A Cross-sectional Study to Evaluate Nuclear Changes in Buccal Mucosa Following Panoramic Radiography

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ABSTRACT

Aim and objective: To evaluate the possible genotoxic effect of X-rays on buccal mucosa while exposing to dental panoramic radiography using micronucleus test.

Materials and methods: The study group comprised of 30 healthy subjects, 15 males and 15 females, aged between 24 years and 65 years. Samples were obtained from the exfoliated oral mucosa cells of buccal mucosa before and 12 days after exposing the patients to panoramic radiography.

Results: The study reported that there was no significant increase in the number of micronuclei cells present before and after panoramic radiography. Positive correlation existed between age with pre- and postexposure micronuclei.

Conclusion: Diagnostic dental panoramic radiograph does not induce micronuclei in the target buccal epithelium cells. A positive correlation between age and micronuclei frequency was established.

Clinical significance: Panoramic radiographs does not induce cytotoxicity but increase frequency may be vulnerable to genotoxic effects in buccal mucosal cells. Hence, dental radiographs should be prescribed only when necessary.

Keywords: Buccal epithelium cells, Micronucleus test, Panoramic radiography, Radiation hazard.

INTRODUCTION

Ionizing radiation has become one of the universal diagnostic and therapeutic tools. With its importance in diagnosis and treatment, ionizing radiation is very potent mutagen which is said to induce mutations of genes and aberrations in chromosome. Ionizing radiation can act directly on the DNA or can form reactive compounds. Thus, alteration or mutation in the DNA is said to be an important aspect in carcinogenesis.1,2

Dentist prefers to request panoramic radiography of dental arches when evaluation of all the teeth is necessary, as it becomes a choice over several periapical radiograph. In order to detect the radiation effects of low-dose diagnostic radiographic exposures, sensitive analysis and specific approaches are needed.3

Chromosomal alterations in the human peripheral lymphocytes and in the exfoliated cells of oral mucosa are evaluated by cytogenetic analysis, and this is one of the most sensitive techniques for monitoring human radiation exposure. Various assays are available and are proposed as potential biomarkers, which include the assay to assess metaphase chromosomal aberrations, sister chromatid exchanges, and host cell reactivation. These methods are laborious and consume lot of time and require highly skilled professionals to accurately read and interpret the slides. All the abovementioned factor have created the need and interest for using the micronucleus test to uncultured exfoliated cells.5,6

One of the most reliable methods to study genetic damage in human is by evaluating the micronuclei in the lymphocytes or in exfoliated cells. Peripheral lymphocytes are inappropriate for evaluation following radiation exposure in the oral cavity. Hence, buccal epithelial cells provide an alternative source of tissue to monitor radiation effects following oral and maxillofacial radiography. In most oral radiographic procedures, the buccal mucosa is a primary target for radiation induced damage. Further, buccal mucosa is easily accessible, and exfoliative cytology is a relatively easy, rapid, and noninvasive procedure.6,7

Micronuclei (MN) are extranuclear bodies that contain damaged chromosome fragments and/or whole chromosomes that were not incorporated into the nucleus after cell division.8-10 Therefore, the aim is to evaluate the possible genotoxic effect of X-rays on buccal mucosa during panoramic dental radiography using micronucleus test, and the objective is to compare and correlate DNA damage
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Materials and Methods

The study was conducted in the Department of Oral Medicine and Radiology, Rajah Muthiah Dental College and Hospital, Annamalai University, Chidambaram, Tamil Nadu, India.

The samples for the study were selected from the Outpatient Department of Oral Medicine and Radiology, Rajah Muthiah Dental College and Hospital. The patients were divided into two groups of 30 each patients. Group I consisted of 30 patients with no mucosal lesion and no previous exposure to dental radiography within 15 days. Group II consisted of 30 patients without any mucosal lesion and exposure to dental radiography 12 days before. A formal ethical clearance to conduct this study was obtained from the ethical committee of the college. All the patients were explained about the study, and a written consent was obtained.

All the patients between the age-group of 24–65 years, without any mucosal lesion were included in the study. Patients who had habit of chewing betal nut/betel leaf/slaked lime/tobacco/gutka, smoking, and alcohol were excluded. Patient with exposure occupational hazards (works exposed to paint, formaldehyde, ethylene oxide and solvent based adhesive like acetone, methyl-ethyl-ketone, hexane and toluene) and patients who received treatment for cancer were also excluded.

Patients were examined in a conventional dental chair, with universal sterile precautions. A detailed case history was recorded for all patients with special reference to their habit (tobacco, smoking and alcohol), previous exposure to dental radiography; occupation and medical history of radiation therapy were excluded from the study.

Preparation of Cytological Smears

The tissue for cytological analysis was taken from the buccal mucosa with the toothbrush. Tissue scraping of the buccal mucosa was taken immediately before exposure to X-ray, and the same procedure was repeated 12 days later.

Slides containing 2 drops of NaCl (0.09%) were used for cytological smear preparation. Methanol and acetic acid (3:1) solution were used to fix the cells. Modified Feulgen—Rossenbeck was used to stain. The prepared slides were kept at room temperature for 15 minutes by placing them in 5 N HCl. The slide was then rinsed in distilled water for 15 minutes, and then Schiff’s reagent was used for staining. After 90 minutes of staining the slide were counterstained for 1 minute using 1% Fast Green stain. Micronuclei in cells were confirmed by cytological analysis which was done under oil immersion at 1,000× magnification (Fig. 1).

After taking detailed history and brush biopsy all the subjects were exposed to panoramic radiograph planmeca proline EC panoramic X-ray machine at 70–74 kV and 10 mA for 18 seconds the patients were recalled for follow-up after 12 days and cytological smear were obtained.

Result

In this study, majority of the patients in the study group were between 20 years and 25 years and the mean age of the group was 28.96 years, with equal number males and females.

In the Pearson correlation between age and pre-exposure micronuclei, the mean was 1.03 and SD was 0.80 (Table 1). While Pearson correlation between age and post exposure micronuclei was 2.20 and SD 0.84 (Table 2). Pearson correlation between gender, pre, and post exposure micronuclei among males the pre-exposure mean is 1.13 and postexposure mean 2.53 among females the pre-exposure mean was 0.33, and postexposure mean was 1.86 (Table 3). Pearson correlation between pre- and postexposure micronuclei for pre-exposure the mean was 1.03 and SD was 0.80 for postexposure the mean was 2.20 and SD of 0.84 (Table 4). From the above result, it is inferred that there was significant difference observed between age, pre-, and postexposure micronuclei (Tables 1 and 2), while inferred there was no significant correlation between gender pre- and postexposure micronuclei (Table 3). There was also no significant observation seen between pre- and postexposure micronuclei (Table 4).

Discussion

Over the years, X-rays have become a universal diagnostic and therapeutic tool. However, X-ray remains a potential mutagenic...
agent which is capable of causing both gene mutations and chromosomal aberrations.\textsuperscript{11}

The primary action of radiation on living systems occurs through direct or indirect effects. When the energy of a photon or secondary electron is transferred directly to the biological molecule, the effect is called direct. Alternatively, the photon may be absorbed by water in a biologic system resulting in ionization of water molecule to be ionized. These ions form free radicals (radiolysis of water) that, in turn, interact with and produce changes in the biologic molecules. This series of events involving water molecules is considered to be indirect.\textsuperscript{2}

Effect of radiation is seen on every living molecule. DNA is considered to be the most important target molecule since they carry genetic information. Any alteration or mutation in DNA is a key step in carcinogenesis.\textsuperscript{12}

There are various methods which are well established for evaluation of mutagenic effect of radiation. Assessing the micronucleus becomes predictive and comparatively rapid method of measuring the abrasion in chromosomes. Their frequency appears to increase in carcinogen-exposed tissues long before any clinical symptoms are evident.\textsuperscript{13}

Dentist prefer to request panoramic radiography of dental arches when evaluation of all the teeth is necessary as it becomes a choice over several periapical radiograph. Till date, the safety or cutoff levels in radiation doses is not made evident. Added to this repetitive exposure to radiograph would result in accumulated biological effects. In order to detect the radiation effects of low-dose diagnostic radiographic exposures, as with panoramic radiograph, a sensitive analysis and specific approaches are needed. Micronuclei appear to be simple markers that can be examined on routinely prepared cytological preparations.\textsuperscript{14}

One of the most reliable methods to study genetic damage in humans is by evaluating the micronuclei in the lymphocytes or in exfoliated cells. Peripheral lymphocytes are inappropriate for evaluation following radiation exposure in the oral cavity. Hence buccal epithelial cells provide an alternative source of tissue to monitor radiation effects following oral and maxillofacial radiography. In most oral radiographic procedures, the buccal mucosa is a primary target for radiation induced damage. Further, buccal mucosa is easily accessible and exfoliative cytology is a relatively easy, rapid, and noninvasive procedure.\textsuperscript{2,8}

Elevated levels of MNC reveal the genotoxic action of carcinogens and may indicate an elevated probability for the formation of particular chromosome changes, which in turn could be associated with neoplastic transformation.\textsuperscript{15}

With all the above fact the present study was designed to evaluate the genotoxic effect of diagnostic dental radiograph on the epithelial cell of buccal mucosa. The aim of the study is to evaluate, compare, and correlate the possible DNA damage (micronucleus) by X-rays on buccal mucosa before and after panoramic dental radiography, using micronucleus test.

Study designed by Popova et al.\textsuperscript{5} considered age and smokers as exclusion criteria, whereas Cerqueira et al.\textsuperscript{3} excluded exposure to other genotoxic agents and regular oral antiseptic solutions in addition to the abovementioned criteria. Our study population comprised of 30 patients, which included 15 male and 15 female with their age ranging between 24 years and 65 years. In the present study, in addition to the abovementioned criteria, previous exposure to radiographs and patients who are receiving any treatment for cancer were also refrained from participating in the study.

This study did not show statistically significant increase in MNC formation, when compared between pre and postexposure. Similar results were stated in the past by Cerqueira et al.\textsuperscript{3} and Popova et al.\textsuperscript{5} thus, it can be firmly stated that panoramic radiographic examination does not induce genotoxic change in target buccal epithelium cells.\textsuperscript{15} Low-dose diagnostic radiographic exposures (9–26 μSv) could be a reason for the phenomena observed.\textsuperscript{16}

On performing Pearson correlation, a positive correlation between age and micronuclei frequency was established which is in agreement with Popova et al.\textsuperscript{5} An increase in frequency may be indicative of an increasing vulnerability of the genome and an increasing deficiency in DNA repair with age. Thus, panoramic dental radiography cannot be considered as a risk-free procedure it should be requested only when necessary.\textsuperscript{2}

Since females appear relatively more vulnerable to neoplasia and immune-related disorders with advancing age, possibly due to a by virtue of drastic hormonal imbalance, we attempted to find correlation between the frequency of MN and gender. Although the results were not significant, it still remains a potential area for research with larger samples.\textsuperscript{5,15}

Till today, no single biomarker can predict the risk of genotoxicity and mutation with precision. Micronucleated cell indexes are thought to reflect genomic instability. Detection of an elevated frequency of micronuclei in a given population indicates an increased risk of cancer.\textsuperscript{5,11}

Further study with larger sample size and biomarkers like micronuclei, nuclear alterations indicative of apoptosis (karyorrhexis, pyknosis and condensed chromatin) and necrosis (karyorrhexis, karyolysis, pyknosis and condensed chromatin), nuclear buds, and broken eggs can also be scored, since increase apoptosis is indicative of cytotoxic damage.\textsuperscript{16} Automated and the use of computerized image analysis systems can be used in counting micronuclei. Micronuclei can be used as an adjuvant for the primary assessment of radiation induced mutation or genotoxicity.

**Conclusion**

There was no statistical significance in the frequency of micronuclei in pre- and post-radiographic exposure. Thus, it is concluded that the radiation exposure from panoramic radiography does not pose significant threat to the buccal epithelial cells. Low radiation exposure during panoramic radiography and time interval of observation could be the possible limitation.

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