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**Research Article**

# Genotoxic effects of panoramic radiation by assessing the frequency of micronuclei formation in exfoliated buccal epithelium

**Vidya KB[1]*, Anupama N. Kalappanavar[2], Muniyappa M[3]**

[1]Department of Oral Pathology, College of Dental Science, Davangere, Karnataka, India
[2]Department of Pathology, Kasturba Medical College, Mangalore, Karnataka, India
[3]Department of Pathology, Shimoga institute of Medical Science, Shimoga, Karnataka, India

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***Correspondence:**
Dr. Vidya KB,
E-mail: medicogen@gmail.com



## ABSTRACT

**Background:** Panoramic radiography is one of the most commonly used radiographic methods to complement clinical examination. Ionizing radiation is a well-known mutagen and carcinogen in the human population. So this study was undertaken to evaluate the possible genotoxic effects of panoramic radiation by assessing the frequency of micronuclei formation in the exfoliated buccal epithelium.
**Methods:** 50 patients of either sex in the age range of 15 to 75 years with apparently normal oral mucosa with no adverse habits and without any oral lesions were included in the present study after their consent. Buccal epithelial cells were obtained from the buccal mucosa by scraping with the toothbrush immediately before and after 10 ± 2 days of exposure to panoramic radiography. Cytological preparations were stained and observed under microscope. Student's paired't' test was used for the comparison between mean frequency of micronuclei in buccal epithelial cells in patients before and after panoramic radiography.
**Results:** Significant increase (P <0.0001) in the frequency of cells with micronuclei and total number of micronuclei after panoramic radiography was detected.
**Conclusion:** The X-radiation emitted during panoramic radiography does induce some genotoxic changes in the form of increased frequency of micronuclei in target buccal epithelial cells.

**Keywords:** Panoramic radiography, Buccal epithelial cells, Genotoxic effects, Micronuclei

## INTRODUCTION

Ionizing radiation is a well-known mutagen and carcinogen in the human population.[1] Carcinogenesis is a multistep process characterized by genetic, epigenetic and phenotypic changes.[2] Many chemical, physical and biological environmental agents are able to interact with deoxyribose nucleic acid (DNA) to induce mutations.[3] A largest man made contribution to human exposure to ionizing radiation comes from its diagnostic and therapeutic uses.[4] Biomarkers of genotoxicity can be used as an indicator of environmental carcinogen exposure.[5,6] So the genetic alterations such as chromosomal aberrations and formation of micronuclei in the cell cytoplasm are the early biological effects of carcinogenesis,[1] and are used as cytogenetic biomarkers.[2] Micronuclei (MN) are derived from both chromosomal fragments and whole chromosomes lagging behind in anaphase,[7,8] and are used to evaluate the magnitude of DNA damage.[2] Hence, the cytogenetic biomarkers are of great concern as they are involved in the mechanism of carcinogenesis and increased cytogenetic damage may be an indicator of an enhanced cancer risk.[9]

To evaluate these micronuclei, two different cells can be chosen which covers the wide range of exposure routes;

the peripheral lymphocytes and exfoliated buccal epithelial cells. Human peripheral blood lymphocytes which are the most frequently used cells for cryptogenic monitoring; appear to be an inappropriate cell system for monitoring the genotoxic effects of radiographic examinations of the oral cavity. As the buccal epithelium is under direct radiation exposure to panoramic radiography examination, it is the primary target for radiation induced damage. Hence, buccal epithelial cells provide an alternative source of tissue for human monitoring to toxic exposures.[1]

The micronucleus assay is a suitable internal dosimeter for revealing tissue specific genotoxic damage in individuals exposed to carcinogens.[10] The analysis of micronuclei has gained popularity as a biomonitoring assay for human genotoxic exposure and effect because, it is non-invasive, the scoring is simple, requires shorter training, less time consuming[8] and the precision is obtained from scoring larger number of cells with better patient acceptance.[2] It is also a sensitive short term assay for the detection of genotoxicants.[11]

The possible genotoxic effects from panoramic dental radiography as assessed by micronucleus occurrence, has not yet been satisfactorily explored since it has been investigated in only a few studies in the exfoliated cells.[3] In the current study, the genotoxic effects induced by x-ray emission during panoramic dental radiography were evaluated in exfoliated buccal epithelial cells that are directly exposed in this procedure by using the micronucleus analysis.

## METHODS

The present study was conducted in the department of oral medicine and radiology, and department of oral pathology, college of dental sciences, Davanagere. 50 patients of either sex in the age range of 15 to 75 years attending the outpatient department of oral medicine and radiology, for routine diagnostic purposes with apparently normal oral mucosa with no history of adverse habits and without any oral mucosal lesions, were included in this study after their informed and written consent. Subjects using tobacco in any form, areca nut, alcohol, antiseptic mouth rinses, Cancerous and precancerous lesions, any oral mucosal lesions were excluded.

### *Collection of buccal cell sample & slide preparation*

After washing the mouth thoroughly with water the exfoliated cells from the buccal mucosa (both right and left) were obtained by scraping with a tooth brush and the material was submersed in 25ml of buffer solution (0.1 M EDTA, 0.01 M tris salt and 0.02 M NaCl). Then the patients underwent panoramic radiographic examination. The exposure parameters used were; 78-80 kVp, 10mA for 12 seconds. The exposed films were processed with visual method of manual processing. The collected cells were washed thrice in the buffer solution by centrifugation. The cell suspension was placed on pre-cleaned slides, and the smear was prepared, and it was fixed in 80% methanol. The fixed slides were stained using 10% Giemsa solution. The frequency of micronuclei in buccal cells were evaluated by scoring 1000 buccal epithelial cells per sample (Cerqueira et al.,[3] Palve et al.) under microscope, at 100 X magnifications by Oral Pathologist in the Department of Oral Pathology. The scoring of micronuclei was done according to the criteria established by Countryman et al. The scrapings from the buccal mucosa (both right and left) were collected after 8-12 days (period required for the turnover of buccal epithelial cells) and micronucleus analysis was done in a similar manner as mentioned above. The buccal epithelial cells were assessed for the number of cells with micronuclei and the total number of micronuclei.

### *Statistical analysis*

Results were represented as Mean ± SD and Range values. Student's paired t' test was used to test the difference in the pair of values and correlation analysis was performed to assess the relationship between measurements. For all the tests, a P value of 0.05 or less was considered for statistical significance.

## RESULTS

The study population comprised of 50 healthy subjects with apparently normal mucosa. The mean age of the patients enrolled in the study was 30.88 ± 12.63 years, with an age range of 15-75 years (Table 1).

**Table 1: Percentage of subjects belongs to the different age groups selected for the study. N=50.**

| Age in years | No of subjects | % |
|---|---|---|
| 15-24 | 21 | 42 |
| 25-34 | 16 | 32 |
| 35-44 | 05 | 10 |
| 45-54 | 02 | 4 |
| 55-64 | 06 | 12 |
| **Mean ± SD** | 30.88 ± 12.63 | |

A total number of 50,000 cells were counted from 50 patients before and 50,000 cells after exposure. There were totally 75 cells with MN before X-ray exposure, and 157 cells with MN post exposure. The total number of cells with micronuclei and total micronuclei was statistically highly significant (P <0.0001) as compared between before and after exposure (Table 2, Figure 1).

Student's paired't' test was used to compare the mean frequency of micronuclei in buccal epithelial cells in patients before and after panoramic radiography. The mean of cells with micronuclei before and after exposure-1 was 0.15% and 0.31% respectively, whereas, mean of total number of micronuclei was 0.16 % and 0.35%

respectively. The mean difference of cells with MN before and after exposure was 0.16 with't' value 7.5 and mean difference for total micronuclei before and after exposure was 0.19 with 't' value 7.7. The frequency of micronuclei scored after the exposure was increased and difference was highly statistically significant (P <0.001) in both the cells with MN and total micronuclei (Table 3).

**Table 2: Descriptive statistics showing the comparison of cells with micronuclei and total number of micronuclei in buccal epithelial cells in patients before and after panoramic radiography.**

| Time of Assessment | Total no. of cells scored | Cells with MN | | | Total MN | | |
|---|---|---|---|---|---|---|---|
| | | Total no. of cells with MN | Mean ± S.D | P Value | Total no. of MN | Mean ± S.D | P Value |
| Before exposure | 50,000 | 75 | 0.15 ± 0.15 | 0.0001 HS | 78 | 0.16 ± 0.16 | 0.0001 HS |
| After exposure | 50,000 | 157 | 0.31 ± 0.17 | | 171 | 0.35 ± 0.21 | |

P = 0.0001: Highly Significant (HS)

**Table 3: Comparison between mean frequency of micronuclei in buccal epithelial cells in patients before and after panoramic radiography.**

| Study param-eters | Mean % | | | | |
|---|---|---|---|---|---|
| | Before exposu-re | After expos-ure | Mean differ-ence | t* value | P Value |
| Cells with MN | 0.15 | 0.31 | 0.16 | 7.5 | <0.001 HS |
| Total MN | 0.16 | 0.35 | 0.19 | 7.7 | <0.001 HS |

*Student's paired t test, P <0.001: Highly Significant (HS)

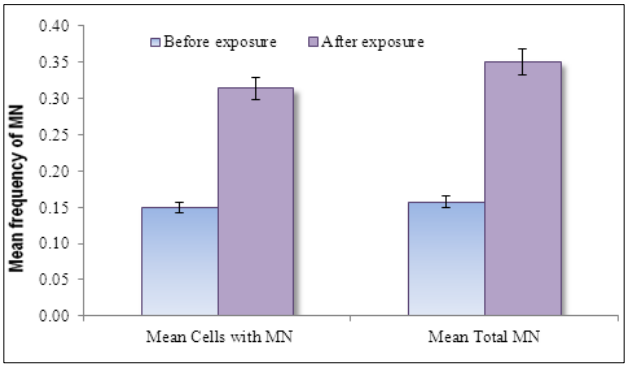


**Figure 1: Comparison between mean frequency of micronuclei in buccal epithelial cells in patients before and after panoramic radiography.**

## DISCUSSION

Ionizing radiation has been described as a double-edged sword. Even though radiation is widely used in diagnostic and therapeutic purpose, there is considerable concern about the potential harmful effects associated with radiation exposure.[12] High doses of ionizing radiation act as a carcinogen, which clearly produce deleterious consequences in humans, on the contrary the effect of very low doses of radiation is not very clear, but its risk is still a societal importance to see its effects on tissues.[13] Panoramic dental radiography is widely used to complement clinical examinations for diagnosing jaws and its diseases. The buccal epithelium is under direct exposure to panoramic radiographic examination, so it is a primary target for the radiation induced damage.[1] Hence, it is essential to implement a reliable biomarker to assess the genetic damage and the micronucleus assay serves as a potential biomarker.

MN formation is undoubtedly an important mechanism for chromosome loss, although it is not the only mechanism.[8] Micronucleus formation has been shown to be a sensitive biomarker for cytogenetic damage due to these radiations.[3] Increased frequencies of micro nucleated cells reflect the exposure with clastogenic and aneugenic modes of action.[5] The possible genetic effect from panoramic 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.[1]

In the present study, the occurrence of micronuclei is highly significant in patients who have undergone panoramic radiography when compared to pre-exposure. The studies by Popova et al.[1] and Angelieri et al.[2] showed statistically insignificant differences between before and after exposures to panoramic radiography whereas, Cerqueira et al.[3] had showed the increased frequency of micronuclei formation which was significantly higher after the exposure. The higher frequency of buccal epithelial micronuclei observed after X-ray exposure corroborates the data in the literature, in which x-ray radiation is reported to be effective in inducing genetic damage which results in the increased formation of micronuclei in these cells. And high radiation absorbed doses could a reason for the positive result.

The micronucleus cell index in buccal epithelial cells may reflect the genomic instability. This index is directly proportional to frequency of micronuclei in a given number of buccal epithelial cells which ultimately indicates an increased risk of cancer. In the our study, 3 out of 50 pre-exposure patients had more than one micronuclei in one cell, whereas 14 post exposure subjects showed more than one micronuclei in each cell. In addition to this, the frequency of total micronuclei scored after radiography was also increased which accounts for high significance. The higher micronucleus frequency in buccal epithelial cells after panoramic radiography can be explained by the direct exposure of these buccal epithelial cells to x-rays, and it is the primary target for radiation induced damage,[1] where radiations are directly absorbed by these cells.

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.

## CONCLUSION

Exposure to x-rays during panoramic radiography can induce genotoxic effects in buccal epithelial cells which cause chromosomal damage and induce apoptosis. Thus, panoramic dental radiography should be advised only when it is necessary because, it cannot be considered a risk-free procedure. It is also recommended that the expanded protocol for the micronucleus test should be adopted, as the procedure is noninvasive, cheap and easy to detect the genotoxic effects of radiation.

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