MATERIALS AND METHODS

The current study was designed to evaluate the possible genotoxic and cytotoxic effect of X-rays on gingival and buccal mucosa cells during full mouth IOPA radiographs, panoramic radiographs, CT scans and CBCT scans for maxilla and mandible. The study was planned in Department of Oral Medicine and Radiology, Pad. Dr. D.Y.Patil Dental College & Hospital, D. Y. Patil Vidyapeeth, Pimpri Pune 18.

Sample size & its characteristics:

The participants were divided in four groups. The samples were selected from Dr. D. Y. Patil Dental and Medical College, Pune 18. The sample size selected for each group was minimum 30 individuals per group.

The study started with 160 individuals (40 per group) and after omitting drop outs, a total of 139 individuals were included in the study.

a) Group I (n=36): Individuals undergoing full mouth IOPA radiographs
b) Group II (n=34): Individuals undergoing panoramic dental radiography (OPG)
c) Group III (n=33): Individuals undergoing CT scan for maxilla and mandible
d) Group D (n=36): Individuals undergoing CBCT scan for maxilla and mandible

A complete personal history was recorded in a predetermined proforma and a written consent was obtained from the patients. Patients were asked in detail about the form of adverse habit, frequency and duration of the same. A formal ethical clearance to conduct this study was obtained from the ethical committee of the college. Informed consent was obtained from all investigated subjects after they received an explanation of the study.
Inclusion criteria:

- Patients who has been advised for any of four radiographic examination (Full mouth IOPA, OPG, CT scan or CBCT scan)
- Age limit between 18-65.
- All healthy patients without any mucosal lesion.

Exclusion Criteria:

- Patient having any soft tissue mucosal lesions
- Patients who had habit of chewing betal nut / betel leaf / Slaked lime / tobacco / gutka, smoking and alcohol
- Previous exposure to dental or general radiography within One month.
- Occupational hazards (works exposed to paint. Formaldehyde, ethylene oxide, solvent based adhesive like acetone, methyl – ethyl – ketone, hexane
- Patient who received treatment for cancer.
- Patients with history of any systemic diseases

Materials:

Conventional dental chair with additional illumination facility
Sterile disposable gloves and disposable mouth mask
Sterile gauze piece and cotton swab
Glass tumbler with water
Sterile Wooden Spatula
Glass Slides
Light microscope
PAP staining kit
Method:

A detail case history was recorded for all patients with special reference to their tobacco, smoking, alcohol habits and occupational risks.

Full mouth IOPA survey (14 films) were performed with PLANMECA Prox machine with at a tube voltage of 70kV, a tube current of 8 mA and exposure time of 0.25 seconds per film. Films used were size 2, E-speed (EKTA films KODAK) IOPA films.

Panoramic radiographs were performed with PLANMECA Proline XC (DIGITAL) machine with tube voltage of 70kV, a tube current of 10 mA and exposure time of 18 seconds.

CT scans were performed with Philips Ingeuity Core 128 slices machine with tube voltage of 70kV and 400mAs per slice. Slices were obtained at 5mm thickness.

CBCT requested by a dentist, were performed with i-CAT CBCT scanner (Imaging Sciences International, LLC) with a minute voxel size of 0.250 mm$^3$. The Maxilla and mandible was imaged with FOV 16X 13 cm at a tube voltage of 120 kV, a tube current of 5 mA and mAs= 37.07.
Cell collection and slide preparation

The material for analysis was obtained from the gingival mucosa of maxillary dental arch and buccal mucosa in molar region by means of gentle scraping with a wooden spatula, immediately before exposure and 10 days later. Cytological smears were prepared on clean slides containing two drops of physiological solution (NaCl 0.09%).

The cells were fixed with BIOFIX SPRAY (Microanatomy Fixative, Biolab Diagnostics Pvt. Ltd.) and stained by means of the Papanicolaou (PAP) Stain - Rapid PAP Stain Kit (Biolab Diagnostics Pvt. Ltd.)

Cytological analysis:

A minimum of 1000 cells was studied by random manner. Abnormalities were identified under the light microscope. Micronuclei in cells, pyknosis, karyorrhexis and karyolysis were confirmed by observing them in oil immersion at X400 magnification.

Counting of micronuclei:

The cells containing micronuclei were counted. The cells were chosen for analysis based on criteria developed by Tolbert et al 1992 which consists of the following parameters.

A. Parameters for cell inclusion in the cells to be scored:

i) Intact cytoplasm and relatively flat cell position on the slide.

ii) Little or no overlap with adjacent cells.

iii) Little or no debris.
iv) Nucleus normal and intact, nuclear perimeter smooth and distinct.

B. Parameters for identifying micronucleus:

i) Rounded smooth perimeter suggestive of a membrane.

ii) Less than a third the diameter of associated nucleus, but large enough to discern shape and color.

iii) Staining intensity similar to nucleus.

iv) Texture similar to nucleus.

v) Same focal plane as nucleus.

vi) Absence of overlap with bridge to nucleus

**Karyolytic (KL) cell**

In order for the cell to be considered karyolytic, it is required to meet the following criteria.\(^{62}\)

- They are angular and flat in shape with cytoplasmic area that is the size of terminally differentiated cell.
- Cell with nuclear dissolution, in which ghost-like image of nucleus remains.

**Karyorrhectic (KH) cell**

In order for the cell to be considered karyorrhectic, it is required to meet the following criteria.\(^{62}\)

- Cell with nuclear disintegration involving loss of integrity of the nucleus.
- Nucleus constitutes more densely aggregated chromatin than that of condensed chromatin cells.
Pyknotic (PK) cell

In order for the cell to be considered pyknotic, it is required to meet the following criteria:\(^{62}\)

- Cell with small and shrunken nucleus with diameter approximately 1/3rd of normal nucleus.
- Nucleus is uniformly and highly stained.
**Fig. 6** Armamentarium used for clinical examination

**Fig. 7** PAP Staining kit
Fig. 8 Photomicrograph of micronucleated cell

Fig. 9 Photomicrograph of cell showing pyknosis
Fig. 10 Photomicrograph of cell showing karyorrhexis

Fig. 11 Photomicrograph of cell showing karyolysis