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RESEARCH ARTICLE

MICRONUCLEUS DETERMINATION IN CHILDREN SUBJECTED TO IOPA RADIOGRAPH DURING DIAGNOSIS OF CARIOUS LESIONS

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ABSTRACT

Background: Radiographic examination has a great value in the detection of carious lesions, especially the diagnosis of the smooth surface caries on the interproximal areas, thereby preventing extensive tooth loss. Periapical radiographs are primarily useful for detecting the extent of the wide carious lesions and changes around the teeth mainly pulp and periodontium. However, it is well known that ionizing radiation is capable of causing genetic damage to cellular systems. One of those genetic alteration is the formation of micronuclei which are not included into the main nuclei of the daughter cells and are used to evaluate the magnitude of DNA damage. ***Objectives:*** To evaluate micronucleus frequency in exfoliated buccal mucosa cells of healthy children before and after the exposure to IOPA radiography during diagnosis and treatment of proximal carious lesion, using Buccal Micronucleus Cytome assay. ***Methods:*** A total of 20 children between the age group of 4-8 years were involved in the study. Cytological smears are taken from the buccal mucosa immediately before the X-ray exposure and 10 ± 2 days after exposure. The cells were stained with fuelgen and evaluated for micronuclei by scoring 1000 cells per sample. ***Results:*** The frequency of micronuclei increases significantly post exposure to IOPAR with a *'p' Value of 0.001.* ***Conclusion:*** The X-ray radiation emitted during IOPAR does induce some genotoxic changes in the form of increased frequency of micronuclei. So, great care to be taken only to advice radiographs if necessary and reduce these cumulated biological effects of radiation exposure.

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INTRODUCTION

The radiographic examination is a fundamental part of comprehensive diagnosis as well as treatment of dental diseases. Over the years x-ray has become an important auxiliary to successful dental practice especially pediatric dentistry, as it provides a pleasant and painless method of introducing the child to dental treatment during their first dental visit. Most of the dentists are generally rely heavily on conventional periapical X-ray films to confirm or supplement their clinical examination. One of the most frequent indication for radiographic examination of the child is the evaluation of carious involvement of the interproximal surfaces of the posterior teeth. However, every X-ray exposure carries with it a risk to the patient, and such risk considerations are even more critical in the radiologic examination of the young patient (Valachovic and Lurie, 1980). 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 .The risk associated with low-level diagnostic exposures could be expected to be low but greater than zero (Agarwal *et al.,* 2015).

Generally in dental radiographic methods, the oral buccal epithelium is directly exposed to ionizing X-ray radiation. Many approaches and techniques have been developed for the monitoring of human populations exposed to various mutagens (Decordier *et al.,* 2011). The micronucleus assay is a suitable internal dosimeter for revealing tissue specific genotoxic damage in individuals exposed to carcinogens (Vidya *et al.,* 2014). Micronuclei (MN) are derived from both chromosomal fragments and whole chromosomes lagging behind in anaphase, (Vidya *et al.,* 2014) and can be induced by substances that cause chromosome breakage (clastogens) as well as by agents that affect the spindle apparatus (aneugens) (Angelieri *et al.,* 2007). Taking into account the strong evidence of a relationship between DNA damage and carcinogenesis (Ribeiro *et al.,* 2008) and the extensive application of intraoral periapical 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. Hence, the present study is carried out to evaluate and compare the DNA damage in oral exfoliated cells from children following single periapical radiograph, using BMCyt assay.

MATERIALS AND METHODS

The present study was conducted after obtaining approval from the Instutional Ethical Committee and signed written informed consent from parents of 20 healthy children, who were advised to undergo intraoral periapical radiograph as a part of their diagnostic procedure and who were referred to the Department of Pedodontics and Preventive Dentistry, Rajarajeswari Dental College and Hospital, Bangalore.

Inclusion criteria

1. Healthy Children who were subjected to single periapical radiograph for diagnosis of proximal caries.
2. Age range- 4 to 8 years.

Exclusion criteria

1. Presence of systemic diseases or being differently abled.
2. Radiographic exposure within the last 6 months.
3. Recent use of antibiotics.
4. Repeated aphthous stomatitis and skin reactions.

Screening and sample collection

In this ex vivo study, 20 children included and were asked to rinse their mouth with normal water to eliminate any unwanted debris before sample collection. Exfoliated buccal mucosal cells were collected by scraping the right/left buccal mucosa with a wooden spatula immediately before the radiographic exposure and 10 ± 2 days after exposure.

Exfoliated buccal cell staining for microscopy

The buccal mucosal cells were transferred to the microscope slides and were allowed to dry. Initial fixation was done using ethanol: acetic acid solution at a ratio of 3:1 followed by serial fixation using 50% (vol/vol) and 20% (vol/vol) ethanol for 1 min, respectively. After washing in distilled water for 2 min, the slides were treated with 5 M Hcl for 20 min and then rinsed with distilled water thoroughly for 3 min. They were then drained, but not allowed to dry out, and were stained with Schiff's reagent for 90 min in the dark at room temperature. The slides were then rinsed in running tap water for 5 min and rinsed well in distilled water. Then the cells were counterstained with 0.2% (wt/vol) Light Green for 20–30 s and rinsed well in distilled water. To remove any residual moisture, the slides were immediately placed face down onto Dr Watts no. 1 filter paper and were allowed to dry for 10–15 min. The efficiency of staining and the presence of cells were examined at × 100 and × 400 magnification, respectively. Thereafter, the slides were dried completely for at least 30 min before placing a coverslip with distrene dibutylphthalate xylene (DPX) and the cells were viewed under fluorescence with a far-red filter and Feulgen-stained DNA appeared bright red in color.

BMCyt assay analysis

Coded slides were examined for 1,000 cells per subject at × 400 magnification. In each slide, 250 intact epithelial cells were scored for the presence of micronuclei. As four slides per subject were scored, a total ssf 1,000 cells were scored per subject. The criteria for designating micro-nuclei was scored according to Tolbert *et al.* (1991) which includes:

a. Rounded smooth perimeter suggestive of a membrane.
b. Less than one third of the diameter of the associated nucleus, but large enough to discern shape and colour.
c. Staining intensity similar to that of the nu-cleus.
d. Texture similar to that of the nucleus.
e. Same focal plane as the nucleus.
f. Absence of overlap with, or bridge to, the nucleus.

Statistical method

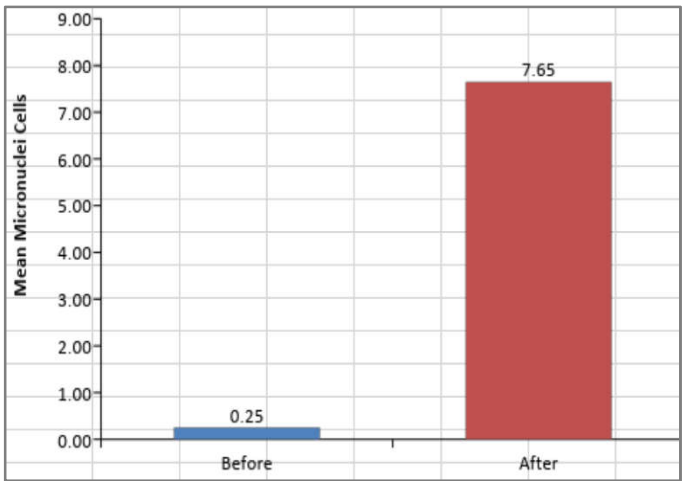
The data obtained were tabulated and subjected to statistical analysis with SPSS software version 20.0. Descriptive Statistics includes expression of micronuclei at before and after radiographic exposure in terms of Mean & SD. Wilcoxon Signed Rank test was used to compare the mean micronuclei between before and after radiographic exposure periods. '*p'-Value of 0.001* were considered statistically significant.

RESULTS

Table 1. Comparison of mean no. of total Micronuclei per 1000 cells between before and after radiographic exposure using Wilcoxon signed rank test

Variable	Time	N	Mean	SD	Mean Diff	Z	P-Value
Total	Before	20	0.25	0.72	-7.40	-3.853	<0.001*
	After	20	7.65	3.01			

*Statistically significant



Graph 1. Comparison of mean no. of Micronuclei per 1000 cells before and after radiographic exposure

In the present study, the genotoxic effects of X-Ray exposure during intraoral periapical radiographic examination were evaluated immediately before and after exposure. A total number of 20,000 cells were analysed for each group. The cells with micronuclei showed presence of both a main nucleus and one or more smaller nuclei. Table 1 shows the frequencies of micronucleated cells in children undergoing IOPAR. The mean frequency of cells with micronuclei before and after exposure were 0.25% and 7.65% respectively. These data are summarized in Graph 1. The difference in frequency of micronuclei was statistically significant (*P-Value of 0.001*) which indicates that the X-Ray can induce some amount of genotoxic changes in the buccal mucosal cells of childen.

DISCUSSION

Carcinogenesis is a multistep process governed by genetic or epigenetic mechanisms and signalling pathways that leads to the change in morphology and cellular behaviour resulting in mutations related to the control of cell division, cell death, and metastatic potential.[6] According to literature review there are various genetic environmental and life style factors which causes DNA damage and hence increases the risk of cancer. Among these agents, ionizing radiation forms the bulk of contribution to human exposure because of its wide use for diagnostic and therapeutic purposes. Ionizing radiation is a potent mutagenic agent, inducing both gene mutations and chromosomal aberrations (Cerqueira *et al.,* 2008). These roentogen rays are indispensable in modern medicine and dentistry as they have a cardinal tool in diagnosis, treatment planning, to assess prognosis and hence to deliver a comprehensive dental care. Even though radiation is widely used in diagnostic and therapeutic purpose, there is considerable concern about the potential harmful effects associated with radiation exposure (Vidya *et al.,* 2014). In general, children are more susceptible to toxic agents than adults, so ionizing radiation can be a mutagenic gent with cumulative action (Evelyn Louise Antonio *et al.,* 2017). Considering the fact that the individual may be repeatedly subjected to various types x-rays over a life time, there may be increase in the frequency of nuclear alteration following such events. Thus the cumulative effect of small doses on sensitive tissues could trigger cytotoxic effects, resulting in chronic cellular aggregation, compensatory cell proliferation, tumor development and carcinogenesis. Our study has demonstrated the genotoxic effects associated with conventional intraoral radiography which is a mandatory supplemental tool in dental practice. This periapical imaging is used mainly for detection of caries, changes around the root and periodontium and to check the status of developing tooth. IOPA radiography is routinely advised in pediatric practice and hence it is important task to study the various changes in and around the cellular system and genetic damages associated with these rays.

Assessment of genotoxicity can be performed at different steps of the interaction as well as the effect of the mutagen on DNA. Today there are several well-established methods for evaluating the mutagenic potential of physical and chemical agents. One of those methods include the detection of micronucleus which is a very reliable noninvasive biodosimeter. The buccal epithelium is under direct exposure to dental radiographic examination, so it is a primary target for the radiation induced damage. In addition, unlike peripheral lymphocytes, epithelial cells can be easily and rapidly sampled, do not have to be cultivated, do not require stimulation or metaphase preparations; therefore, application of the micronucleus test in epithelial cells is considered as a sensitive tool to biomonitor genetic damage in populations exposed to several genotoxic agents. The possible genetic effect from IOPA radiography, by observing micronucleus occurrence in the buccal epithelial cells, has been assessed in our study. It is evaluated before and 8-12 days after exposure. This time period following exposure is enough for the formation of micronucleus on the basis of turnover in the epithelial cell kinetics (Popova *et al.,* 2007). Micronuclei are the microscopically visible round or oval cytoplasmic chromatin mass in the extranuclear vicinity. They originate from mitosis and consist of eccentric chromosomes, chromatid fragments, or whole chromosomes, which failed to reach spindle poles during mitosis (Neville *et al.,* 2002; Agarwal *et al.,* 2014). When the basal stem cells divide, damaged and fragmented chromosomes can lag during mitotic division and appear in the cytoplasm of the daughter cells as a small nuclear particle. It is essential to know that genetic alteration/response also depends on the individual's environmental exposure (to carcinogen), occupational exposure, diet etc. It is also important to believe the fact that, the genotoxic response to mutagenic agent depends on individual genetic variability, tolerance of the target cells, alterations in the immune system and other constitutional factors such as age and gender (Vidya *et al.,* 2014). Tolbert *et al.* (1991) developed the criteria for scoring the cells and it is the most widely used criteria for evaluation of MN frequency till today. Several staining methods have been used for the evaluation of micronucleus. Although DNA-specific stains are preferred for staining MN, the most commonly used staining procedure for identifying DNA of the nucleus and MN is the feulgen stain.

In our study we have considered the formation of micronuclei before and after exposure to single IOPA radiograph [Figure 2 & 3]. The mean frequency of micronucleus in buccal mucosa before exposure was 0.25 and after exposure was 7.65 respectively; this diference was statistically significant. These results are fully in line with Mohan *et al.* (2016) who compared the genotoxic effect induced by periapical radiography and panoramic radiography. The mean value of micronuclei, condensed chromatin, karyorrhectic cells, pyknotic cells, karyolytic cells, and cells with nuclear bud was found to be increased after IOPAR and panoramic radiographic exposure.The mean value of micronuclei after exposure was significantly (P=0.008) 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 by Preethi et al. the number of micronuclei increases significantly post exposure to both bitewing and digital dental panoramic radiography in children, but the frequency was higher in bitewing radiographs (Preethi *et al.,* 2016). Many other studies Cerqueira *et al.* (2008), Waingade (2012), Arora *et al*. (2014) and Silva *et al.* (2007) 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.

The studies by Popova *et al.* (2007), and Madhavan *et al*. (2011) found overall increase in mean micronuclei number after exposure to panoramic radiography but the increase in number was statistically insignificant. Other studies conducted by Ribeiro (2008) showed no alteration in frequency of micronuclei after radiographic exposure. On the other hand Angeleri *et al.* (2007) have conducted studies in children who were undergoing panoramic radiography and compared the cytotoxicity and genotoxicity. Lorenzoni *et al.* (2011) also evaluated DNA damage and cellular death in exfoliated buccal mucosa cells from children undergoing orthodontic radiographs. Both the study have concluded that that panoramic dental radiography might not induce chromosomal damage, but may be cytotoxic. Recent studies by Carlin *et al.* (2010) with CBCT have shown that mutagenicity was not induced by CBCT but in contrast cytotoxicity can be appreciated. The successive occurrence of these micronuclei may delay the renewal of the epithelium lining in the oral cavity. Increase in Micronuclei formation increases the predisposition to malignant transformation in an individual over a cumulative period (Carrad *et al.,* 2007).

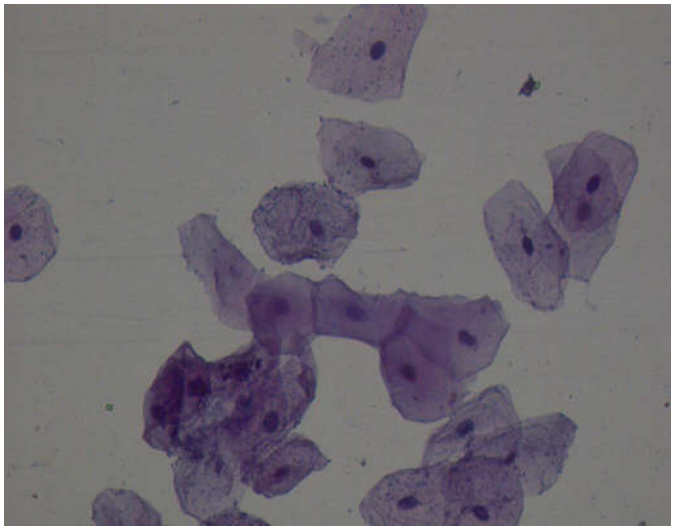


Figure 1. Exfoliative buccal mucosal cells showing cytoplasm and nucleus under H & E Stain for confirmation of presence of cells (400x and x10 to 100x).

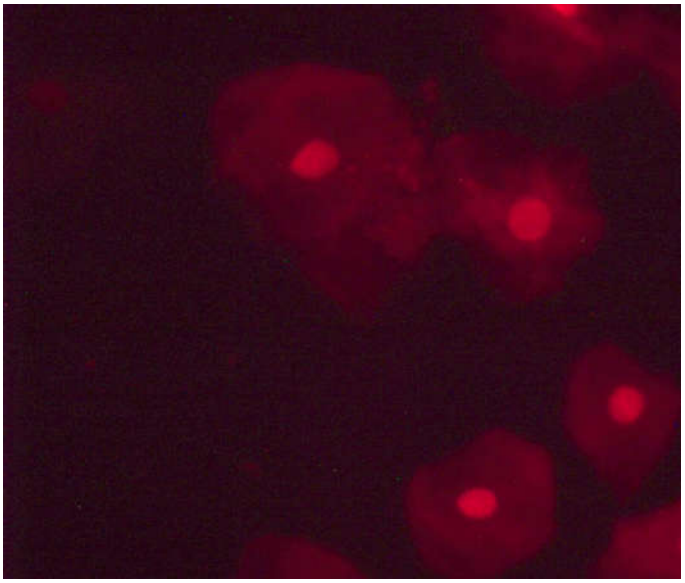


Figure 1. Preexposed exfoliative buccal mucosal cells showing cytoplasm and nucleus under fluorescence microscopy (400x and x10 to 100x)

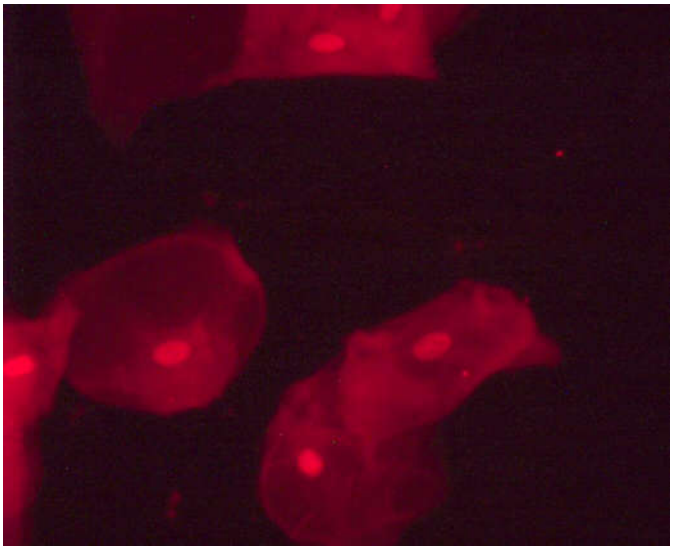


Figure 1. Postexposed exfoliative buccal mucosal cells showing cytoplasm, nucleus and micronuclei under fluorescence microscopy (400x and x10 to 100x 0)

It was repeatedly emphasized that this noninvasive method might be a suitable biomonitoring approach for the detection of genotoxicity and hence increased cancer risk in humans in a regular basis. But the processing of the collected samples was time-consuming, and identification of MN proper was tedious, 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 required.

Conclusion

- The present ex vivo study was conducted to evaluate the extent of genetic changes on the basis of MN frequency in exfoliated buccal mucosa cells before and after exposure to intra oral periapical radiographs in children.
- It was concluded that the frequency of micronuclei increases post exposure to IOPA radiographs. So, it is important know more about these miniature nuclear offshoots which may cause cumulated biological effects of radiation exposure.
- This study will contribute to a better understanding of radiation induced changes seen on the oral mucosa after diagnostic radiography.
- By using an accurate radiographic technique and following the current radioprotection criteria, we can avoid unnecessary repetition and thus reducing the risk of promoting chromosomal changes at each exposure.

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Conflicts of interest: The authors declare no conflict of interest

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Abbrevations

BMCyt assay- Buccal Micronucleus Cytome assay
CBCT-Cone Beam Computed Tomography
DNA- Deoxyribonucleic acid
IOPAR- Intraoral periapical radiographic
MN - Micronuclei

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