

# Alteration of mucosa cell maturation pattern after exposure to different radiographic imaging methods

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**Aim:** Dental imaging has been widely used for diagnosis in dentistry. However, dental X-ray may induce cytotoxicity leading to apoptosis in oral mucosa cells. The present study aimed to observe the maturation pattern of buccal and gingival cells after exposure to X-ray radiation from analog/digital panoramic scanning and cone beam computed tomography (CBCT). **Methods:** The research samples were 40 subjects who fulfilled the inclusion and exclusion criteria. The subjects were divided into the exposed (patients who received analog/digital panoramic radiography or CBCT) and controlled (patients who had no radiography examinations) groups, with 10 subjects in each group. Exfoliative cytology smears were obtained from buccal mucosa and gingiva before exposure (or on day 0 for the control group) and 10 days later. The cells were stained with the Papanicolaou method. Then, the superficial, intermediate, and parabasal cells were counted in each glass slide. **Results:** No significant differences ( $p > 0.05$ ) were observed among all cell types between day 0 and 10 in the control group. Meanwhile, after exposure to three kinds of radiography examinations, the frequency of intermediate cells in buccal mucosa and gingiva increased ( $p < 0.05$ ), but that of superficial cells decreased ( $p < 0.05$ ) significantly. No significant difference was found in the parabasal cells ( $p > 0.05$ ). The frequency differences between intermediate and superficial cells showed no significant difference between the buccal mucosa and gingiva. **Conclusion:** Analog/digital panoramic radiography and CBCT exposure can induce cytotoxicity by altering the maturation pattern of buccal mucosa cells and gingiva, so it is strongly recommended to only perform these procedures if necessary and avoid repeated exposure to the same patient.

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## Introduction

The use of ionizing radiation in imaging science in dentistry has been rapidly increasing. The essential diagnostic method in dental practice is radiography, but X-ray provokes genotoxic and cytotoxic outcomes in cells. The genotoxic effects of X-rays are still debatable, whereas its cytotoxic effects are more proven. X-ray exposure through panoramic scanning initiates a cytotoxic consequence by increasing cell apoptosis<sup>1,2</sup>. The patients exposed to a series of radiographs had a significant increase in cellular death marked by pyknosis, karyolysis, and karyorrhexis in buccal mucosa smears. Cone beam computed tomography (CBCT) is a widely used oral-maxillofacial imaging modality which provides accurate 3D imaging of hard tissue structure<sup>3</sup>. Radiation exposure from CBCT also acts as a cytotoxicant to oral mucosa cells due to its high radiation dose. The patients submitted to CBCT show higher cell death than conventional radiographs group<sup>4</sup>. Cytotoxicants act by disrupting the molecules involved in cell growth and cell death<sup>5</sup>.

The oral epithelium has been widely used to analyze X-ray effects because it is directly exposed to radiation, and the tissue is easily collected by scraping the mucosa. The effects of X-ray exposure can be studied using exfoliative cytology because the method is simple, low-cost, and non-invasive<sup>6</sup>. Papanicolaou staining is a cytology method that analyzes the proportion of exfoliated cells as the maturation pattern. Thus, this method was applied in this study. The oral epithelium is continuously renewed; new cells migrate from the parabasal layer to the outer tissue layer, causing epithelial maturation<sup>7</sup>. Normally, oral mucosa smears consist of cells from the superficial, intermediate, and rare cases, the parabasal layer. Normal maturation pattern indicates the predominance of intermediate cells accompanied by superficial cells and rarely parabasal cells. Lesions in oral mucosal cells such as leucoplakia and oral squamous cell carcinoma have been associated with the maturation pattern changes indicated by a greater number of intermediate or parabasal cells<sup>8</sup>.

Studies have evaluated the influence of extrinsic factors, such as tobacco and alcohol consumption, on the process of oral epithelium maturation, given that they have been suggested as risk factors for oral cancer development<sup>8-10</sup>. Dental X-rays stimulate cytotoxic consequences in oral mucosa cells, leading to cellular death, and may be considered as a nongenotoxic mechanism of carcinogenesis<sup>1,2</sup>. However, whether X-ray radiation exposure affects epithelial maturation's normal process in the oral cavity remains uncertain. Thus, this study aimed to evaluate the outcomes of X-ray exposure from analog/digital panoramic radiography and CBCT on the maturation patterns of buccal and gingival cells.

## MATERIAL AND METHODS

### Study design and sample

This cross-sectional observational study evaluated patients aged 25 years or older and who had analog or digital panoramic radiography or CBCT examinations requested

by a dentist independent of this study at Dental Hospital, Universitas Gadjah Mada, Indonesia and were considered eligible for the study. The subjects of this study fulfilled the following inclusion criteria: (1) non-cigarette smoker and/nor alcohol consumer; (2) no exposure to radiographic imaging for the last 3 weeks; (3) no visible lesions in the oral mucosa; (4) not using orthodontic and prosthodontic appliances. Ethical clearance had been granted by the Research Ethics Commission of the Faculty of Dentistry, Universitas Gadjah Mada, Indonesia. The informed consent for each patient was obtained. The sample consisted of ten subjects for each radiological exam selected by purposive sampling design based on the previous study conducted by Shantiningsih and Diba<sup>11</sup> (2018). The control group included an equal number of subjects who had no radiography examination and were observed under the same research protocol.

Analog panoramic radiographs were obtained using a Yoshida Panoura Deluxe System (The Yoshida Dental MFG. CO., LTD., Tokyo, Japan) with the following exposure parameters: 90 kVp, 8–10 mA, and 20 s. A digital system using a Pax-i (Vatech, Gyeonggi-do, Republic of Korea) was operated with the following exposure parameters: 72 kVp, 10 mA, and 16 s. The CBCT images were obtained using a Volux 3D Dental CT System (Genoray, Gyeonggi Province, Republic of Korea) with parameter settings of 85 kVp, 6 mA, and 16.6 s.

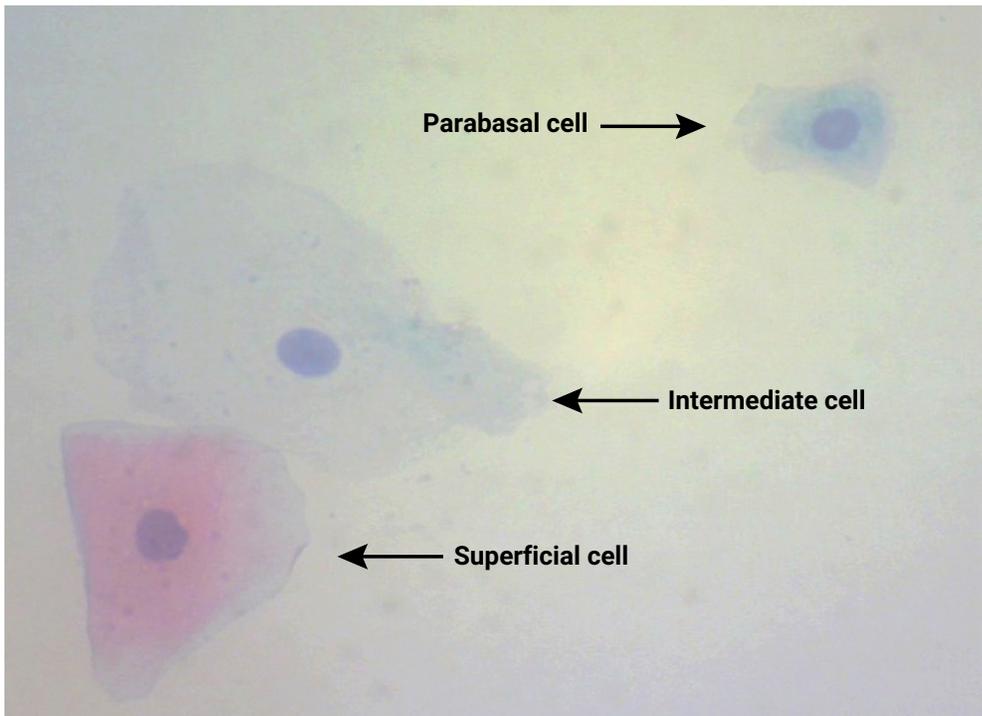
### Sample collection and cytopathological analysis

Smears were collected immediately from buccal mucosa and gingiva cells before X-ray exposure and 10 days later. Before smear collection, the patients were instructed to rinse their mouths with water for 30 s. After rinsing, a cytobrush was used to collect smears over the buccal mucosa and gingiva. Both buccal and gingival mucosa samples were taken twice, before the X-ray exposure on the left region and after the procedure on the right region. The smears were spread onto a glass slide and fixed in 96% alcohol. The slides were stained by the Papanicolaou method. The stained slides were analyzed under a light microscope (YS100, Nikon, Japan) and Optilab Viewer 2.1.

All slides were analyzed by an experienced and blinded observer. Cells were classified as superficial, intermediate, and parabasal according to the criteria described by Montgomery<sup>6</sup>. The cells were counted until 100 visible, and non-overlapping cells were obtained at 400x magnification on each slide horizontally, from left to right (Figure 1). The results are expressed in percentages. Similar analyzes have been established in previous published studies<sup>8,10,12</sup>.

### Statistical data analysis

All data were presented as mean  $\pm$  standard deviation (S.D.). Statistical analysis was performed using IBM® SPSS® Statistics 25 version software (IBM Corporation, Armonk, New York). The normality criterion was evaluated using the Shapiro–Wilk test. Based on the Shapiro–Wilk test results, the normally distributed data were further analyzed using a paired *t*-test, otherwise using Wilcoxon signed-rank test to examine the difference in the number of superficial, intermediate, and parabasal cells, before and after X-ray exposure. A paired *t*-test was also used to measure



**Figure 1.** Papanicolaou staining showing three types of oral mucosa cells. Magnification: 400 $\times$ .

the differences between buccal mucosa and gingiva of the oral cavity in the study group. The statistical differences were significant if  $P < 0.05$ . The intraclass correlation coefficient (ICC) was used to assess the reproducibility of measurements. Intra-rater reliability statistics for cell numbers were calculated after the same examiner re-evaluated a randomly selected subset of 8 slides after 2 weeks. Based on the 95% confidence interval of the ICC estimate, the values were interpreted as follows: poor for less than 0.5, moderate for 0.5–0.75, good for 0.75–0.9, and excellent reliability for values greater than 0.9<sup>13</sup>.

## RESULTS

Intra-rater reliability measurement was excellent for parabasal cells (ICC = 1) and good for intermediate and superficial cells (ICC = 0.8). The cell number was normally distributed by Shapiro–Wilk test, except for parabasal cells. Tables 1 and 2 show the mean frequency differences in percentages of parabasal, intermediate, and superficial cells of buccal mucosa and gingiva, respectively, before and after obtaining analog/digital panoramic or CBCT radiographs and in the control group. A small number of parabasal cells were detected after radiation exposure, but the number was not significant ( $p > 0.05$ ) by Wilcoxon signed-rank test. The paired  $t$ -test showed a significant increase in intermediate cells ( $p < 0.05$ ), while superficial cells were significantly decreased ( $p < 0.05$ ) after radiation exposure using the three types of radiography methods. No significant differences were observed among all cell types in the control group ( $p > 0.05$ ).

Table 3 shows the frequency differences between the intermediate and superficial cells in buccal mucosa and gingiva after X-ray exposure under the three types of radiographic methods. No significant differences were observed between the buccal mucosa and gingiva in any radiography methods ( $p > 0.05$ ).

**Table 1.** Comparison of mean frequencies (in percentages) of parabasal, intermediate, and superficial cells before and after exposure to each radiography imaging in the buccal mucosa.

Variable	Parabasal	p <sup>a</sup>	Intermediate	p <sup>b</sup>	Superficial	p <sup>b</sup>
Control						
Before	0	Ref.	67.80 + 3.88	Ref.	32.20 + 3.88	Ref.
After	0	1	69.8 + 5.31	0.254	30.20 + 5.31	0.254
Analog Panoramic						
Before	0	Ref.	69.50 + 4.42	Ref.	30.50 + 4.42	Ref.
After	0.083 + 0.29	0.32	75.17 + 6.89	0.036	24.83 + 6.89	0.036
Digital Panoramic						
Before	0	Ref.	69.70 + 6.74	Ref.	30.50 + 8.26	Ref.
After	0.33 + 0.65	0.10	81.20 + 6.37	0.003	17.83 + 7.41	0.000
CBCT						
Before	0	Ref.	67.80 + 3.88	Ref.	32.89 + 3.41	Ref.
After	0.5 + 0.71	0.059	77.6 + 9.80	0.013	22.67 + 9.51	0.018

<sup>a</sup> Wilcoxon signed-rank test

<sup>b</sup> Paired t-test

\*Comparison of the reference group (Ref.); differences were considered to be statistically significant when  $p < 0.05$ .

**Table 2.** Comparison of mean frequencies (in percentages) of parabasal, intermediate, and superficial cells before and after exposure to each radiography imaging in the gingiva.

Variable	Parabasal	p <sup>a</sup>	Intermediate	p <sup>b</sup>	Superficial	p <sup>b</sup>
Control						
Before	0	Ref.	58.10 + 7.49	Ref.	41.9 + 7.49	Ref.
After	0	1	63.00 + 9.59	0.186	37.00 + 9.59	0.186
Analog Panoramic						
Before	0	Ref.	61.08 + 6.71	Ref.	38.92 + 6.71	Ref.
After	0.083 + 0.29	0.32	67.75 + 5.24	0.039	31.42 + 5.55	0.017
Digital Panoramic						
Before	0	Ref.	60.60 + 8.06	Ref.	41.83 + 9.28	Ref.
After	0.33 + 0.65	0.10	79.40 + 6.42	0.000	20.83 + 7.48	0.000
CBCT						
Before	0	Ref.	58.1 + 7.49	Ref.	43.33 + 6.32	Ref.
After	0.5 + 0.71	0.059	73.70 + 7.36	0.001	26.11 + 9.02	0.001

<sup>a</sup> Wilcoxon signed-rank test

<sup>b</sup> Paired t-test

\*Comparison of the reference group (Ref.); differences were considered to be statistically significant when  $p < 0.05$ .

**Table 3.** Comparison of frequency differences of intermediate and superficial cells in buccal mucosa and gingiva before and after exposure to each radiography imaging between buccal mucosa and gingiva

Cell types	Buccal	Gingival	p-value <sup>a</sup>
Analog Panoramic			
Intermediate	5.67	6.67	0.773
Superficial	-5.67	-7.50	0.577
Digital Panoramic			
Intermediate	11.50	18.80	0.069
Superficial	-12.75	-21.00	0.085
CBCT			
Intermediate	9.80	15.60	0.252
Superficial	-11.30	-14.10	0.584

<sup>a</sup> Paired *t*-test

\*Differences were considered to be statistically significant when  $p < 0.05$ .

## DISCUSSION

The cytotoxic effects of panoramic and CBCT lead to cell death<sup>3,4,14,15</sup>. Cellular death is believed to be a nongenotoxic mechanism induced by carcinogenesis<sup>1,2</sup>. Genotoxic and cytotoxic X-ray effects on exfoliated buccal mucosal and gingival cells were detected when a series of X-ray dental imaging exams, including full mouth radiograph, panoramic, lateral cephalometric radiographs, posteroanterior cephalometric radiographs, and CBCTs, were performed<sup>14-16</sup>. Further, analog panoramic radiographs showed a significant increase in post-exposure micronuclei relative to digital panoramic radiographs<sup>12</sup>. Exposure to carcinogens also affects the maturation pattern of the oral mucosa, as detected by Papanicolaou<sup>10</sup>. This study aimed to evaluate whether X-ray radiation from dental imaging affects buccal mucosa and gingiva epithelium maturation patterns. This research is the first study to assess the maturation patterns after dental imaging by cytopathology.

Oral epithelium maturation involves several widely studied confounding factors, such as mouth rinse utilization, oral lesion, the use of orthodontic and prosthodontic appliances, smoking, and alcohol usage<sup>8,10,17-19</sup>. In this study, the patient with those confounding factors was excluded. The first cell count represented any consequences of cytotoxic agents before radiation exposure. Therefore, any differences emerging between before and after radiation exposure can be attributed to radiation.

This study showed that normal mucosa smears contained no parabasal cell, and no significant change was noted in the intermediate and superficial cell numbers for 10 days. Burzlauff et al. have confirmed that cells in the superficial and intermediate layers and, in rare cases, the parabasal layer are normally observed in oral mucosal smears<sup>8</sup>. Normal maturation pattern shows the balanced proportions of cells categorized as superficial, intermediate, and parabasal in exfoliated cells. The alteration in oral epithelial cell composition is related to the abnormal composition of cell

types. Cytopathological studies to assess the maturation pattern of oral mucosal cells of patients exposed to tobacco and alcohol found fewer superficial cells and an increased number of intermediate cells<sup>8</sup>. These findings are in line with our results that more intermediate cells were detected after radiation exposure, implying the alteration of maturation pattern.

In the present study, the number of intermediate cells increased ( $p < 0.05$ ), whereas that of superficial cells decreased ( $p < 0.05$ ) after exposure to analog/digital panoramic or CBCT. This finding may correlate with the cytotoxicity effect of dental imaging on oral mucosa cells. X-ray exposure during panoramic dental radiography triggers a cytotoxic effect by increasing apoptosis<sup>1</sup>. The number of karyolytic, karyorrhexic, and pyknotic cells showed a significant increase after panoramic radiography and CBCT scan<sup>3,14</sup>. These types of cells represent the cell death process, which enables the elimination of defected cells, resulting in a massive discharge of superficial cells followed by increased mitosis on the parabasal layer as replacement<sup>20</sup>. The homeostatic mechanism of the parabasal layer that produces new cells replaces the loose cells; the more severe the lesion, the more parabasal cells are found<sup>8,21</sup>. Moreover, parabasal cells were detected after X-ray radiation, although the statistic calculation was not significant ( $p > 0.05$ ) compared with that before radiation. We consider that the cytobrush swab may not reach the deepest layers of the oral mucosal epithelium. Cytohistological techniques showed a limited sensitivity of between 79 and 97%<sup>22</sup>.

Buccal mucosa and gingiva showed no difference in the number of cells before and after analog/digital panoramic radiography and CBCT ( $p > 0.05$ ). Yang et al. verified that the number of nuclear changes in both sites, including those in karyorrhexis, condensed chromatin, pyknosis, and karyolysis, showed no significant increase before and after CBCT scan<sup>14</sup>. However, Kesidi et al. compared the differences in micronuclei and other nuclear alterations in buccal mucosa and gingiva after exposure by conventional full mouth radiograph procedure, which revealed a substantial difference with the mean difference being high in the buccal mucosa compared to the gingiva<sup>14</sup>. This condition may be explained by the differences in radiography, indicating variations in the dosage. Differences in radiation dose and repeated exposure may increase the radiation effect on the body, as studies have proven that cellular death increases with radiation dose<sup>23</sup>.

There may be some possible limitations in this study. The first limitation is the reliability of cell frequency measurement within an observer. To avoid this limitation, we assessed intra-rater reliability that reflects the variation of data measurement performed by the observer. Intra-rater measurement showed good to excellent reliability in this study. The second limitation concerns the absence of anucleated superficial cell measurement; hence the keratinization index cannot be determined. The keratinization index may be established in future research to provide additional information as a marker to detect early cell changes in oral cancer.

The results from the present study suggest that routine X-ray dental imaging exams, such as analog/digital panoramic and CBCT, can induce changes in the maturation pattern of buccal mucosa and gingiva epithelium.

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