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ORIGINAL RESEARCH


# Radiation Risk Assessment in Professionals Working in Dental Radiology Area using Buccal Micronucleus Cytome Assay

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## ABSTRACT

**Objective:** The aim of this study was to assess the incidence of micronuclei (MN) in buccal mucosal cells of professionals working in radiology area to determine the risk of stochastic effects of radiation.

**Materials and methods:** All the professionals and students working in King Khalid University - College of Dentistry radiology area were included in the Risk Group (RG = 27). The Control Group (CG = 27) comprised of healthy individual matching the gender and age of the RG. Buccal mucosal scraping from all the 54 subjects of RG and CG were stained with Papanicolaou stain and observed under oil immersion lens (×100) for the presence of micronuclei (MN) in the exfoliated epithelial cells.

**Results:** There was no significant difference between the incidence of MN in RG and CG (p = >0.05) using t-test.

**Conclusion:** Routine radiation protection protocol does minimize the risk of radiation induced cytotoxicity, however, screening of professionals should be carried out at regular intervals.

**Keywords:** Micronucleus, Buccal Epithelial cells, Stochastic, Radiation.

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## INTRODUCTION

Micronuclei (MN) are small extra nuclear bodies formed at anaphase during nuclear division that are included in the new cell alongside the daughter nuclei. They are composed of chromosome fragments or whole chromosome that is present in the cellular cytoplasm in addition to the main nucleus.[1] Increased frequency of MN demonstrates cytogenic effect of environmental, occupational and lifestyle factors and dietary deficiencies. A strong relationship exists between cytogenic damage and carcinogenesis. Formation of MN is believed to be an early biomarker of carcinogenesis.[2] They have been found early in the progression of head and neck squamous cell carcinoma[3] and peripheral blood lymphocytes during carcinogenesis.[4]

In the early part of 20th century Howell and Jolly were the first to report nuclear bodies in reticular cytoplasm that were positive to Feulgen stain. Later *in vitro* irradiated cells demonstrated Howell-Jolly like nuclear bodies indicating nuclear aberration. In 1970 Boller, Schmidt and Heddle[5] named it the Micronucleus test after employing it on bone marrow erythrocytes of experimental animals. In the 1980's Buccal micronucleus cytome assay (BMCA) was introduced to determine the presence of MN in buccal mucosal cells. This test involves the collection of exfoliated buccal epithelial cells from the inner side of the cheek and observing the stained smear under light microscope. Buccal epithelium cells provide an excellent source of tissue for human monitoring to occupational and environmental toxic exposures. Minimal invasiveness, ease of storing of samples and slide preparation makes it a good choice for large biomonitoring studies. It can be effectively employed to detect the effects of exposure to inhaled or ingested genotoxic agents especially in pediatric population.

Another application of BMCA is monitoring the risk of ionizing radiation, a well-known mutagen and carcinogen. As the buccal epithelial tissue is under direct exposure during head and neck radiography it is a prime target for radiation induced stochastic and deterministic effects. Stochastic effect has been detected in low dose medical exposure to patients[6] and exposed workers.[7] This effect poses significant health risks leading to cancer or genetic damage transmitted

**Table 1:** Distribution of individuals in risk and control group

| | | | | |
|---|---|---|---|---|
| | | *Radiology lab technicians* | *Students* | *Doctors* |
| *RG* | Males | 4 | 17 | 3 |
| | Females | 3 | 0 | 0 |
| | Total | 7 | 17 | 3 |
| | | *Dental lab technicians* | *Students* | *Doctors* |
| *CG* | Males | 10 | 11 | 3 |
| | Females | 3 | 0 | 0 |
| | Total | 13 | 11 | 3 |

**Table 2:** Incidence of micronuclei in RG and CG

| | *N* | *Mean* | *Std. deviation* | *Std. error mean* |
|---|---|---|---|---|
| RG | 27 | 1.52 | 0.89 | 0.17 |
| CG | 27 | 1.07 | 1.04 | 0.2 |

to subsequent generations of exposed individuals. The association between the dose of ionization radiation and increased frequency of MN has been well established.[1] Therefore, the objective of this study was to determine the radiation risk by assessing the incidence of MN in buccal mucosal cells of professionals working in radiology.

## MATERIALS AND METHODS

### Sampling (Table 1)

All the professionals and students working in KKU-College of Dentistry, radiology area for at least 1 year before the start of the study were included in the risk group (RG, n = 27). RG included seven radiology technicians, three radiologist doctors and 17 students working in the radiology area and radiology lab. A questionnaire was distributed among College staff and students that had never worked in the radiology area to select the control group (CG). Twenty-seven individuals were selected matching the gender and age of the RG. The other inclusion criteria for CG were individuals using normal oral hygiene maintenance methods (tooth brushing only and not using any form of mouth rinses). Not wearing orthodontic appliance or have discontinued orthodontic appliance 1 year before the start of the study. Smokers and individuals having red or white oral mucosal lesions or using any topical agents were excluded from the study. The age range of the study population was 21 to 40 years. Ethics approval was obtained from Scientific Research Committee, College of Dentistry and King Khalid University before the start of the study.

### Staining

Buccal mucosal scraping from all the 54 subjects of RG and CG were taken with sterile wooden tongue depressors. The scraping was immediately smeared on the surface of a clean microscopic glass slide. Two slides were smeared for each subject and coded. The slides were stained with Papanicolaou stain. The staining procedure closely followed Ayyad SB et al[8] method. The smears were fixed in 95% ethanol for 10 minutes followed by 10 dips in 70% ethanol. Hydration was done in running tap water. Nuclear staining (DNA specific) was done with Gill's hematoxylin for 5 minutes and rinse with stream of running tap water for another 5 minutes. Dehydration was then performed with 5 to 8 dips in 70% ethanol. Cytoplasmic stain orange green (OG6) was applied for 5 minutes, followed by rinsing with 95% ethanol for 10 minutes. Cytoplasm and nuclear stain (RNA specific) by EA polychrome (EA 50) was applied for 5 minutes, followed by 10 dips in 95% ethanol. The Smear was dehydruted using absolute ethanol by dipping the slides 5 to 10 times. Finally clearing with xylene was done for 1 minute. The preparation was then mounted with mounting medium.

### Micronuclei

Stained slides were observed under oil immersion lens (×100) for the presence of MN in the epithelial cells. Two observers with prior experience in MN examination sat together for observation. The stained field of slide was projected onto a screen and both the observers viewed the cells simultaneously. At least 1000 epithelial cells with clear cytoplasm and nucleus distinction were counted for each slide. Care was taken to exclude overlapping and clusters of stained epithelial cells. An average of 2000 epithelial cells was observed for each subject. The epithelial cells were identified as either normal differentiated cells or cells with MN. Criteria for identifying and scoring MN cells were done according to established protocol.[9] Description of MN cells from this protocol is as follows: (1) Contains both main nucleus and micronucleus, (2) Micronuclei are round or oval with similar stain intensity as main nucleus, (3) Micronuclei usually have 1/3-1/16 diameter of main nucleus, (4) Micronuclei must be located in cellular cytoplasm, (5) Scored in basal and differentiated cells only.[9]

## RESULTS

The data obtained was analyzed using t-test analysis (SPSS 17.0). The mean value of MN cells observed in RG was 1.52 compared to 1.07 MN cells in CG. Table 2 details the mean and standard deviation of the analysis. The mean of CG

**Table 3:** Differences between RG and CG

| | Mean | 95% confidence interval of the difference | | t | df | Significance (two tailed) |
|---|---|---|---|---|---|---|
| RG and CG | –0.44 | Lower | Upper | 1.69 | 52 | 0.0972 |
| | | –3.37 | 0.33 | | | |

minus RG was –0.44 and two-tailed significance value was 0.09 (Table 3). Hence, the incidence of MN cells in RG when compared to CG was statistically insignificant ($p > 0.05$).

## DISCUSSION

DNA damage and cell death are considered to be prime mechanisms during carcinogenesis.[10] There is an established correlation between long-term exposure to very low radiation doses and DNA damage.[11] Stochastic effects following low dose ionizing radiation pose a significant health hazard to professionals working in radiology area. There has always been a need for a reliable and fast method both for screening groups of workers at risk and for estimation of damage in cases of radiation induced biological changes. The radio sensitivity of buccal mucosal cells and its relation to increased MN frequency provides a strong rationale for screening vulnerable individuals through BMCA. The fate of MN cells after exposure to ionization irradiation was examined by Yun huang et al.[12] They found that more MN are formed in radiosensitive cells than in radio resistant cells. Therefore, it is imperative to select the right cells for risk assessment studies. They also reported that cells with MN are more likely to die when compared with MN-free cells after irradiation. Additionally, DNA damage was more severe in MN cells than in non-MN cells. They concluded that the micronucleus index is a valuable biomarker for radio sensitivity. Greater sensitivity of buccal mucosal cells over peripheral blood lymphocytes in demonstrating MN formation was observed in plywood and fiber glass workers.[13] Suruda A et al[14] reported significantly higher MN in buccal mucosal cells than peripheral blood in students exposed to formaldehyde in mortuary.

Apart from monitoring radiation risk through BMCA, the MN frequency has been widely used as an indicator in analyzing risk of various genotoxic factors in exposed individuals. Hair dressers,[15] painters, battery renovation workers,[16] fire fighters,[17] petrol station attenders[18] and workers exposed to calcite dust[19] have been reported to be at risk. Oral habits utilizing tobacco, smokeless tobacco, betel quid,[20] snuff,[21] gutkha, pan masala,[22] areca nut[23] and gasp[24] have also placed the users in high risk group for developing oral cancer. Genetic disorders like Downs syndrome exhibit increased MN in buccal mucosal cells.[25] BMCA done with epithelial cells of patients undergoing fixed orthodontic appliances[26] and using mouth rinses[27] has indicated an increase in MN formation.

However, panoramic radiography did not show an increase in MN frequency in tongue epithelial cells.[28] Similarly BMCA done on buccal mucosal cells before and after exposure to panoramic radiography resulted in insignificant difference in MN cells indicating the probable safety of the X-ray technique.[29] Significant cytogenic changes were not observed in a study conducted on blood samples of dentists exposed to low doses of ionizing radiation.[30] In the present study too, the MN cells frequency in the risk group was not significantly different from the control group. Routine radiation protection measures are followed in the area where this study was carried out. They include wearing of leaded apron (0.35 mm equivalent lead), leaded walls and glasses around the X-ray units, following the position-and-distance rule and patient selection. Strict implementation of Quality assurance procedures ensures regular maintenance of processing systems, proper X-ray exposure factors and regular change of solutions in the X-ray unit set up. This along with the low radiation doses could be a reason for the insignificant result.

Results of this study validate the perception that radiation protection methods minimize the genotoxic risk of ionization radiation. It can be a probationary if not definitive indication of the effectiveness of the radiation protection protocol. However, stochastic effects of radiation should not be ruled out at any point of time. Instead a plan should be formulated for periodic radiation risk assessment at the cellular level using MN and more DNA specific tests like Comet assay.

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