Biomonitoring of Genotoxic Effect in Children Exposed to Dental Radiographs during Pulpectomy Procedure—BMCyt Assay

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Abstract

Background: Radiography is one of the most valuable diagnostic tools used in comprehensive dental care. Radiation from dental radiographs was thought to cause cytogenetic changes and its plausible effects can remain for some hours, months, or generations especially in children.

Aims and objectives: To evaluate and compare the possible genotoxic effect of routinely used intraoral periapical radiographic exposure and radiovisiographic exposure in exfoliated epithelial cells as measured by the formation of micronuclei during single visit pulpectomy procedure using Buccal Micronucleus Cytome (BMCyt) assay in children.

Materials and methods: Study comprised 60 healthy children who has undergone either intraoral periapical radiography (IOPAR; group 1, n = 30) or radiovisiography (RVG; group 2, n = 30) during various steps of single visit pulpectomy procedure. Cytological smears were taken from the buccal mucosa immediately before the X-ray exposure and 10 ± 2 days after exposure. The cells were stained with Feulgen and evaluated for micronuclei by scoring 1,000 cells per sample.

Results: The genotoxic effect of radiation exposure from intraoral periapical radiography higher than that of RVG showing significant increase in micronucleus (MN) formation.

Conclusion: The X-ray radiation emitted during IOPAR or RVG does induce genotoxic changes in the form of increased frequency of micronuclei. So, great care and standard protocol should be followed to advice radiographs if necessary and reduce the cumulated biological effects of radiation exposure.

Keyword: Taking into account the strong evidence of a relationship between DNA damage and carcinogenesis and the extensive application of intraoral radiographs in pediatric dentistry, it would be useful to know to what extent these dental X-rays cause genotoxic effects resulting in DNA damage on oral mucosa.

Keywords: Buccal micronucleus cytome assay, Fluorescence microscope, Intraoral periapical radiography, Micronuclei, Intraoral periapical radiography, Radio visiography.

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Introduction

Roentgenography has become an unavoidable diagnostic tool in successful dental practice. It plays vital role in treatment planning of the disorders of oral cavity. In pediatric dentistry, radiography has a unique role in daily practice by introducing a child for a dental treatment and most of the dentists generally rely heavily on conventional periapical or digital imaging to confirm or supplement their clinical examination.

Pulpectomy is the ideal treatment option for preserving a pulply involved primary tooth. It ensures proper eradication of bacteria and their products so that the primary teeth can complete its function until normal exfoliation without harming the successor or affecting the health of the patient. Generally pulpectomy requires radiographs for assessing condition of the tooth preoperatively, working length of the tooth intraoperatively, and finally postoperatively to assess quality of obturation resulting in multiple radiation exposure. During intraoral periapical radiography or RVG the epithelium of buccal mucosa is directly exposed to ionizing X-ray radiation.

Ionizing radiation is a widely known mutagen and carcinogen owing to its ability to deposit energy within the cells. Ionizing radiation has been described as a double-edged sword, and there is no doubt about the risk that exposure to high doses of ionizing radiation poses for human health. There is no margin of safety for dosage as the risk linked with low-level diagnostic exposures could be expected to be low but greater than zero. One of the reason which brings about cytotoxicity is the ionizing radiation which acts primarily on the DNA molecule or incidentally through the formation of reactive compounds that interact with the DNA molecule. Moreover, intercellular outcomes of ionizing radiation are progressive and budding and young and rapidly growing immature tissues are more radiosensitive than mature tissues; hence, children are at greater risk for cytotoxic effects and DNA damage. Hence it is mandatory to detect the early

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changes caused by these ionizing radiation exposures, to know the level of genetic damage induced. Copious biomarkers are utilized at the present time to evaluate DNA-induced genetic destruction. One among them is the BMCyt assay that discloses the genotoxic injury with the identification of the presence of micronucleus (MN). 3

The study of MNs has gained ground as a biomonitoring evaluation for human genotoxic exposure and its consequences because it is noninvasive, the scoring is simple, it requires shorter training, it is less time-consuming, and accuracy is procured from scoring a large number of cells with finer patient adoption. 4 It can be used as an index of DNA damage, instability of chromosome, cell death, and the regenerative potential of human buccal mucosa. 4

Taking into account the strong evidence of a relationship between DNA damage and carcinogenesis 5 and the extensive application of intraoral periapical radiographs and radiovisiographs in pediatric dentistry, it would be functional to know to what degree these dental X-rays cause genotoxic effects resulting in DNA damage on oral mucosa. The feasible genotoxic effects from these dental radiations in children during pulpectomy procedure as assessed by MN formation, has not yet been satisfactorily probed in the literature. Hence the present study is aimed to assess the genotoxic effect in the exfoliated epithelial cells of buccal mucosa from children following dental radiography during pulpectomy procedure by using BMCyt assay.

**Materials and Methods**

**Source of Data**

The present study was conducted on 60 healthy children, who were advised for intraoral periapical radiography and RVG as a part of diagnosis and treatment for deep carious lesions and are referred to the Department of Pediatric and Preventive Dentistry, RajaRajeswari Dental College and Hospital, Bengaluru.

Healthy children aged between 5 and 9 years who were selected for single-visit pulpectomy procedure in primary molars were included in this study. Exclusion criteria include children with systemic diseases, physically/mentally disabled, prior radiographic exposure in the previous 6 months, recent use of antibiotics, and repeated aphthous stomatitis or any other skin reactions.

**Sample Collection**

Sixty children, who fulfilled the inclusion criteria along with signed informed consent were selected for the study. Before sample collection subjects were asked to rinse their mouth thoroughly with normal water to remove unwanted debris and the exfoliated buccal mucosa cells was collected by scraping the right/left buccal mucosa with a wooden spatula (Fig. 1). Immediately before the X-ray exposure, first sample was obtained. Then the subjects underwent single visit pulpectomy procedure for the deep carious lesion during which they have exposed to multiple radiograph preoperatively, during working length determination and finally postobturation radiograph. Thirty children were advised for intraoral periapical radiographs and 30 children were advised for radiovisiographs. Second sample was collected 10 (±2) days after exposure.

**Group 1** ($n=30$)—Subjects exposed to IOPAR
- S1—sample collected immediately before the X-ray exposure
- S2—sample collected 10 (±2) days after the X-ray exposure

**Group 2** ($n=30$)—subjects exposed to RVG
- R1—sample collected immediately before the X-ray exposure
- R2—sample collected 10 (±2) days after the X-ray exposure

**Exfoliated Buccal Cell staining for Microscopic Evaluation**

After centrifugation collected buccal mucosal cells were first stained with hematoxylin and eosin (H&E) stain and confirmed the presence of adequate amount of cells under 100× microscope (Fig. 2). Then rest of the collected samples were smeared over the microscopic slides and initial fixation was done using in ethanol: acetic acid (3:1) for 10 minutes. Then the slides were air dried and coded according to group and subject. Then serial fixation was done by immersing in 50% (vol/vol) and 20% (vol/vol) ethanol for 1 minute each , respectively. Then washed for 2 minutes using Milli-Q water and transferred into a Coplin jar with 5 M HCl for 20 minutes and washed out in running tap water for 3 minutes. The slides were drained, but not allowed to dry out and placed in a Coplin jar containing Schiff’s reagent for 90 minutes in the dark at room temperature. The slides were then rinsed in running tap water for 5 minutes and then in Milli-Q water. Light green 0.2% (wt/vol) was used to counter stain the cells for 20–30 seconds and

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**Fig. 1:** Collection of exfoliated buccal mucosal cells using sterile wooden sticks

**Fig. 2:** Exfoliative buccal mucosal cells showing cytoplasm and nucleus (H&E stain 100x)
rinsed well in Mill-Q water. The slides were immediately placed facedown onto Dr. Watts no.1 filter paper to remove any residual moisture. Then the slides were placed on a slide tray and allowed to dry for about 10 to 15 minutes. The efficiency of staining and the density of the cells were seen under 100x and assessed under 400× magnification. Before placing coverslip with DPX, the slides were dried completely for at least 30 minutes and the slides were stored in slide boxes at room temperature. The slides were assessed first using transmitted light microscopy under 200x and 400×, the nuclei and the micronuclei were magenta in color, whereas the cytoplasm appeared pale blue/green (Figs. 3 and 4). And later the cells were viewed under fluorescence using Olympus BX41 research microscope with a far-red filter.

Buccal Micronucleus Cytome Assay Analysis
A minimum of 1,000 cells will be studied by blind analysis for each individual. Two hundred fifty intact epithelial cells were scored in each slide for the presence of micronuclei. Since 4 slides per subject was scored, a total of 1,000 cells were scored per subject. Slides are evaluated using criteria for nuclear abnormalities by Tolbert et al.6 to determine the MN frequencies.

Determination of Micronucleus Frequencies
Criteria for inclusion in the total cell count are the following:
- Cytoplasm intact and lying relatively flat
- Little or no overlap with adjacent cells
- Little or no debris
- Nucleus normal and intact, nuclear perimeter smooth distinct

The inclusion criteria for MN include the following:
- Rounded, smooth perimeter suggestive of membrane
- Less than a third the diameter of nucleus, but large enough to discern shape and color
- Feulgen positive (i.e., Brightfield illumination)
- Staining intensity similar to that of nucleus
- Texture similar to nucleus
- Same focal plane as nucleus
- Absence of overlap with/or bridge to nucleus.

Statistical Analysis
The comparison between the groups were done using Mann-Whitney U test and within the groups was done using Wilcoxon signed rank test. The tests were done using SPSS software version 20.2. and “p” value less than 0.05 was taken to be statistically significant.

RESULTS
- Intraoral periapical radiography showed more genotoxicity in terms of micronuclei which is 1.5-fold higher than that of RVG (Table 1 and Fig. 5).
- Intraoral periapical radiography causes increase in micronuclei frequency with postexposure (group 1B) compared to preexposure (group 1A) with a statistically significant “p” value (p < 0.001) (Table 2 and Fig. 6).
- Radiovisiography also causes increase in micronuclei frequency with postexposure (group 2B) compared to preexposure (group 2A) with a statistically significant “P” value (p < 0.001) (Table 2 and Fig. 7).
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Children have higher amenability to the threatening effects of ionizing radiation because their maturing tissues are inherently more radiosensitive and they have more remaining years of life during which a radiation induced changes could develop and express.

The prime objective of current concepts of dentistry is to maintain the integrity of the primary dentition until their normal exfoliation for the purpose of promoting function, esthetics, and phonetics. Pulpectomy is one of such treatment options for maintaining primary teeth with radicular pulpal tissue inflammation which requires multiple radiographs. IOPAR or RVG is routinely advised in pediatric practice during endodontic procedure and hence it is important to study the various changes in and around

## Discussion

Genomic damage is probably considered as the prime root of cause for developmental and degenerative diseases. According to literature review there are various genetic, environmental, and lifestyle factors which causes genotoxicity through direct or indirect effects on the DNA. Among these etiologic agents, ionizing radiation is the prime input to human exposure because of its wide usage in diagnostic and therapeutic areas. Exposure to low-level radiations/diagnostic X-rays can destruct living cells by either causing cell death or by mutations or other collective changes in the DNA that can accumulate to the point where the normal controls on cell division is lost and the cell becomes anomalous and abnormal. However, children have higher amenability to the threatening effects of ionizing radiation because their maturing tissues are inherently more radiosensitive and they have more remaining years of life during which a radiation induced changes could develop and express.

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### Table 2: Comparison of mean number of micronuclei/1,000 cells between pre- and postexposure time period within intraoral periapical radiography (IOPAR) and radiovisiography (RVG) groups using Wilcoxon signed rank test

| Group | Time   | N  | Mean | SD  | Mean diff | Z     | p       |
|-------|--------|----|------|-----|-----------|-------|---------|
| IOPAR | Before | 30 | 0.70 | 0.84| -23.57    | -4.799| <0.001* |
|       | After  | 30 | 24.27| 2.68| -14.66    | -4.845| <0.001* |
| RVG   | Before | 30 | 0.57 | 0.73| -14.66    | -4.845| <0.001* |
|       | After  | 30 | 15.23| 1.07|           |       |         |

*Statistically significant.
the cellular system and genetic damages associated with these rays. Periapical radiography and RVG describe under intraoral radiographic techniques designed to provide detailed information about pathologic changes associated with primary teeth, pulp calcification, or root resorption and also root-end condition and environment. It also used to evaluate pulp treatment, to detect development abnormalities, and analyze space in primary and mixed dentition.

Thus our study has evaluated and compared the MN frequency in exfoliated buccal mucosal cells pre- and postexposure to intraoral periapical radiography and radiovisiography during single visit pulpectomy procedure by using BMCyt assay. A total of 60 subjects submitted to either intraoral periapical radiographs or radiovisiographs during different phases of pulpectomy procedure were investigated in this study.

The results of our study suggested that intraoral radiographic exposure can induce a discernable increase in the number of micronuclei in buccal epithelial cells in the postexposure when compared to preexposure period (Figs 8 to 10). Intraoral periapical radiographs showed a statistically significant increase in MN frequency with postexposure compared to preexposure with "p" value \( p < 0.001 \) (Table 2; Figs 7 and 9). These results are comparable to a study which compared the genotoxic effect induced by periapical radiography and panoramic radiography which concluded that the frequency of micronuclei after exposure was significantly higher in patients who underwent periapical radiography rather than panoramic radiography indicating of more genotoxic effects induced by intraoral periapical radiograph. According to yet another study, the frequency of micronuclei increases significantly postexposure in bitewing and digital dental panoramic radiography in children, but the frequency was higher in bitewing radiographs.

In our study RVG also showed statistically significant increase in MN frequency with postexposure compared to preexposure with "p" value \( p < 0.001 \) (Table 2; Figs 7 and 10). So far in the literature there are no studies have been done with respect to radiographic exposure changes using radiovisiograph. The present study also indicated that significant genotoxic effects were induced by intraoral periapical radiography when compared to radiovisual radiography. This can be explained by higher radiation dose with targeted time of 0.8 seconds and also the buccal mucosal site of interest is the direct point of focus of cone beam radiation with comparatively decreased amount of scattered radiation in intraoral periapical radiation exposure. In contrast, in RVG is radiation dosage is 50–80% less than conventional radiography because of the use of digital detectors and less exposure time resulting could be the reason for less number of micronuclei. As per our knowledge, this is the first study in the literature, which has dealt with the MN formation between radiovisiographic exposure and periapical radiographic exposure using fluorescent microscopy.

Manpreet (2016) defined MN as a microscopically visible, round, or oval cytoplasmic chromatin mass next to the nucleus which are derived from both chromosomal fragments and whole chromosomes lagging behind in anaphase. The two basic phenomena responsible for the formation of MN in mitotic cells are dysfunction of the mitotic spindle apparatus—aneugenic event or chromosomal breakage—clastogenic event. X-rays are clastogenic agents that induce the formation of micronuclei, in addition to other nuclear alterations. The criteria developed by Tolbert et al. for identification and classification of the nuclear

Fig. 8: Normal exfoliative buccal mucosal cells: Before exposure (Fuelgen stain under fluorescent microscopy 400x)

Fig. 9: Exfoliative buccal mucosal cells with micronucleus after exposure to IOPA radiography (Fuelgen stain under fluorescent microscopy 400x)

Fig. 10: Exfoliative buccal mucosal cells with micronucleus after exposure to radiovisiography (Fuelgen stain under fluorescent microscopy 400x)
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anomalies is most widely used for identification of micronuclei. Even though peripheral blood lymphocytes, fibroblasts, erythrocytes, and alveolar macrophage, can be used for the determination of MN, exfoliated buccal epithelial cells represent a preferred site in children as the buccal epithelium is under direct radiation exposure to IOPAR and RVG. Advantages such as noninvasive, simple scoring, shorter training, explain that they do not have to be cultivated and do not require stimulation or metaphase preparation to identify micronuclei and the results shown were more accurately reflects genomic instability events in epithelial tissues. In our study the genotoxic effects of the dental radiation was assessed in exfoliated buccal mucosal cells using BMCyt assay.

The oral epithelium maintains lifecycle by continuous cell renewal, where new cells are generated in the basal layer by mitosis and migrate to the surface of those and shed which takes about 7–21 days. Thus, in the design of our study required a time period of 10 ± 2 days to detect the maximum effect of radiation exposure. Metaphase chromosomal aberrations, sister chromatid exchanges, and host cell reactivation assays can be also used for monitoring, but these methods are typically strenuous, time consuming, and require highly trained technicians to interpret slide.

In the present study, fluorescent microscopy was used to accurately identify and visualize the cell nuclei and MN using Feulgen staining method, and also to minimize the incidence of false positives or false negatives. Advanced research methods have an upper hand as a sensitive indicator of low-dose radiation exposure in children. It includes activated histone 2AX (Y-H2AX) and activated checkpoint kinase 2 (pChk2), which are DNA damage response molecules in irradiated cells and fluorescence in situ hybridization analysis.

In literature various studies have done using micronuclei as a biomarker for assessing postexposure changes to dental radiography including panoramic radiography. There are many studies which have concluded that the frequency of formation of micronuclei was statistically significant after exposure to panoramic radiography and also some other authors have showed significant increase in the frequencies of other nuclear alterations. Few other studies have found overall increase in mean micronuclei number after exposure to panoramic radiography, but the increase in number was statistically insignificant. On the contrary, studies in children concluded that panoramic radiography might not induce chromosomal damage, but may be cytotoxic. Recent studies with cone-beam computed tomography (CBCT) have shown that mutagenicity was not induced by CBCT but in contrast cytotoxicity can be appreciated.

The BMCyt assay can identify a 16-fold increase in MN in oral cancer patients after completion of treatment with photons. The buccal mucosa also has the potential to be utilized to identify inherited genomic instability such as Bloom’s syndrome. Other study has also conducted and defined MN as an early diagnostic tool of leukoplakia and squamous cell carcinoma.

It is also wise to note that, in our study micronuclei found in group 1 and group 2 pre-X-ray radiation may be due to diverse environmental factors, age, oral hygiene, lifestyle factors such as nonvegetarian diet. This is in light with the other studies, which proved that lifestyle factors including smoking, alcohol consumption, and diet especially vitamin deficiencies and supplementation have direct influence on increase in MN. The detection of micronuclei in the preexposure stage along with the postexposure to X-ray radiation has significant influence in rise of MN. But children are minimally affected by confounders which are of great concern in adults.

Regarding the demographic details of study population, the present study showed female predominance in micronuclei formation compared to their counter parts and a marginal increase in the frequency of micronuclei with age in both the study population (Table 3 and Fig. 6). Thus our study also concluded exposure to intraoral periapical radiography showed more genotoxicity in terms of micronuclei which is 1.5-fold higher than that of RVG (Table 1 and Fig. 5).

The limitations that were encountered in our study were the time-consuming laboratory procedures, and proper spotting of MN was strenuous, as we performed manual staining and a visual examination count. Although the study concluded statistically significant results, further studies with large sample sizes, which are epidemiological in nature and conducted under different clinical scenarios and with different radiographic exposure, with different age groups and using automatic counting are suggested. Further studies are also needed to investigate the health effects of dental diagnostic X-rays in dental practitioners, who may be frequently exposed to high levels of radiation exposure. Advanced research using various biomarkers such as Y-H2AX and pChk2, fluorescence in situ hybridization (FISH) analysis may also be recommended for analyzing radiation effects.

Thus radiography should only be performed when a child’s history and/or symptoms and objective finding lead to the conclusion that further useful information might be obtained. Also an expanded protocol for the MN test should be adopted, as the procedure is noninvasive, cheap, and easy to detect genotoxic effects of radiation in exfoliated buccal cells in children. Keywords for good practice are appropriate selection criteria for

Table 3: Comparison of age and gender distribution among study subjects between two groups using Chi-square test

| Variables | Category | IOPAR | RVG | \( \chi^2 \) | p |
|-----------|----------|-------|-----|----------------|---|
| Age       | 5 years  | 3     | 10.0| 4   | 13.3| 0.567| 0.97 |
|           | 6 years  | 5     | 16.7| 6   | 20.0|     |     |
|           | 7 years  | 5     | 16.7| 5   | 16.7|     |     |
|           | 8 years  | 10    | 33.3| 10  | 33.3|     |     |
|           | 9 years  | 7     | 23.3| 5   | 16.7|     |     |
| Sex       | Males    | 17    | 56.7| 18  | 60.0| 0.069| 0.79 |
|           | Females  | 13    | 43.3| 12  | 40.0|     |     |

IOPAR, intraoral periapical radiography; RVG, radiovisiography
use of radiography, using an accurate radiographic technique, optimized radiation protection, and utilization of the total amount of information in each radiograph in order to avoid unnecessary repetition thus reducing more exposure to each patient.

CONCLUSION
Roentgenography may induce detectable amount of genotoxicity in terms of micronuclei at each exposure. The present study also indicated that significant genotoxic effects were induced by intraoral periapical radiography when compared to RVG due to substantially decreased radiation dose. So radiographs should be indicated only when necessary, using an accurate radiographic technique and follow current radioprotection criteria, in order to avoid unnecessary repetition. Although dental radiography contributes to a small dose—but not necessarily to insignificant portion, dental X-rays should always follow the concept of maximum benefit with minimum risk.

CLINICAL SIGNIFICANCE
Among various genetic, environmental, and lifestyle factors which cause genotoxicity, ionizing radiation forms the bulk of contribution to human exposure because of its wide use for diagnostic and therapeutic purposes. Children have higher vulnerability to radiation effects because of the more radiosensitive cells.

Pulpectomy is one of the treatment options used to maintain primary teeth with radicular pulpal tissue inflammation which requires multiple radiographs for assessing condition of the tooth preoperatively, working length of the tooth intraoperatively and finally postoperatively to assess quality of obturation. IOPAR or RVG is routinely advised in pediatric practice during endodontic procedure and hence it is important to study the various changes in and around the cellular system and genetic damages associated with these rays.

In the literature various studies have done using micronuclei as a biomarker for assessing postexposure changes to dental radiography including panoramic radiography. The present ex-vivo study was conducted to evaluate and compare the extent of genotoxic changes using MN frequency in exfoliated buccal mucosal cells related to before and after exposure to intraoral periapical radiography and RVG in children. Our study is the only study in the literature so far which assessed and compared the genotoxic effects of conventional and digital imaging, which are commonly used in daily practice. It is the first clinical study which is based on our routine clinical procedure like pulpectomy and we got the knowledge of extent of effects of frequent radiation exposure. Study concluded that roentgenography may induce detectable amount of genotoxicity at each exposure. So radiographs should be indicated only when necessary, using an accurate radiographic technique and follow current radioprotection criteria, in order to avoid unnecessary repetition. Keywords for good practice are technique and follow current radioprotection criteria, in order to avoid unnecessary repetition. Although dental radiography contributes to a small dose—but not necessarily to insignificant portion, dental X-rays should always follow the concept of maximum benefit with minimum risk.

REFERENCES
1. Fukus AB. Pulp therapy for the primary dentition. In: Pinkham JR, ed. Pediatric Dentistry: Infancy Through Adolescence. 1st ed. Philadelphia, PA: Elsevier;2005 pp. 333–351.
2. Agarwal P, Vinuth DP, Haranal S, et al. Genotoxic and cytotoxic effects of X-ray on buccal epithelial cells following panoramic radiography: A pediatric study. J Cytol 2015;32:102–106. DOI: 10.4103/0970-9371.160559
3. Preeti N, Chikkanarasaih N, Bethur SS. Genotoxic effects of X-rays in buccal mucosal cells in children subjected to dental radiographs. BDJO Open 2016;2:16001. DOI: 10.1038/bdopen.2016.1
4. Thomas P, Holland N, Bolognesi C, et al. Buccal micronucleus cytome assay. Nat Protoc 2009;4:825–837. DOI: 10.1038/nprot.2009.53
5. Ribeiro DA, De Oliveira G, De Castro GM, et al. Cytogenetic biomonitoring in patients exposed to dental X-rays: comparison between adults and children. Dentomaxillofac Radiol 2008;37:404–407. DOI: 10.1259/dmfr/58548698
6. Tolbert PE, Shy CM, Allen JW. Micronuclei and other nuclear anomalies in buccal smears: methods development. Mutat Res 1992;271:69–77. DOI: 10.1016/0165-1161(92)90033-i
7. Cerqueira EM, Meireles JR, Lopes MA, et al. Genotoxic effects of X-rays on keratinized mucosa cells during panoramic dental radiography. Dentomaxillofac Radiol 2008;37:398–403. DOI: 10.1259/dmfr/56848097
8. Ingle JJ, Bakland LK, Baumgartner JC. Ingle’s, Bakland, Baumgartner. Ingles Endodontics, 6th Ed. Endodontic Therapy for Primary Teeth. Hamilton: BC Decker Inc.; 2008. pp. 1400–1430.
9. Mathewson RJ, Robert E. Radiographic Assessment. In: Primosch. Fundamentals of Pediatric Dentistry, 3rd ed. Carol Stream: Quintessence Books; 1995. pp. 35–55.
10. Mohan N, Ravikumar PT, Madhumitha C. Genotoxic and cytotoxic effects following dental and panoramic radiography. Indian J Oral Sci 2016;7:92–98. DOI: 10.4103/0970-9371.160559
11. Ingle JJ, Bakland LK, Baumgartner JC. Ingle’s Endodontics, 6th ed. Digital Imaging; 2008. pp. 580–589.
12. Shashikala R, Indira AP, Manjunath GS, et al Role of micronucleus in oral exfoliative cytology. J Pharm Bioallied Sci 2015;7:5409–413. DOI: 10.4103/0975-7406.163472
13. Jain V, Lohra P, Priya B, Singh D. Buccal cell micronuclei assay: a non-invasive genotoxic marker. UCMR 2017;4:100–104. ISSN (Online): 2393-915X; (Print): 2454-7379
14. Manpreet SD, Sonal G, Jatinder B, et al. Micronucleus as a non-invasive biomarker—a review. JAMPS 2016;6:1–9. DOI: 10.9734/JAMPS/2016/24136
15. Jois HS, Kale AD, Mohan Kumar KP. Micronucleus as potential biomarker of oral carcinogenesis. IJDA 2010;2:197–202. DOI: 10.4103/0975-7406.163472
16. Neri M, Fuccia A, Knudsen LE, Lando C, Merlo F, Bonassi S. Micronuclei frequency in children exposed to environmental mutagens: a review. Mutat Res 2003;544:243–254. DOI: 10.1016/j.mrrev.2003.06.009
17. Popova L, Kishkiulova D, Hadjidekova VB, et al. Micronucleus test in buccal epithelial cells from patients subjected to panoramic radiography. Dentomaxillofac Radiol 2007;36(3):168–171. DOI: 10.1259/dmfr/29195361
18. Arora P, Devi P, Wazir SS. Evaluation of genotoxicity in patients subjected to panoramic radiography by micronucleus assay on epithelial cells of the oralmucosa. J Dent 2014;11:47–55. pubmed id 24910676.
19. Vidya KB, Kalapannavar AN, Muniyappa M. Genotoxic effects of panoramic radiation by assessing the frequency of micronuclei formation in exfoliated buccal epithelium. Int J Res Med Sci 2014;2:541–544. DOI: 10.4103/2320-6012.154033
20. Veeracharil U, Venkatesh S, Yadav A, et al. Biomonitoring genetic instability in normal healthy population using a simple cytogenetic marker- micronucleus test. Internat Multidiscipl Res J 2011;1:1–9. corpus ID 82420054
21. Madhavan R, Kumarswamy M, Kum SM. Genetic damage in exfoliated cells from oral mucosa of individuals exposed to rays after panoramic

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22. Yoon AJ, Wu HC, Angelopoulos C, et al. Expression of activated checkpoint inase 2 and histone 2AX in exfoliative oral cells after exposure to ionizing radiation. Radiat Res 2009;171:771–775. DOI: http://dx.doi.org/10.1667/R08560.1

23. Waingade M, Medikeri RS. Analysis of micronuclei in buccal epithelial cells in patients subjected to panoramic radiography. Indian J Dent Res 2012;23(5):574–578. DOI: 10.4103/0970-9290.107329

24. Sandhu M, Mohan V, Kumar JS. Evaluation of genotoxic effect of X-rays on oral mucosa during panoramic radiography. J Indian Acad Oral Med Radiol 2015;27:25–27. DOI: 10.4103/0972-1363.167070

25. Angelieri F, de Oliveira GR, Sannomiya EK, et al. DNA damage and cellular death in oral mucosa cells of children who have undergone panoramic dental radiography. Pediatr Radiol 2007;37:561–565. DOI: 10.1007/s00247-007-0478-1

26. El-Ashiry E, Abo-Hager E, Gawish A. Genotoxic effects of dental panoramic radiograph in children. J Clin Pediatr Dent 2010;35:69–74. DOI: 10.17796/jcpd.35.1.y613824735287307

27. Lorenzoni DC, Fracalossi ACC, Carlin V, et al. Cytogenetic biomonitoring in children submitting to a complete set of radiographs for orthodontic planning. Angle Orthodont 2012;82:585–590. DOI: 10.2319/072311-468.1

28. Haghgoo R, Sedaghat R, Ahmadvand M, et al. Cytological Changes of oral mucosa following lateral cephalometry and panoramic radiograph. Int J Dent Clin 2014;6:1–3.

29. Carlin V, Artioli AJ, Matsumoto MA, et al. Biomonitoring of DNA damage and cytotoxicity in individuals exposed to cone beam computed tomography. Dentomaxillofac Radiol 2010;39(5):295–299. DOI: 10.1259/dmfr/17573156

30. Rosin MP, German J. Evidence for chromosome instability in vivo in Bloom syndrome: increased numbers of micronuclei in exfoliated cells. Hum Genet 1985;71:187–191. DOI: 10.1007/BF00284570

31. Kamboj M, Mahajan S. Micronucleus - an upcoming marker of genotoxic damage. Clin Oral Invest 2007;11:121–126. DOI: 10.1007/s00784-006-0075-y