatin cells, pyknosis cells, karyolysis cells and karyorrhexis cells were scored (×400 magnifications). The scoring criteria for the various distinct cell types and nuclear anomalies were based on the criteria described in the article by Thomas *et al.*<sup>15</sup> A micronucleated cell must meet the following criteria: (1) boundary of the micronuclei is round/oval and smooth; (2) the diameter of micronuclei less than a third of the main nucleus but large enough to discern shape and colour; (3) the main nucleus and the micronuclei are Feulgen positive (*i.e.* pink in bright field illumination); (4) staining intensity of micronuclei is similar to that of main nucleus; (5) texture of micronuclei is similar to that of main nucleus; (6) focal plane of micronuclei is the same as the main nucleus; and (7) the micronuclei is absence of overlap with or bridge to the main nucleus. The certainty with which cells are believed to be micronucleated is noted. Cells with objects amply meeting all of the above micronucleation criteria are assigned high certainty. Those with objects slightly different from the micronucleation criteria 4, 5 or 6 and meeting all of the other criteria are assigned medium certainty.<sup>16</sup> Example images of the cells are shown in [Figure 1](#).

The frequency difference was defined as the frequency of the observed cells after the CBCT scan minus the frequency of the observed cells before the CBCT scan.

*Inter- and intraobserver variances*

To determine the inter- and intraobserver variances, the second observer evaluated 16 slides chosen from all of the subjects, which were randomly numbered. The second observer did not know when and where the cells were obtained from. For each slide, the first observer identified 1000 cells. During this period, the microscope was connected with the computer, therefore the cells could be scored on the big screen. An open accessed grasp screen software Camtasia® Studio 8.6 (TechSmith Corp., Okemos, MI) was used to capture the video in the whole process. After that, the second observer scored the cells by viewing the video. The results of the two observers were compared and the interobserver variance was determined. Moreover, the same set of 16 slides were analyzed once again a month later by the two observers for analyzing intraobserver variances.

*Data analysis*

The Wilcoxon test for dependent samples was used to compare the frequencies of micronucleated cell and other types of cells among the samples before and after CBCT scan. It was also used to compare the consistency within observer and between the two observers. The Friedman test was applied to compare the sensitivity of exfoliated buccal mucosa cells, cells of the tongue and epithelial gingival cells to the same CBCT scan. Statistical analysis was conducted using SPSS® v. 19.0 (IBM Corp., New York, NY; formerly SPSS Inc., Chicago,

IL). The difference among groups was found to be significant when  $p < 0.05$ .

**Result**

*Sample characteristics*

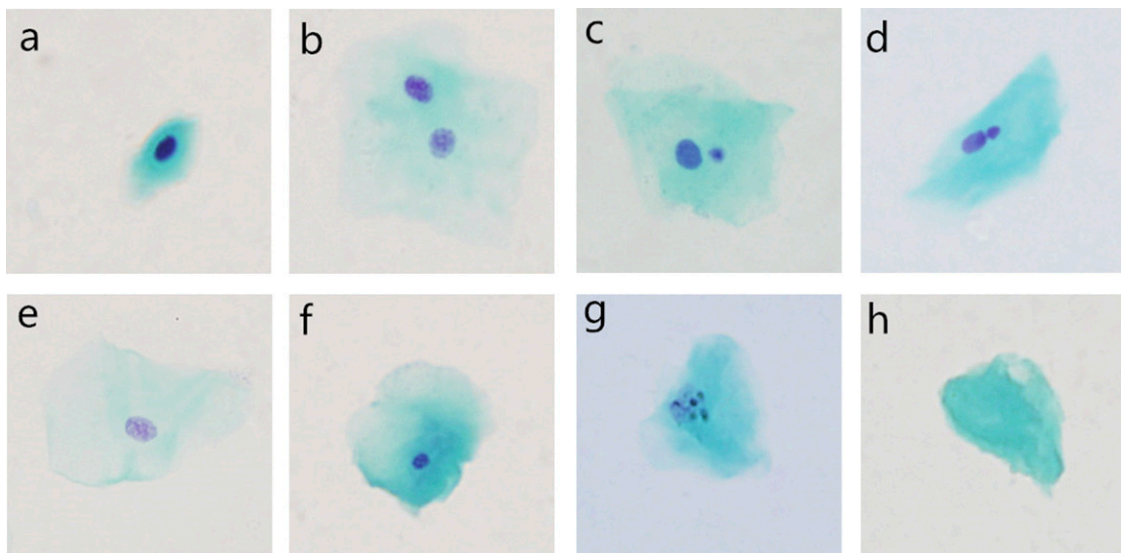
The sample included 39 females and 7 males. The median age of the subjects was 27 years (range 23–42 years).

*Data analysis*

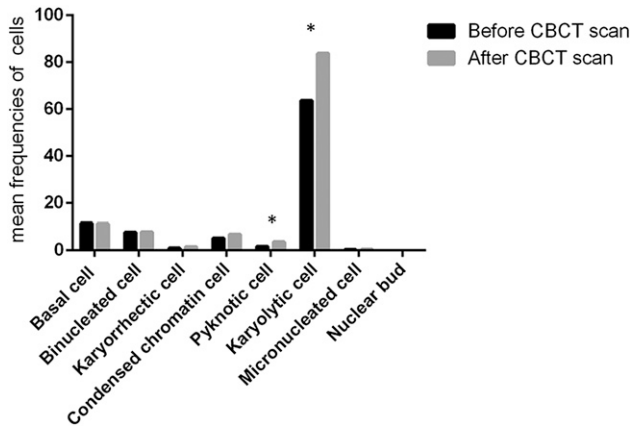
The number of the exfoliated cells collected from each area is about  $1-10 \times 10^5$ . Cell viability was always found to be  $>70\%$ .

The statistical analysis for the three groups' micronucleated cells and other nuclear alterations is shown in [Figures 2–4](#). With regard to the frequency of micronucleated cells, no significant statistical differences were noticed after CBCT scan in exfoliated buccal mucosa cells, cells of the tongue and epithelial gingival cells, respectively. However, CBCT scan did increase the frequencies of other nuclear alterations such as karyorrhexis cell, condensed chromatin cell, pyknosis cell and karyolysis cell in all the three groups. Among them, the frequencies of pyknosis cell and karyolysis cell increased significantly.

[Table 1](#) shows the frequency differences of micronucleated cells and other nuclear alterations in the three kinds of oral exfoliated cells before and after taking the same CBCT scan. There were no significant differences among exfoliated buccal mucosa cells, cells of the tongue and epithelial gingival cells in any kind of nuclear alterations.



**Figure 1** Images of the different cell types stained using Schiff's reagent and fast green. (a) Example images of basal cells. (b) Example images of binucleated cells. (c) Example images of micronucleated cells. (d) Example images of cells with nuclear buds. (e) Example images of condensed chromatin cells. (f) Example images of pyknosis cells. (g) Example images of karyorrhexis cells. (h) Example images of karyolysis cells.



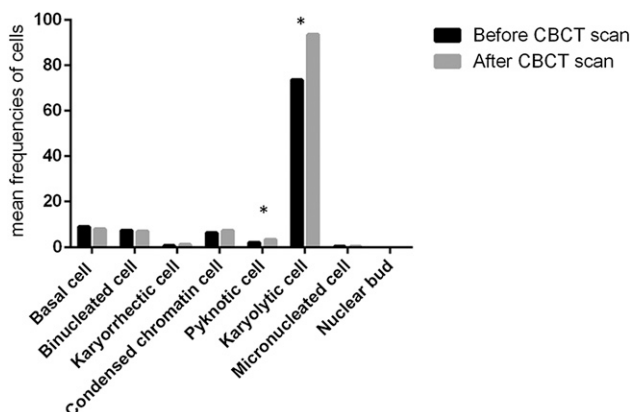
**Figure 2** Comparison of mean frequencies of micronucleated cells and other nuclear alterations in buccal mucosa cells before and after CBCT scan. \*Significant difference between values before and after CBCT scan.

The interobserver ( $p = 0.624$ ) and intraobserver ( $p = 0.193$ ) variances were not significant.

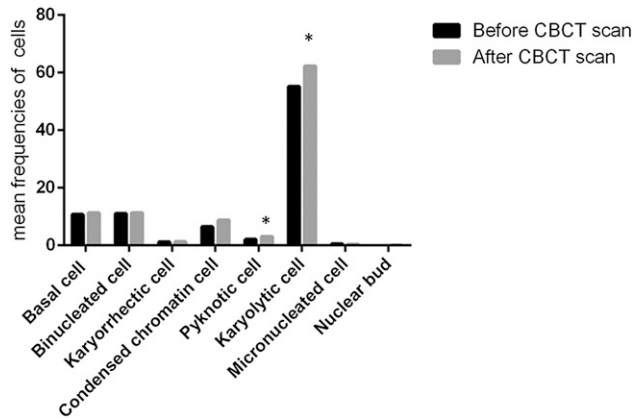
## Discussion

The aim of this study was to evaluate chromosomal damage and cytotoxicity in exfoliated buccal mucosa cells, cells of the tongue and epithelial gingival cells collected from adults exposed to CBCT and to compare them to determine which type of cells is more sensitive to ionizing radiation. As far as we know, there was no such study reported in the literature.

The buccal micronucleus cytome assay is a minimally invasive method for monitoring chromosomal instability, cell death and the regenerative potential of human buccal mucosal tissue. This method is increasingly used in molecular epidemiological studies for investigating the impact of genotype,<sup>17</sup> lifestyle factors<sup>18</sup>



**Figure 3** Comparison of mean frequencies of micronucleated cells and other nuclear alterations in cells of the tongue before and after CBCT scan. \*Significant difference between values before and after CBCT scan.



**Figure 4** Comparison of mean frequencies of micronucleated cells and other nuclear alterations in epithelial gingival cells before and after CBCT scan. \*Significant difference between values before and after CBCT scan.

and external environment factors.<sup>19</sup> The key advantage of the micronucleus assay is the relative ease of scoring, limited cost and person-time required.

Micronucleated cells are characterized by the presence of a main nucleus together with one or more small nuclear structures that are called micronuclei. The micronuclei are round or oval in shape and their diameter should range between one-sixth and one-third of the main nucleus. Micronuclei have the same staining intensity and texture as the main nucleus. In the present study, the mean frequencies of micronucleated cells were 0.37‰, 0.43‰ and 0.54‰ for exfoliated mucosa cells, cells of the tongue and gingival cells, respectively, before a CBCT scan. After the scan, the frequencies of the micronucleated cells were not significantly increased. This result was in line with the result from the study by Popova et al for the exfoliation mucosa cells,<sup>7</sup> whereas it was contrary to the results from some of the studies performed for panoramic radiography, in which the micronucleated cells were significantly increased after one panoramic examination.<sup>10–12</sup> In theory, panoramic radiography has a lower radiation dose than a large FOV CBCT scan, and therefore should have little impact on mucosa cells than a large FOV CBCT scan.

**Table 1** Comparison of frequency differences of micronucleated cells and other nuclear alterations in buccal mucosa cells, cells of tongue and epithelial gingival cells after CBCT scan

Cell types	Buccal	Tongue	Gingiva	p-value
Basal cell	-0.26	-0.98	0.50	0.073
Binucleated cell	0.13	-0.46	0.28	0.344
Karyorrhectic cell	0.54	0.52	0.07	0.538
Condensed chromatin cell	1.61	0.98	2.24	0.740
Pyknotic cell	1.74	1.33	1.02	0.646
Karyolytic cell	20.09	19.98	13.07	0.196
Micronucleated cell	0.09	0.02	-0.13	0.668
Nuclear bud	0.04	0.00	0.02	0.741

No significant difference among buccal mucosa cells, cells of tongue and epithelial gingival cells in any kind of nuclear alterations ( $p > 0.05$ ).



Apoptosis, believed to be the major mode of cellular death in living tissues, appears to be under physiological control and effects orderly cell death such as that which occurs during embryogenesis and normal cell turnover. Because it is stimulated both by ionizing radiation and by chemicals that bind to DNA, apoptosis may also act as a surveillance mechanism, eliminating cells with genetic damage; thus, apoptosis, in excess of normal levels, may be an indicator of genotoxic insult.<sup>16</sup> Pyknosis, condensed chromatin and karyorrhexis were probably the early stages of apoptosis, whereas karyolytic cells had no nucleus and represented a very late stage in the cellular death process.<sup>15</sup> Pyknosis, condensed chromatin, karyorrhexis and karyolysis could present cytotoxicity. In the present study, the frequencies of karyolysis cell and pyknosis cell had increased significantly. This indicates that the radiation from a CBCT scan might promote cytotoxicity in oral mucosa cells.

The basal cells, binucleated cells and cells with nuclear buds were also scored in the present study. Basal cells were located in the basal layer and they could differentiate into normal cells. Basal cells have a large nucleus : cytoplasm ratio relative to differentiated cells. The oral epithelium maintains itself by continuous cell renewal whereby new cells produced in the basal layer by mitosis migrate to the surface replacing those that are shed.<sup>4</sup> Binucleated cells are cells containing two main nuclei instead of one. Binucleation probably does not involve direct interaction with DNA but rather involves interference with events occurring late during cell division.<sup>16</sup> In the present study, there were no significant differences for basal cell and binucleated cell before and after a CBCT scan. Moreover, cells with a small amount of genetic material adhered to the main nucleus were classified as nuclear buds. The mechanism leading to nuclear bud formation is not known, but it may be related to the elimination of amplified DNA or DNA repair.<sup>15</sup> The frequency of nuclear buds in exfoliated buccal mucosa cells and epithelial gingival cells increased after a CBCT scan but the increase was not significant.

It is well known that different individuals have different radiation sensitivity. Some of them are very sensitive to ionizing radiation and some of them are less. Thus, to investigate the radiation sensitivity of different

mucosa cells, the frequency difference before and after CBCT scan was employed. The results from the present study demonstrate that there are no significant differences between the three kinds of mucosa cells. This is consistent with the results from the study by Arora *et al*,<sup>12</sup> where the percentage of micronucleated cells in the buccal mucosa and the keratinized gingiva was not significantly increased before and after radiographic exposure.

One of the limitations in the practical application of the micronucleus assay is the large variability of cell frequencies scored within observer and between observers.<sup>20</sup> This variability might be caused by the subjective factors, differences in the scoring criteria and technical factors such as the differences in microscopes that were used, the different illumination setup and the variations of the computer screen resolution. To avoid these limitations, we identified the cells based on the unified scoring criteria strictly, and the second observer scored the cells by viewing the video captured by the first observer. In this way, the same FOV was observed and the machine parameters were consistent. This ensured the counting results of the cells to be as accurate and precise as possible. This also provides one method for further preserving and documenting the slides. In the present study, there were no significant differences between and within the observers.

## Conclusions

The results from the present study suggest that a NewTom CBCT scan could induce cytotoxicity but not chromosomal damage in the oral mucosa cells including buccal mucosa cells, cells of the tongue or epithelial gingival cells. Further studies are necessary to monitor the influence of the larger dose of radiation. With regard to the biological effects of radiation, the expression and the definite mechanism at the molecular gene level should be further investigated. Future studies should also explore the relationship of micronucleated cell expression with changes in DNA methylation and the associated transcriptome, metabolome and proteome profiles to unravel the underlying molecular mechanisms that correlate with this DNA damage biomarker.

## References

1. Arai Y, Tammisalo E, Iwai K, Hashimoto K, Shinoda K. Development of a compact computed tomographic apparatus for dental use. *Dentomaxillofac Radiol* 1999; **28**: 245–8. doi: <https://doi.org/10.1038/sj/dmfr/4600448>
2. Li G. Patient radiation dose and protection from cone-beam computed tomography. *Imaging Sci Dent* 2013; **43**: 63–9. doi: <https://doi.org/10.5624/isd.2013.43.2.63>
3. Heddle JA, Hite M, Kirkhart B, Mavournin K, MacGregor JT, Newell GW, *et al*. The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat Res* 1983; **123**: 61–118.
4. Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, *et al*. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: the HUMN project perspective on current status and knowledge gaps. *Mutat Res* 2008; **659**: 93–108. doi: <https://doi.org/10.1016/j.mrev.2008.03.007>
5. Berrington de Gonzalez A, Mahesh M, Kim KP, Bhargavan M, Lewis R, Mettler F, *et al*. Projected cancer risks from computed tomographic scans performed in the United States in 2007. *Arch Intern Med* 2009; **169**: 2071–7. doi: <https://doi.org/10.1001/archinternmed.2009.440>
6. Kanagaraj K, Abdul Syed Basheerudeen S, Tamizh Selvan G, Jose MT, Ozhimuthu A, Panneer Selvam S, *et al*. Assessment of

- dose and DNA damages in individuals exposed to low dose and low dose rate ionizing radiations during computed tomography imaging. *Mutat Res Genet Toxicol Environ Mutagen* 2015; **789–790**: 1–6. doi: <https://doi.org/10.1016/j.mrgentox.2015.05.008>
7. 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**: 168–71.
  8. 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**: 561–5. doi: <https://doi.org/10.1007/s00247-007-0478-1>
  9. da Silva AE, Rados PV, da Silva Lauxen I, Gedoz L, Villarinho EA, Fontanella V. Nuclear changes in tongue epithelial cells following panoramic radiography. *Mutat Res* 2007; **632**: 121–5.
  10. Waingade M, Medikeri RS. Analysis of micronuclei in buccal epithelial cells in patients subjected to panoramic radiography. *Indian J Dent Res* 2012; **23**: 574–8. doi: <https://doi.org/10.4103/0970-9290.107329>
  11. Cerqueira EM, Meireles JR, Lopes MA, Junqueira VC, Gomes-Filho IS, Trindade S, et al. Genotoxic effects of X-rays on keratinized mucosa cells during panoramic dental radiography. *Dentomaxillofac Radiol* 2008; **37**: 398–403. doi: <https://doi.org/10.1259/dmfr/56848097>
  12. Arora P, Devi P, Wazir SS. Evaluation of genotoxicity in patients subjected to panoramic radiography by micronucleus assay on epithelial cells of the oral mucosa. *J Dent (Tehran)* 2014; **11**: 47–55.
  13. Carlin V, Artioli AJ, Matsumoto MA, Filho HN, Borgo E, Oshima CT, et al. Biomonitoring of DNA damage and cytotoxicity in individuals exposed to cone beam computed tomography. *Dentomaxillofac Radiol* 2010; **39**: 295–9. doi: <https://doi.org/10.1259/dmfr/17573156>
  14. Lorenzoni DC, Fracalossi AC, Carlin V, Ribeiro DA, Sant'anna EF. Mutagenicity and cytotoxicity in patients submitted to ionizing radiation. *Angle Orthod* 2013; **83**: 104–9. doi: <https://doi.org/10.2319/013112-88.1>
  15. Thomas P, Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, et al. Buccal micronucleus cytome assay. *Nat Protoc* 2009; **4**: 825–37. doi: <https://doi.org/10.1038/nprot.2009.53>
  16. Tolbert PE, Shy CM, Allen JW. Micronuclei and other nuclear anomalies in buccal smears: methods development. *Mutat Res* 1992; **271**: 69–77.
  17. Thomas P, Harvey S, Gruner T, Fenech M. The buccal cytome and micronucleus frequency is substantially altered in Down's syndrome and normal ageing compared to young healthy controls. *Mutat Res* 2008; **638**: 37–47.
  18. Reis SR, do Espirito Santo AR, Andrade MG, Sadigursky M. Cytologic alterations in the oral mucosa after chronic exposure to ethanol. *Braz Oral Res* 2006; **20**: 97–102. doi: <https://doi.org/10.1590/s1806-83242006000200002>
  19. Suruda A, Schulte P, Boeniger M, Hayes RB, Livingston GK, Steenland K, et al. Cytogenetic effects of formaldehyde exposure in students of mortuary science. *Cancer Epidemiol Biomarkers Prev* 1993; **2**: 453–60.
  20. Bolognesi C, Roggieri P, Ropolo M, Thomas P, Hor M, Fenech M, et al. Buccal micronucleus cytome assay: results of an intra- and inter-laboratory scoring comparison. *Mutagenesis* 2015; **30**: 545–55. doi: <https://doi.org/10.1093/mutage/gev017>