Research article

Does dental x-ray cause oral keratosis?

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ABSTRACT

Introduction and Aim: Ionizing radiation in dental imaging technology may cause cytotoxicity by means of changing the buccal mucosa cells and gingiva maturation pattern. Alteration of maturation pattern was often accompanied by increasing anucleated cells number that indicates keratosis. This study aimed to evaluate whether superficial keratosis also occurred after exposure to dental X-ray radiation.

Methods: The study samples consisted of 40 participants and were divided into two groups: exposed (patients who came in for taking analog/digital panoramic radiography or cone beam CT) and controlled (no radiography examinations). Each group contained ten individuals. Exfoliative cytology smears have been taken from the gingival and buccal mucosa before (or on day 0 for the control group) and 10 days post-exposure. The Papanicolaou approach was used to stain the cells. The anucleated cells in each glass slide have been then counted.

Results: No significant differences (p > 0.05) were observed in anucleated cell numbers between days 0 and 10 in both the controlled and exposed groups. The anucleated cell numbers also confirmed no significant difference between the gingiva and buccal mucosa.

Conclusion: Analog/digital panoramic radiography and CBCT exposure do not cause increases in anucleated cell numbers, so keratosis may not occur in patients exposed to dental X-ray radiation.

Keywords: Papanicolaou test; keratosis; buccal mucosa; cone-beam computed tomography; panoramic radiography.

INTRODUCTION

Anucleated squamous cells (ANSC) are a symptom of superficial epithelial keratosis, which can lead to squamous intraepithelial lesions or squamous cell carcinoma (1). Increased keratin layer thickness (hyperkeratosis or keratosis) may have been caused by local frictional irritation, cigarettes, parafunctional behaviors, or more critical processes such as premalignant or malignant transformation. Tobacco smoking is a prevalent cause of mild keratosis, particularly on the palate, lip, and commissures. Parafunctional behaviors such as sucking, biting, or rubbing the oral mucosa against the teeth can cause keratoses on the tongue, lip, and buccal mucosa (2).

As a defensive strategy against a range of stressors, the surface of the squamous epithelium may develop a granular layer and multiple layers of anucleated cells, a condition known as hyperkeratosis. The most frequent oral keratotic lesion is hyperkeratosis, which manifests clinically as an area of leukoplakia (2).

Clinically, oral leukoplakia appears as a white plaque and histologically as a hyperkeratotic lesion with variable degrees of keratinization. Two kinds of hyperkeratosis may be distinguished microscopically: hyperkeratosis with dysplasia or carcinoma and hyperkeratosis without dysplasia or parakeratosis (3). The cytopathological pattern of the epithelium changes in leukoplakia. The number of intermediate or parabasal cells rises as the disorder of epithelial maturation worsens. The oral mucosal cell maturation pattern in individuals exposed to alcohol and tobacco revealed alterations that might be linked to increased cell numbers in the deeper epithelial layers. Those with leukoplakia with dysplasia showed a higher number of intermediate and parabasal cells than patients with leukoplakia without dysplasia (4).

This maturation pattern might also be found in the gingiva and buccal mucosa of people exposed to dental X-ray radiation. Intermediate cell frequency in the gingiva and buccal mucosa rose after analog/digital panoramic radiography and CBCT, but superficial cells decreased (5). Panoramic radiography is frequently used to examine bone changes in the oral cavity because it gives the dentist a broad view of the whole maxillomandibular complex, is simple to interpret and is a low-cost, easily accessible technique. The superimposition of anatomical features, on the other hand, is a fundamental limitation of the extensive use of panoramic radiography (6). Cone-beam computed tomography (CBCT) is a popular oral-maxillofacial imaging technique that produces precise 3D images of hard tissue structure (3). CBCT is becoming more popular because it offers considerable advantages over conventional panoramic radiography, such as simplicity of storage, improved contrast, and brightness control, and lower effective
Yanuaryska and Gracea: Does dental x-ray cause oral keratosis?

radiation dosage (6). In comparison to E-speed film, digital panoramic radiography requires 90% less dosage (7). Nonetheless, radiographic imaging techniques can cause cytotoxicity by changing the maturation pattern of gingival and buccal mucosa cells (5). However, whether dental X-ray radiation exposure causes oral keratosis is yet unknown. Thus, the objective of this study was to evaluate the number of ANSC that indicate a keratosis in gingival and buccal cells after X-ray exposure from analog/digital panoramic radiography and CBCT.

MATERIALS AND METHODS

Study design and sample

This cross-sectional observational study assessed individuals aged 25 and older who underwent analog or digital panoramic radiography or CBCT exams ordered by a dentist independent of this study at the Dental Hospital, Universitas Gadjah Mada, Indonesia, and was judged suitable for the study. The subjects of this study met the following inclusion criteria: (1) non-smoker and/or alcoholic drinker; (2) no radiographic imaging in the previous 3 weeks; (3) no apparent lesions in the oral mucosa; and (4) no use of orthodontic and prosthodontic equipment.

Ethical clearance had been granted by the Research Ethics Commission of the Faculty of Dentistry, Universitas Gadjah Mada, Indonesia. Each patient’s informed consent was acquired. Based on the previous study conducted by Shantiningsih and Diba (8), ten participants were chosen at random for each radiological test using a purposive sampling approach. The control group included an equal number of patients who did not receive a radiographic examination and were observed using the same research methodology. Analog panoramic radiographs were acquired using a Yoshida Panoura Deluxe System (The Yoshida Dental MFG. CO., LTD., Tokyo, Japan) with exposure parameters: 8–10 mA, 90 kVp, and 20 s.

A digital system using a Pax-i (Vatech, Gyeonggi-do, Republic of Korea) with parameter settings of 85 kVp, 6 mA, and 16.6 s.

Sample collection and cytopathological analysis

Smears were taken immediately before X-ray exposure and 10 days later from the gingival and buccal mucosa cells. Patients were asked to rinse their mouths with water for 30 seconds before smear collection. Following washing, smears were collected using a cytobrush over the gingival and buccal mucosa. Both gingival and buccal mucosa samples were taken twice, before the X-ray exposure and after the treatment on the right. The smears were placed on a glass slide and immersed in 96% alcohol. The Papanicolaou technique was used to stain the slides. A light microscope (YS100, Nikon, Japan) and Optilab Viewer 2.1 were used to examine the stained slides. A trained and blinded observer reviewed all of the slides. Cells were classified as ANSC according to the criteria described by Izadi-Mood et al. (1). The results were expressed in percentages.

Statistical data analysis

All data were performed as mean ± standard deviation (S.D.). Statistical analysis was carried out using IBM® SPSS® Statistics 25 version software (IBM Corporation, Armonk, New York). The normality criteria were assessed using Shapiro–Wilk test. Paired t-test and Wilcoxon signed-rank test were used to assess the difference in ANSC numbers before and after X-ray exposure. The statistical differences were significant if \( p < 0.05 \). The intraclass correlation coefficient (ICC) was utilized to determine the measurement's reproducibility. After the same examiner re-evaluated a randomly selected sample of 8 slides after 2 weeks, intra-rater reliability statistics for cell counts were obtained. The values were interpreted as poor (less than 0.5), moderate (0.5–0.75), good (0.75-0.9), and excellent (greater than 0.9) based on the 95% confidence interval (9).

RESULTS

As low doses of X-ray radiation have been found to affect maturation patterns, this study investigated whether dental X-ray radiation exposure may produce keratosis in gingival and buccal mucosa cells.
Fig. 1. Papanicolaou staining shows anucleated, superficial with nuclei and intermediate cells of oral mucosa. Magnification: 400×

Fig.1 depicts typical photomicrographs of anucleated, superficial with nuclei, and intermediate cells from a patient's buccal mucosa smears. Large polygonal cells with abundant thin pink to green cytoplasm and central pyknotic nuclei characterize the superficial cells. The intermediate cells are nearly identical in size and form to the surface cells, but they have a lot of greenish cytoplasm. The nucleus distinguishes superficial cells from intermediate cells. Compared to pyknotic nuclei of superficial cells, the nuclei in intermediate cells are relatively big and vesicular (10). ANSC appears as the commonly folded cells and pale yellowish pink with a central clear zone called "nuclear ghosts" (1). Intra-rater reliability measurement was excellent for ANSC (ICC = 1). The Shapiro–Wilk test revealed that the cell number was not normally distributed. Wilcoxon signed-rank test was performed to compare the number of ANSC before and after X-ray exposure.

Table 1 demonstrates the mean frequency differences in percentages of ANSC in gingival and buccal mucosa cells before and after receiving analog/digital panoramic or CBCT radiographic and the control group. There were no significant changes in any cell types in the control group between days 0 and 10 (p > 0.05). The Wilcoxon signed-rank test revealed that a modest number of ANSC were identified following radiation exposure; however, the number was not significant (p > 0.05).

DISCUSSION

Exfoliative cytology is a straightforward, low-cost, and non-invasive technique for studying the effects of X-ray exposure (6). Papanicolaou staining is a cytology technique for determining the percentage of exfoliated cells. Thus, this method was applied in this study. ANSC, which can be single or in sheets, detects hyperkeratosis on Papanicolaou smears1. Maturation abnormalities are often associated with increased keratinization (4). Abnormal mitotic figures were commonly found under hyperkeratotic lesions (10). Moreover, there was a change in the maturation pattern in patients with leukoplakia without dysplasia accompanied by an increase in ANSC of about 41% (4). A prior study revealed that dental X-ray radiation altered the gingival and buccal mucosa maturation pattern (5). Therefore, this study assessed whether X-ray radiation from dental imaging also induces oral keratoses.

Oral mucosa differs significantly from other body mucosa and is more like skin in that it is covered by a stratified epithelium built up of several cell layers with varying maturation patterns. Keratinocyte bodies lose their cytoplasmic organelles and nucleus during maturation after producing keratin (11). The presence of anucleated cells in smears from the oral cavities has been well documented in studies related to repeated trauma to the mucosa or the use of tobacco (2,12,13).

This study showed that normal mucosa smears contained no ANSC, and no significant change was noted in the cell numbers for 10 days. This condition may be explained by the fact that dental X-ray radiation is not an agent that is constantly in contact with the oral mucosa, hence it does not cause chronic damage. This argument is based on a prior study that found that
hyperkeratosis is caused by prolonged physical or chemical damage, such as friction or tobacco use (10). Preclinical changes in epithelial cells caused by orthodontic equipment included a decrease in nuclear area and increased cell keratinization (13). Oral mucosa smears from smokers and alcohol drinkers had a higher number of keratinized cells (2,10,12). Repeated injury to the epidermis causes the proliferative rate of keratinocytes to rise, increasing keratin production (10).

The results from the present study suggest that routine X-ray dental imaging examinations, such as analog/digital panoramic and CBCT, may not induce oral keratosis. Further studies are recommended for risk groups such as tobacco smokers, alcoholics, or patients with orthodontic appliances who undergo repeated radiographic examinations.

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**CONFLICT OF INTEREST**

The authors declare that there are no conflicts of interest.

**REFERENCES**

Yanuaryska and Gracea: Does dental x-ray cause oral keratosis?