# GENETIC DAMAGE IN EXFOLIATED CELLS FROM ORAL MUCOSA OF INDIVIDUALS EXPOSED TO X-RAYS AFTER PANORAMIC RADIOGRAPH

*Dissertation submitted to*

**THE TAMILNADU Dr.M.G.R.MEDICAL UNIVERSITY**

*In partial fulfillment for the Degree of*

**MASTER OF DENTAL SURGERY**

**BRANCH VII**

**ORAL MEDICINE AND RADIOLOGY**

**MARCH 2010**

# CERTIFICATE

This is to certify that this dissertation titled "**Genetic Damage In Exfoliated Cells From Oral Mucosa Of Individuals Exposed To X-rays After Panoramic Radiograph"** is a bonafide record of work done by **Dr. M.Ramalakshmi** under my guidance during her postgraduate study period between 2007-2010.

**This Dissertation is submitted to THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, in Partial fulfillment for the Degree of Master of Dental Surgery in Branch VII –Oral Medicine & Radiology.**

It has not been submitted (partial or full) for the award of any other degree or diploma.

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***"The mediocre teacher tells. The good teacher explains. The superior teacher demonstrates. The great teacher inspires"***

***- William Arthur Ward***

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# LIST OF ABBREVIATIONS

| S.NO | ABBREVIATION | EXPANSION |
|---|---|---|
| 1. | µSv | Micro Sievert |
| 2. | BC | Binucleated cells |
| 3. | BE | Broken eggs |
| 4. | CC | Condensed Chromatin |
| 5. | DNA | Deoxyribonucleic acid |
| 6. | DPR | Dental panoramic radiography |
| 7. | DSB | Double strand breaks |
| 8. | Gy | Gray |
| 9. | hr | Hour |
| 10. | ICRP | International Commission on Radiation Protection |
| 11. | keV | Kilo electron voltage |
| 12. | KL | Karyolysis |
| 13. | KR | Kayorrhexis |

| | | |
|---|---|---|
| **14.** | **kVp** | Kilo voltage potential |
| **15.** | **MN** | Micronuclei |
| **16** | **mSv** | Milli Sievert |
| **17** | **NB** | Nuclear buds |
| **18** | **NP** | Nuclear projections |
| **19** | **OH** | Hydroxyl ions |
| **20** | **PN** | Pyknosis |
| **21** | **RER** | Rough endoplasmic reticulum |
| **22** | **ROS** | Reactive Oxygen Species |
| **23** | **SD** | Standard deviation |
| **24** | **Sv** | Sievert |
| **25** | **TLD** | Thermoluminescent dosimeters |

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# *Introduction*

***"Radiation cloud over medicine!"***

***"Tests show radiations bad effects!"***

***"Single dose of 'safe' radiation found harmful"***

***"Diagnostic x-rays deserve that negative reaction"***

**- White,Pharaoh[85].2006**

These headlines have appeared in newspapers across the world over the years. Before an appointment with the dentist, a patient may read one of these articles and understandably form a negative opinion concerning the use of x-rays for diagnostic purposes. Practitioners must be prepared to discuss intelligently the benefits and possible hazards involved with the use of x-rays and are able to describe the steps taken to reduce the hazard.

Practitioners who administer ionizing radiation must become familiar with the magnitude of radiation exposure encountered in medicine and dentistry, the possible risk that such exposure entails, and the methods used to affect exposure and radiation dose. This information provides the necessary background for explaining to concerned patients the benefits and possible hazards involved with the use of x-rays.

In the last decades x-rays have been used widely for diagnosis in medical and dental practitioners. However, it is well known that ionizing radiation damages DNA, including single and double strand breaks, and DNA protein cross links. X-rays are a

potent mutagenic agent capable of inducing both gene mutations and chromosomal aberrations. They act directly on the DNA molecule or indirectly through the formation of reactive compounds that interact with this molecule. In spite of their mutagenic potential, this kind of radiation is an important tool for diagnosing diseases and is used in medical and dental practice.

Taking the strong evidence for the relationship between DNA damage and carcinogenesis into consideration it will be useful to know if and to what extent adults are more susceptible to the harmful effects induced by radiation particularly because of the lack of previous reports. Today there are several well established methods for evaluating chromosomal damage. It can be performed on lymphocytes or on interphasic exfoliated cells from many tissues.

Panoramic dental radiography is used for diagnosing dental arch and tooth diseases. It has been widely used to compliment clinical examinations and is considered less harmful than performing several periapical radiographs. As a result and because of inadequate in vivo evidence; the present study is aimed to investigate genetic damage from the exfoliated epithelial cells of oral mucosa in patients exposed to panoramic dental radiographs.

To evaluate the magnitude of DNA damage and genetic effect from panoramic dental radiography, the micronucleus test is used. Micronuclei arise from accentric

fragments or whole chromosomes which are not included in the main nuclei of the daughter cells.The formation of the micronuclei can be induced by substances that cause chromosome breakage (clastogens) as well as by antigens that affect the spindle apparatus (aneugens).

According to **Tobert[73] at al.,1991** the sensitivity of micronucleus test is increased by recording degenerative nuclear alterations indicative of apoptosis and necrosis such as karyorrhexis, karyorlysis, pyknosis, nuclear bud and condensed chromatin, in addition to micronucleus. In order to monitor cytotoxic effects, micronucleus, pyknosis, karyolysis, karyorrhexis, nuclear bud and condensed chromatin are evaluated in this study in patients exposed to panoramic radiography.

# *Introduction*

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Different laboratories have reported variable normal background MN frequency in human oral epithelial cells: 0.16% (**Tolbert[73] et al. 1991**), 0.04% (**Karahalil[47] et al. 1999**), 0.1-0.3% (**Fenech[28] et al. 1999**) and 0.08% (**Burgaz[9] et al. 1999**).

**Tolbert[74] et al., 1992** defined morphologic scoring criteria for micronuclei and other confounding factors, such as age and gender of the study groups. However, only little attention has been, until now, given to the effect of different staining procedures on the results of micronuclei assays. An evaluation of the literature shows that a variety of different stains is used in micronuclei studies.

**Application of oral exfoliative cytology in measuring damage from radiation:**

Evaluation of radiation-induced cellular changes with a view to predict radiosensitivity has interested many investigators since such changes were first found in biopsy material in 1935. Cytologic evaluation of irradiation effects on oral mucosa was first reported in 1957 and on oral cancer in 1959. By the 1960s the nuclear morphologic changes that were to be evaluated by cytology became well-established and included pyknosis, karyorrhexis, karyolysis, enlargement, crenation of the nuclear membrane and multinucleation Even though micronucleation had been reported as a radiation-related change and later came to be accepted as a reliable indicator for measuring radiation exposure, but cytologists had not incorporated it in their evaluation of smears.

**Cerqueira EM, Gomes-Filhoet[11] al 2004**, studied the genotoxic effects of X-ray emitted during dental panoramic radiography in exfoliated cells from oral epithelium through a differentiated protocol of the micronucleus test. Thirty-one healthy individuals participated in this study. All of them answered a questionnaire before the examination. Cells were obtained from both sides of the cheek by gentle scrapping with a cervical brush, immediately before the exposure and after 10 days. Cytological preparations were stained according to Feulgen-Rossenbeck reaction and analyzed under light and laser scanning confocal microscopies. Micronuclei, nuclear projections (buds and broken eggs) and degenerative nuclear alterations (condensed chromatin, karyolysis and karyorrhexis) were scored. The frequencies of micronuclei, karyolysis and pycnosis were similar before and after exposure ($P > 0.90$), whereas the condensation of the chromatin and the karyorrhexis increased significantly after exposure ($P < 0.001$). In contrast, both bud and broken egg frequencies were significantly higher before the examination ($P < 0.005$), suggesting that these structures are associated to the normal epithelium differentiation. The results suggested that the X-ray exposure during panoramic dental radiography induces a cytotoxic effect by increasing apoptosis. The score of other nuclear alterations in addition to the micronucleus improves the sensitivity of genotoxic effects detection.

**Angelieri F, de Oliveira GR[1], et al 2007**, evaluated DNA damage (micronucleus) and cellular death (pyknosis, karyolysis and karyorrhexis) in exfoliated buccal mucosa cells taken from 17 healthy children following exposure to

radiation during dental radiography. They found no statistically significant differences ($P > 0.05$) between micronucleated oral mucosa cells in children before and after exposure to radiation. On the other hand, radiation did cause other nuclear alterations closely related to cytotoxicity including karyorrhexis, pyknosis and karyolysis. They conclude that these results indicate that panoramic dental radiography might not induce chromosomal damage, but may be cytotoxic. Overall, the results reinforce the importance of evaluating the health side effects of radiography and contribute to the micronucleus database, which will improve our understanding and practice of this methodology in children.

**da Silva AE, Rados PV[14] et al 2007**, aimed to investigate the effect of radiation from panoramic radiographs on the cells of the lateral border of the tongue by evaluating nuclear changes. Forty-two patients were included: 22 had one radiograph (Group I), and 20 required a repeat radiograph due to error in the first exposure (Group II). Material for the cytopathologic evaluation was collected before radiographs and 10 days later. Smears were stained with the Feulgen reaction and micronuclei, buds, broken eggs, karyorrhexis and binucleate cells were scored. The comparison of nuclear changes before and after radiation exposure in both groups revealed a statistically higher number of broken eggs, buds, karyorrhexis and binucleate cells 10 days after exposure ($P=0.01$). The number of karyorrhexis and binucleate cells was greater in group II ($P=0.01$). There was no change in the frequency of micronuclei before and after the radiographs. Radiation emitted during

panoramic radiographs increased the number of nuclear anomalies (except micronuclei) in exfoliated cells of the lateral border of the tongue. This effect was more pronounced when the patients were exposed to a repeat radiograph, without however implying increased risk of irreversible tissue damage.

**Popova L, Kishkilova[60] et al 2007**, evaluated the possible genotoxic effect of radiation exposure for dental diagnostic purposes as measured by the formation of micronuclei. The micronucleus test was applied to buccal epithelium cells, which are target cells for dental radiography. Specimens of exfoliated buccal cells were collected from patients subjected to panoramic radiography. Samples were obtained from 32 patients, 12 male and 20 female, aged from 24 years to 73 years, before and 10+/-2 days after panoramic radiation exposure. No significant increase in the frequency of cells with micronuclei and total number of micronuclei after panoramic tomography was detected. Mean values of buccal cells with micronuclei+/-standard deviation (SD) before and after radiation examination were 2.34+/-1.49% and 2.81+/-1.64%, respectively. A significant correlation between the age of investigated subjects and the initial frequency of micronuclei in buccal cells was observed ($r=0.60$, $P<0.01$).They concluded panoramic radiographic examination does not induce micronuclei in target buccal epithelium cells.

**Cerqueira EMM, JRC Meireles[12] et al 2008,** evaluated the genotoxic effects of X-rays on epithelial gingival cells during panoramic dental radiography using a differentiated protocol for the micronucleus test on 40 healthy individuals

immediately before exposure and 10 days later. Cytological preparations were stained according to the Feulgen-Rossenbeck reaction, counterstained with fast green 1% for 1 min and analysed under a light microscope. Micronuclei, nuclear projections (broken eggs) and degenerative nuclear alterations (pyknosis, karyolysis, karyorrhexis and condensed chromatin) were scored. The frequency of micronuclei was significantly higher after exposure (P= 0.05), as were the frequencies of nuclear alterations indicative of apoptosis (P=0.001). These results indicate that X-ray radiation emitted during panoramic dental radiography induces a genotoxic effect on epithelial gingival cells that increases the frequency of chromosomal damage and nuclear alterations indicative of apoptosis.

**Ribeiro DA, Angelieri F[64] 2008**, conducted a study to evaluate DNA damage (micronucleus) and cellular death in exfoliated buccal mucosa cells from healthy individuals (smokers and nonsmokers) following dental X-ray exposure. A total of 39 healthy people who had submitted to panoramic dental radiography were included in the study: 9 smokers and 30 nonsmokers. The results indicated no significant statistically differences (P>0.05) in micronucleated oral mucosa cells before and after dental X-ray exposure. On the other hand, X-ray exposure did increase other nuclear alterations closely related to cytotoxicity, such as karyorrhexis, pyknosis, and karyolysis. It seems that cigarette smoke did not affect X-ray outcomes induced in buccal cells. They concluded that these data indicate that dental panoramic radiography may not induce chromosomal damage, but it is able to promote

cytotoxicity. Because cellular death is considered a prime mechanism in nongenotoxic mechanisms of carcinogenesis, dental X-ray should be used only when necessary.

**A Ribeiro, G de Oliveira[64], 2008** comparatively evaluated the DNA damage (micronucleus) and cellular death (pyknosis, karyolysis and karyorrhexis) of exfoliated buccal mucosa cells from children and adults following dental X-ray exposure in 17 adults and 17 children. The results indicated no statistically significant differences (P=0.05) in children's micronucleated oral mucosa cells before and after dental X-ray exposure. In the same way, no mutagenic effects were observed in adults following X-ray exposure. On the other hand, X-rays increased other nuclear alterations closely related to cytotoxicity such as karyorrhexis, pyknosis and karyolysis in both groups. The comparative analysis between children and adults demonstrated no statistically significant differences in micronucleus frequency or cytotoxicity (P= 0.05). In summary, these data indicate that dental paroramic radiography may not be a factor that induces chromosomal damage, but it is able to promote cytotoxicity. It seems that children are not more susceptible to the noxious activities induced by X-rays when compared with adults.

**Liu, Cao et al[49], 2009** in their study of dose estimation by chromosome aberration analysis and micronucleus assays in victims accidentally exposed to 60Co radiation summarized that the results from their study indicate that both chromosome aberration analysis and the micronucleus assay provide a reliable estimate for

biological exposure to radiation, which is demonstrated a critical role in estimating the radiation dose and facilitating an accurate clinical diagnosis. This may enable faster and more reliable estimation of radiation exposure, leading to better treatment for patients.

**Conclusions Regarding Health Risk:**

We assume that any radiation exposure, no matter how small, carries with it some risk. However, we know that on an average, these risks are comparable to or smaller than risks we encounter in other activities or occupations that we consider safe. Since we have extensive control over how much radiation exposure we receive on the job, we can control and minimize this risk. The best approach is to keep our dose As Low As Reasonably Achievable, or ALARA - a term we will discuss in detail later. Minimizing the dose minimizes the risk. The development of digital radiography and the related advantages should not lead to increasing the number of radiographs. The prescribed and performed types of examinations, and their number, should always be selected based on the clinical situation and on sound clinical judgment and experience in order to solve the raised medical problem.

**Study design:**

Cross- sectional study conducted between August 2008 to July 2009 to determine the genetic changes in oral mucosa of healthy individuals subjected to orthopantamography.

**Study site:**

Department of Oral Medicine and Radiology in Ragas Dental College and Hospital, Uthandi, Chennai-600119.

**Study population:**

The study population consisted of healthy individuals subjected to orthopantamographs attending the extra oral radiology department in Ragas Dental College and Hospital, Uthandi, Chennai-600119.

**Obtaining approval from the authorities:**

Permission from the ethical committee of the Ragas Dental College and hospital was obtained before the starting of the study for examining and interpretation of patients. Also an informed consent was obtained from the patients forming the study sample, to participate in the study.

**Selection criteria:**

**Inclusion criteria:**

Normal healthy individuals who were subjected to orthopantamogram for various diagnostic purposes other than pathological conditions were included in the study.

**Exclusion criteria:**

1. Presence of other mucosal lesions like Leukoplakia, Oral Submucous Fibrosis, Lichen Planus.
2. Presence of intra oral swellings, ulcers.
3. Presence of malignant lesions.
4. Patients who have the habit of smoking, chewing tobacco products, betel nut or consuming alcohol.
5. Patients not willing for buccal scrapping.
6. History of previous exposure to radiation within past 3 months.
7. History of any systemic illness or immunodeficiency state.

**Sample Size**

Cells from buccal mucosa were obtained from 35 healthy individuals who were submitted to panoramic dental radiographic examination.

**MATERIALS**

1. **For examination of the patient:**

a. Physiological dental chair with provision for halogen light illumination.

b. Mouth mask

c. Sterile glove

d. Dental Mouth mirrors

e. Dental Explorer

f. Stainless Steel Kidney tray

g. Sterile Cotton and Gauze Pieces

**2. Materials for Radiographic procedures:**

a. Sattelac dental panoramic radiographic unit with specifications of 70 Kv, 10 mA, magnification factor.

b. Lead apron.

**3. Materials for Obtaining Buccal Smear:**

a. Pair of sterile gloves

b. Sterile disposable tooth brush

c. Sterile cotton

d. Isotonic saline solution

e. Glass slides

**3. Materials Used for Slide preparation:**

a. Methanol- acetic acid (3:1) solution as fixer solution.

b. 5M Hydrochloric acid.

c. Distilled water

d. Schiffs reagent

e. 0.2% light green

**4. Material for cytological analysis:**

a. Light microscope

**METHODOLOGY:**

**PROCEDURE:**

Cells from oral mucosa were obtained from thirty-five healthy individuals (18 males and 17 females) submitted to a panoramic dental radiography examination. They answered a questionnaire before the X-ray examination. The main features computed were age, consumption of tobacco and alcohol, exposure to other genotoxic agents and regular oral antiseptic solutions. The panoramic dental radiographies were performed with Sattelac X mind panoceph equipment, system 250—71 kV/15 mA/14 s/110 mGy cm2, effective dose 21.4 μSv. A detailed history of the patient and thorough clinical examination was done and findings recorded in the enclosed Performa.

**1. Interrogation:**

Detailed medical histories of the subjects were taken before clinical examination.

**2. Examination of the patients:**

The patients were made to sit comfortably on the dental chair with artificial illumination. The following findings such as Demographic details, Chief Complaint and Duration, Past Medical, Past Surgical and Past Dental History, Habits and reason for obtaining panoramic radiography as per criteria were recorded in a specialized proforma with regard to the study using Dental Mouth Mirrors, Dental Explorer and Williams Periodontal Probe.

**3. Collection of cells and slide preparation:**

Cytological smears were prepared immediately before the X-ray exposure and after 10 days.

**Preparation:**

Before subjecting the patient to panaromic radiography the subjects were seated in the dental chair with halogen light illumination and the sample material for analysis was collected in the following manner. The subjects were instructed to gargle with normal saline. The oral mucosa was dried with gauze swab to remove surface debris and excess saliva.

**Cell collection:**

The material for analyses was obtained from both sides of the buccal mucosa through gentle scrapping with a cervical brush and it was smeared over clean slides.

**Fixation and staining:**

Cells were fixed in a methanol–acetic acid (3:1) solution for 15-30 minutes. Slides were air-dried for 10 min prior to staining.

The common fixative used in cytogenetics (usually a combination between methanol and acetic acid) helps keep the cells in a "swollen" state. The fixative solution makes the cell membrane more fragile and suitable for spreading flat on the slide.

**Fuelgen reaction** :

It is a reaction in which an aldehyde combines with a modified Schiff's reagent to produce a purplish compound: used especially to test for the presence of DNA. Mild acid hydrolysis by using 5 M Hydrochloric acid makes the aldehyde group of deoxyribose available to react with Schiff's reagent to give a purple colour.

**Schiff's reagent** :

It is a colorless solution of fuchsin and sulfurous acid used as a reagent to identify an aldehyde from a ketone from the shade of reddish purple produced, to stain DNA.

**Feulgen staining:**

Slides were treated in 5 M hydrochloric acid for 30 min and then washed in running tap water for 3 min. Slides were drained but not allowed to dry out before being treated in room temperature Schiff's reagent (Sigma 3259016) in the dark for 60 min. Slides were washed in running tap water for 5 min and rinsed well in distilled water for 1 min. Slides were stained for 30 sec in 0.2% light green (Sigma L-1886) and rinsed well in distilled water for 2 min.Slides were allowed to air-dry.Nuclei and MNs are stained magenta, while the cytoplasm appears green. Slides were scored using a light microscope.

After 10 days of radiation exposure the samples were collected and smears were stained and studied in the same way.

**4. Cytological analysis**

Analysis was performed in a blind fashion in 2000 cells. The scoring was done according to the criteria established by Tolbert et al 1991. The following nuclear alterations were considered: micronucleus, nuclear projections as broken eggs and buds, pycnosis, karyorrhexis, karyolysis and condensed chromatin. Binucleated cells were excluded from analysis. The alterations were identified under light microscope.

The various distinct populations used in the buccal cytome assay were determined based on criteria outlined by **Tolbert et al[73] 1991**. These criteria are intended to classify BCs into categories that distinguish between 'normal' cells and

cells that are considered 'abnormal', based on nuclear morphology. These abnormal nuclear morphologies are thought to be indicative of DNA damage or cell death.

**Detailed descriptions of the various cell types are given below:**

**Basal cells:**

These are the cells from the basal layer. The nuclear to cytoplasm ratio is larger than that in differentiated BCs that are derived from basal cells. Basal cells have a uniformly stained nucleus and they are smaller in size when compared to differentiated BCs. Basal cells can contain MNs and were scored.

**Normal differentiated cells:**

These cells have a uniformly stained nucleus that is usually oval or round in shape. They are distinguished from basal cells by their larger size and by a smaller nuclear to cytoplasmic ratio. No other DNA containing structures apart from the nucleus are observed in these cells. These cells are considered to be terminally differentiated relative to basal cells because no mitotic cells are observed in this population.

**Cells with MNs:**

These cells are characterized by the presence of both a main nucleus and one or more smaller nuclei called MNs.The MNs are usually round or oval in shape and their diameter may range between 1/3 and 1/16 the diameter of the main nucleus.

Cells with MNs usually contain only one micronucleus. It is possible but rare to find cells with more than six MNs. The nuclei in micronucleated cells may have the morphology of normal cells or that of dying cells (i.e. condensed chromatin cells). The MNs must be located within the cytoplasm of the cells. The presence of MNs is indicative of chromosome loss or fragmentation occurring during previous nuclear division. MNs were scored only in basal and differentiated cells with uniformly stained nuclei. Cells with condensed chromatin or karyorrhectic cells were not scored for MNs.

For the scoring of micronuclei the following criteria were adopted from **Fenech[28] et al, 2003:**

1. the diameter of the MN should be less than one-third of the main nucleus
2. MN should be separated from or marginally overlap with main nucleus as long as there is clear identification of the nuclear boundary.
3. MN should have similar staining as the main nucleus.

**Binucleated differentiated cells:**

These cells have two nuclei instead of one. The nuclei are usually very close to each other and may be touching. The nuclei usually have the same morphology as that observed in normal cells. The significance of these cells is unknown but they may be indicative of failed cytokinesis following the last nuclear division.

**Condensed chromatin cells:**

These cells have nuclei with regions of condensed or aggregated chromatin exhibiting a speckled or striated nuclear pattern. In these cells, it is apparent that chromatin is aggregating in some regions of the nucleus while being lost in other areas. When chromatin aggregation is extensive, the nucleus may appear to be fragmenting. These cells may be undergoing early stages of apoptosis although this has not been conclusively proven. These cells may appear to contain MNs but should not be scored for MNs in the assay.

**Karyorrhectic cells:**

These cells are characterized by the more extensive appearance of nuclear chromatin aggregation (relative to condensed chromatin cells) leading to fragmentation and eventual disintegration of the nucleus. These cells may be undergoing a late stage of apoptosis but this has not been conclusively proven.

**Pyknotic cells:**

These cells are characterized by a small shrunken nucleus, with a high density of nuclear material that is uniformly but intensely stained . The nuclear diameter is usually one- to two- thirds of a nucleus in normal differentiated cells. The precise biological significance of pyknotic cells is unknown but it is thought that these cells may be undergoing a form of cell death; however, the precise mechanism is unknown.

**Karyolytic cells:**

In these cells, the nucleus is completely depleted of DNA and apparent as a ghost-like image that has no Feulgen staining. These cells thus appear to have no nucleus. It is probable that they represent a very late stage in the cell death process but this has not been conclusively proven.

**Cells with broken eggs:**

Broken eggs are considered as a fragmented nucleus, the little one corresponding to one third of the larger nucleus diameter and are connected with the main nucleus by means of thread or stalk like structure.

**Cells with nuclear buds:**

These cells have nuclei with an apparent sharp constriction at one end of the nucleus suggestive of a budding process, i.e. elimination of nuclear material by budding. The nuclear bud and the nucleus are usually in very close proximity and are apparently attached to each other. The nuclear bud has the same morphology and staining properties as the nucleus; however, its diameter may range from a half to quarter of that of the main nucleus. The mechanism leading to this morphology is not known but it may be due to elimination of amplified DNA or DNA repair complexes.

**5. Statistical analysis:**

Differences were statistically analyzed using the following method

**Independent t-test/ unpaired t-test**

When we compare the means two independent sample groups, we can use the student independent t-test . It is obtained using the following formula

When the difference between the means is divided by this standard error the result is *t*.

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{\sqrt{\left(\frac{s_p^2}{n_1} + \frac{s_p^2}{n_2}\right)}}$$

$sp^2$ **is** the pooled variance

$$s_p^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}$$

The standard error of the difference between the means is

$$SE(\bar{x}_1 - \bar{x}_2) = \sqrt{\left(\frac{s_p^2}{n_1} + \frac{s_p^2}{n_2}\right)}$$

Where $n_1$ is the sample size of first sample

$n_2$ is the sample size of second sample

$s_1$ is the standard deviation of first sample

$s_2$ is the standard deviation of first sample

$x_1$ is the mean of first sample

$x_2$ is the mean of second sample

**Paired / Matched /Dependent t-test**

When we use same group of samples in the pretest and in post test then we can analyze the data using paired t-test using the following formula.

- Find the mean of the differences, $\bar{d}$.
- Find the standard deviation of the differences, SD.
- Calculate the standard error of the mean $SE(\bar{d}) = SD/\sqrt{n}$
- To calculate *t*, divide the mean of the differences by the standard error of the mean

$$t = \frac{\bar{d}}{SE(\bar{d})}$$

where $\bar{d}$ is the mean of the differences, SE is the standard deviation of the differences, N is the number of pairs.

P- value: probability of differences

P> 0.05 = Difference is not significant (NS)

P≤ 0.05 = Difference is significant (S)

P≤ 0.01 = Difference is highly significant (S)

P≤ 0.001= Difference is very highly significant (HS)

**RAGAS DENTAL COLLEGE & HOSPITAL**

2/102, East Coast Road, Uthandi, Chennai - 600119

**DEPARTMENT OF ORAL MEDICINE & RADIOLOGY**

## Case Sheet Performa

Serial no: O.P No: Date:

1. **Name** :
2. **Age** :
3. **Sex** :
4. **Address** :

**5. Occupation :**

i. Unemployed ☐
ii. Employed ☐
iii. Business ☐
iv. Student ☐

**6. Income :**

i. < Rs. 1000 /-month ☐
ii. > Rs.1000-5000 /-month ☐
iii. > Rs. 5000 /-month ☐

**7. Religion :**

**8. Chief complaint:**

**9. History of presenting illness:**

**10. Past medical history:**

a. Presence of any systemic disease

(i)Yes ☐

(ii)No ☐

If yes specify

b. History of medication

(i)Yes ☐

(ii)No ☐

If yes specify

**11. Past surgical history:**

**12. Personal history:**

a. Food habits -

b. Brushing habits -

c. Use of oral mouth rinses-

**13. Previous exposure to X-rays:**

(i)Yes ☐

(ii)No ☐

If yes specify

**14. Indication for OPG exposure :**

**15. Investigation**:

Cytological analysis (pre-exposure)

[Scraping taken from right & left buccal mucosa before OPG radiograph]

i. Micronuclei (%) :
ii. Apoptosis (%) :
iii. Pycnosis (%) :
iv. Broken eggs (%) :
v. Nuclear bud (%) :

Cytological analysis (post-exposure)

[Scraping taken from right & left buccal mucosa after OPG radiograph]

i. Micronuclei (%) :
ii. Apoptosis (%) :
iii. Pyknosis (%) :
iv. Broken eggs (%) :
v. Nuclear bud (%) :

**Figure-1 Armamentarium for clinical examination**

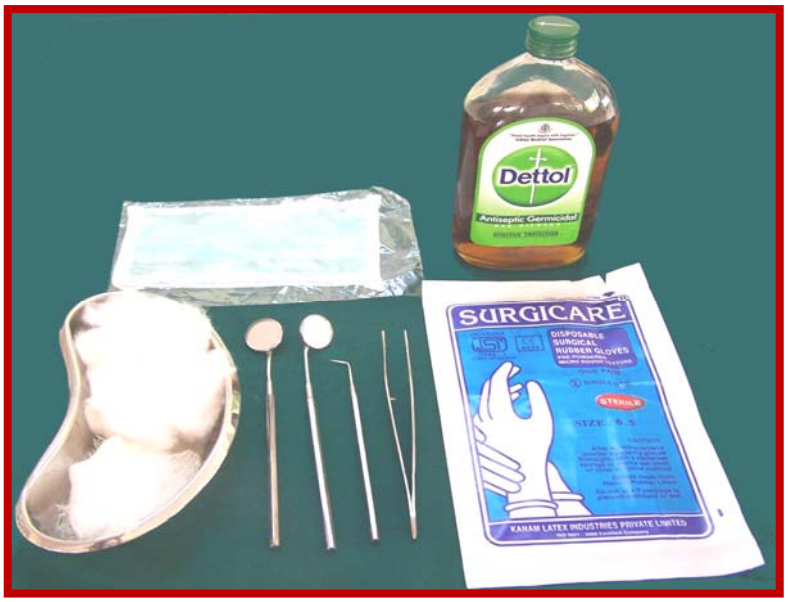



**Figure-2 Armamentarium for Buccal Cell Collection**

**Figure-3 Armamentarium for making panoramic radiograph**


**Figure-4 Armamentarium for staining the smears**

**Figure-5 Buccal Cell Collection**

**Figure- 6 Slide Preparation**

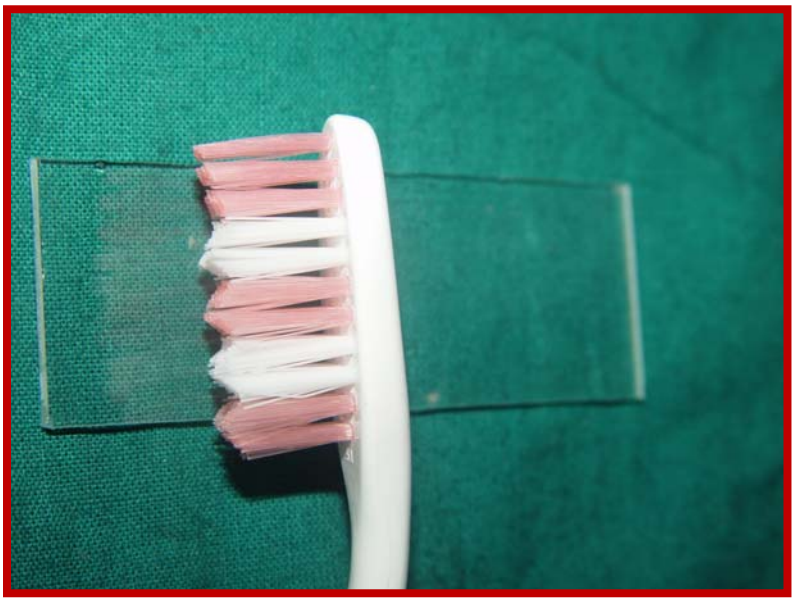


**Figure- 7 Patient positioning for panoramic radiograph.**

**Figure- 8 Prepared Slide**

**Figure- 9 Flourescent Microscope**

**Figure- 10 Cells in 10X Magnification**

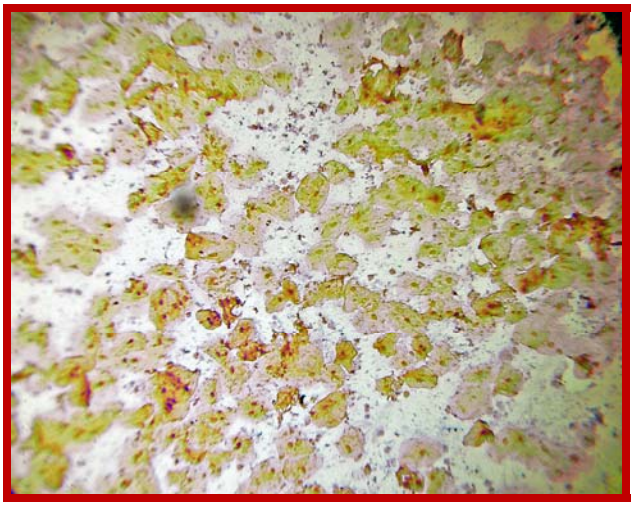


**Figure- 11 Normal Cells in 100X Magnification**

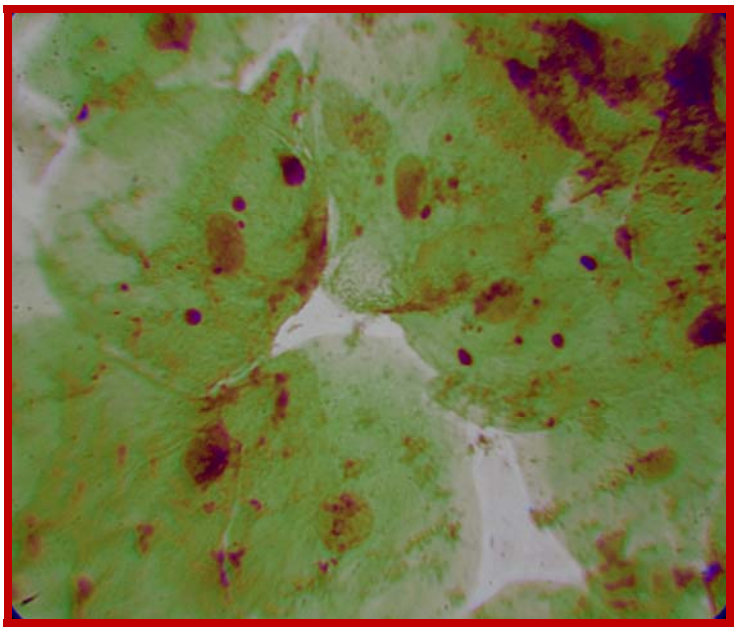


**Figure- 12 Normal Cells in 1000X Magnification**

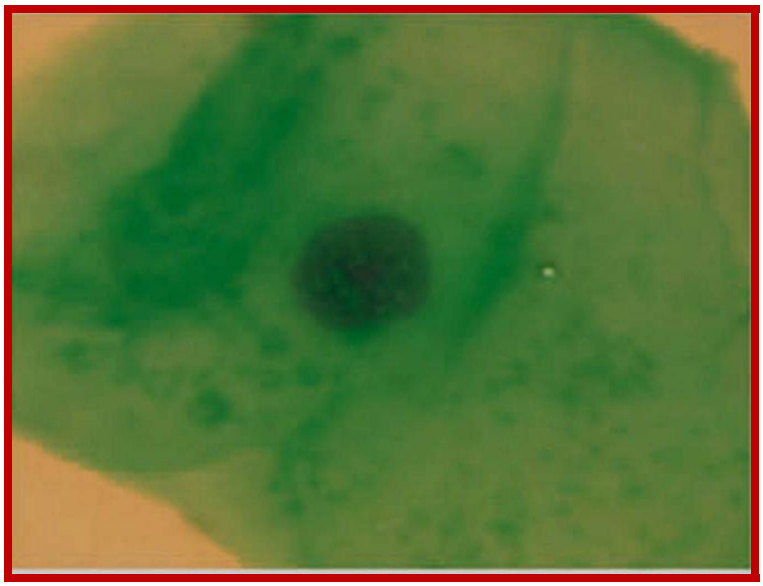


**Figure- 13 Differential Cells with micronuclei in 1000X Magnification**

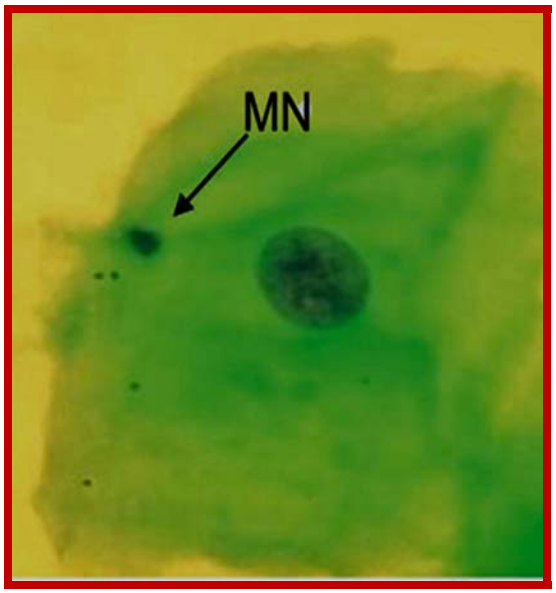



**Figure- 14 Karyorrhexis in 1000X Magnification**

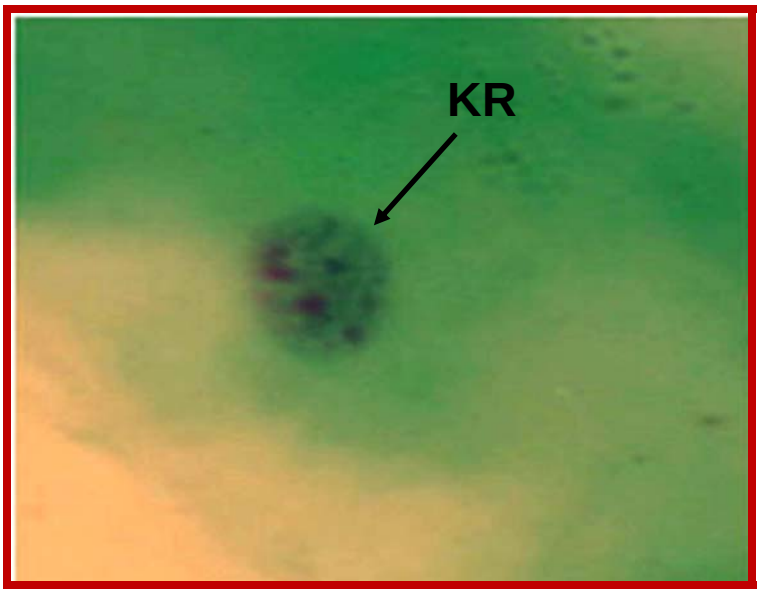


**Figure- 15 Condensed chromatin in 1000X Magnification**

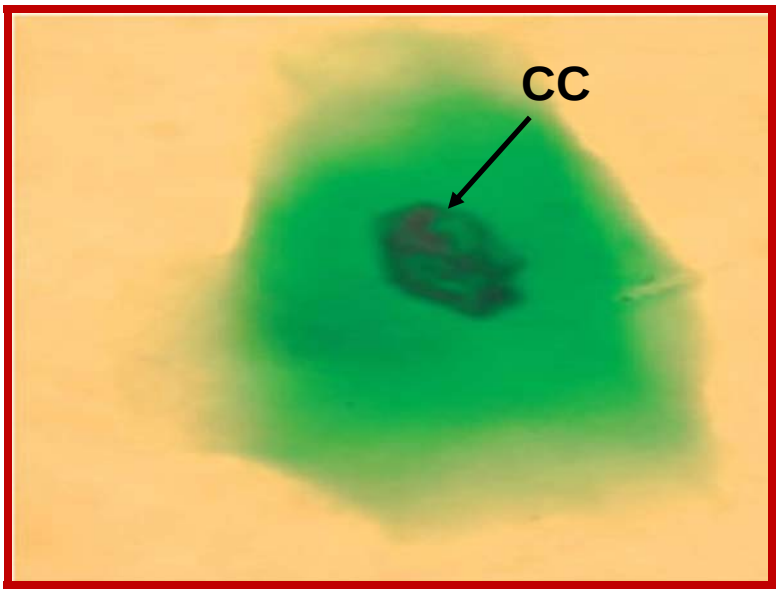



**Figure- 16 Pyknosis in 1000X Magnification**

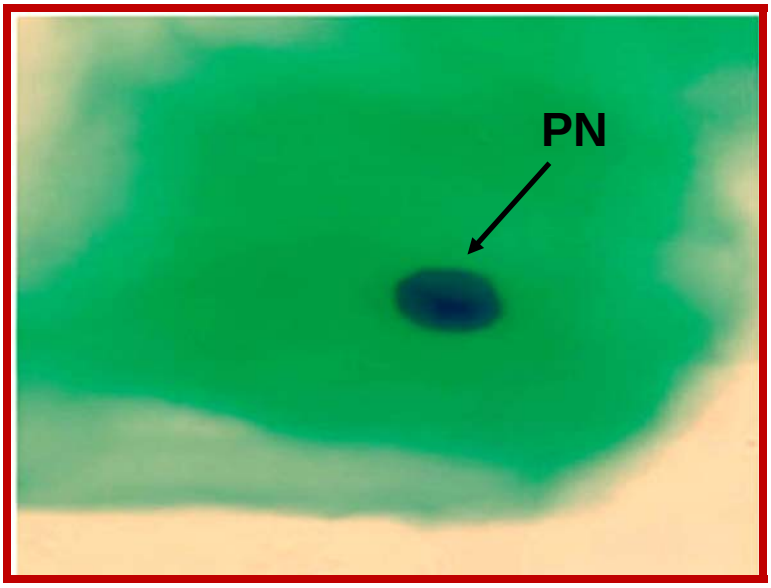


**Figure- 17 Karyolysis in 1000X Magnification**

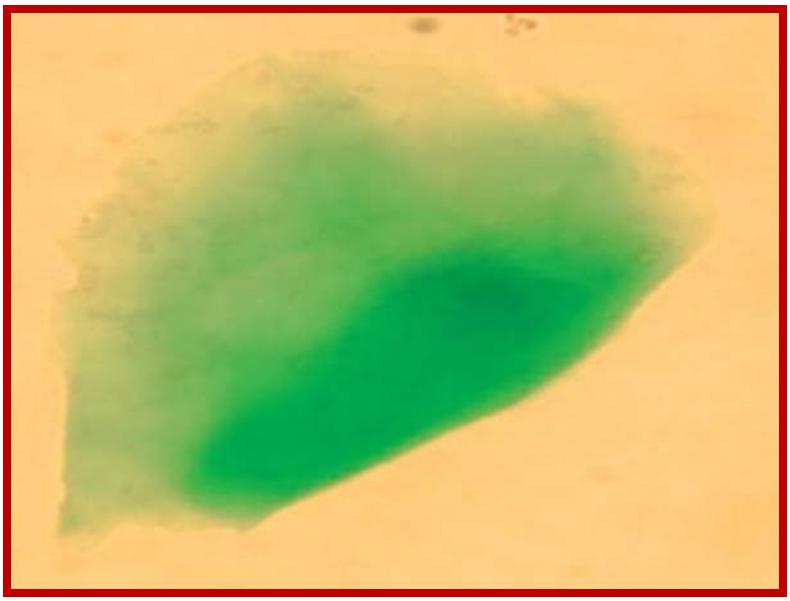
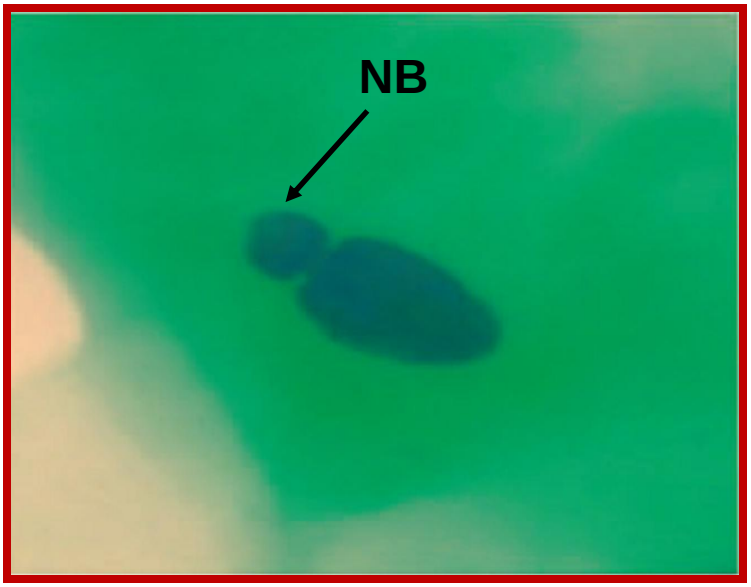


**Fig – 18 Nuclear Bud in 1000X Magnification**



The study population consisted of 35 normal healthy subjects attending the radiology department in Ragas Dental College and hospital. Data were collected to assess the genetic damage before and after exposure to panoramic dental radiography.

**Table 1-Graph 1: Gender wise distribution of subjects:**

The study comprised of 18(51.43%) males and 17(48.57%) females.

**Table 2-Graph 2: Age wise distribution of subjects:**

Table 2 shows age wise distribution of subjects in which 11 (31.43%) were between 16-20 years, 6 (17.14%) were between 21 - 24 years, 9(25.71%) were between 25 - 28 years, 6(17.14%) were between 29 - 32 years, 3(8.57%) were above 33 years.

**Table 2A-Graph 2A: Age wise distribution of subjects with distribution of male and female:**

Table 2A shows age wise distribution of subjects with distribution of male and female in which 4 (22.22%) males and 7 (41.17%) females were between 16-20 years, 1 (5.56%) males and 5 (29.41%) females were between 21 - 24 years, 6 (33.33%) males and 3 (17.65%) females were between 25 - 28 years, 5 (27.78%) males and 1 (5.88%) female were between 29 - 32 years, 2(11.11%) males and 1 (5.88%) female were above 33 years.

**Table 3-Graph 3 : Occupation wise distribution of subjects:**

Table 3 shows age wise distribution of subjects in which 3(8.57%) were unemployed, 10(28.57%) were employed, 5(14.29%) were business people, and 17(48.57%) were students.

**Table 3A-Graph 3A : Occupation wise distribution of subjects with distribution of male and female:**

Table 3A shows age wise distribution of subjects with distribution of male and female in which 0 (0.00%) male and 3(17.65 %) females were unemployed, 8(44.44%) males and 2(11.76%) females were employed, 5(27.78%) males and 0(0.00 %) female were business people, and 5(27.78%) male and 12( 70.59%) females were students.

**Table 4-Graph 4: Prevalence of micronuclei in subjects:**

Table 4 shows the prevalence of micronuclei in subjects before and after exposure to OPG.

**Pre exposure:** The total number of micronuclei counted was 39 (0.06%) and the number of micronuclei counted per subject were between 0-4 with 7(20%) had no micronuclei,18(51.43%) had 1 micronuclei, 9(25.71%) had 2 micronuclei and 1(2.86) had 3 micronuclei.

**Post exposure**: The total number of micronuclei counted was 41(0.06%) and the number of micronuclei counted per subject were between 0-4 with 9(25.71%) had no micronuclei,14(40%) had 1 micronuclei, 10(28.57%) had 2 micronuclei, 1(2.86) had 3 micronuclei and 1(2.86) had 4 micronuclei.

Since the p- value was 0.54, the micronuclei count is not statistically significant after exposure.

**Table 5 -Graph 5: Prevalence of karyorrhexis in subjects:**

Table 5 shows the prevalence of karyorrhexis in subjects before and after exposure to OPG.

**Pre exposure:** The total number of karyorrhexis counted was 2125(3.04%) and the number of karyorrhexis counted per subject were between 0 to 100 and above 100, with 4(11.43%) were between 0-20, 6(17.14%) were between 21-40, 8(22.86%) were between 41-60, 9(25.71%) were between 61-80, 6(17.14%) were between 81-100, and 2(5.71%) were above 100.

**Post exposure:** The total number of karyorrhexis counted was 2915(4.16%) and the number of karyorrhexis counted per subject were between 0 to 100 and above 100 with 0(0.0%) were between 0-20, 6(17.14%) were between 21-40, 6(17.14%) were between 41-60, 6(17.14%) were between 61-80, 8(22.86%) were between 81-100, and 9(25.71%) were above 100.

Since the p- value was 0.001, the karyorrhexis count is statistically significant after exposure.

**Table 6-Graph 6: Prevalence of condensed chromatin count in subjects:**

Table 6 shows the prevalence of condensed chromatin in subjects before and after exposure to OPG.

**Pre exposure:** The total number of condensed chromatin counted was 1650(2.36%) and the number of condensed chromatin counted per subject were between 0 to 100 and above 100 with 7(20.00%) were between 0-20, 8(22.86%) were between 21-40, 9(25.71%) were between 41-60, 9(25.71%) were between 61-80, 1(02.86%) was between 81-100 and 1(2.86%) was above 100.

**Post exposure:** The total number of condensed chromatin counted was 2435(3.48%) and the number of condensed chromatin counted per subject were between 0 to 100 and above 100 with 4(11.43%) were between 0-20, 5(14.29%) were between 21-40, 7(20.00%) were between 41-60, 6(17.14%) were between 61-80, 8(22.86%) were between 81-100 and 5(14.29%) were above 100.

Since the p- value was 0.001, the condensed chromatin count is statistically significant after exposure.

**Table 7 -Graph 7: Prevalence of pyknosis in subjects:**

Table 7 shows the prevalence of pyknosis in subjects before and after exposure to OPG.

**Pre exposure:** The total number of pyknosis counted was 429(0.61%) and the number of pyknosis counted per subject were between 1 to 40 with 17(48.57%) were between 1-10, 15(42.86%) were between 11-20, 2(5.71%) were between 21-30 and 1(2.86%) was between 31-40.

**Post exposure:** The total number of pyknosis counted was 516(0.74%) and the number of pyknosis counted per subject were between 1 to 50 and above 50 with

10(28.57%) were between 1-10, 20(57.14%) were between 11-20, 4(11.43%) were between 21-30, 0(0%) was between 31-40 and 1(2.86%) was above 50.

Since the p- value was 0.001, the pyknosis count is statistically significant after exposure.

**Table 8 -Graph 8: Prevalence of karyolysis in subjects:**

Table 8 shows the prevalence of karyolysis in subjects before and after exposure to OPG.

**Pre exposure:** The total number of karyolysis counted was 31(0.04%) and the number of karyolysis counted per subject were between 0 to 3 with 9(25.71%) had no karyolysis, 21(60%) had 1, and 5(14.29%) had 2.

**Post exposure:** The total number of karyolysis counted was 46(0.07%) and the number of karyolysis counted per subject were between 0 to 3 with 7(20%) had nil, 14(40%) had 1 ,10(28.57%) had 2, and 4(11.43%) had 3.

Since the p- value was 0.009, the karyolysis count is statistically significant after exposure.

**Table 9-Graph 9: Prevalence of broken eggs in subjects:**

Table 9 shows the prevalence of broken eggs in subjects before and after exposure to OPG.

**Pre exposure**: The total number of broken eggs counted was 37(0.05%) and the number of broken eggs counted per subject were between 0 to 3 with 10(28.57%) had no broken egg,15(42.86%) had 1, 8(22.86%) had 2 and 2(5.71) had 3.

**Post exposure:** The total number of broken eggs counted was 67(0.1%) and the number of broken eggs counted per subject were between 0 to 4 with 3(8.57%) had no broken egg,10(28.57%) had 1, 13(37.14%) had 2 , 6(17.14%) had 3 and 3(8.57) had 4.

Since the p- value was 0.001, the broken egg count is statistically significant after exposure.

**Table 10 -Graph 10: Prevalence of nuclear buds in subjects:**

Table 10 shows the prevalence of nuclear buds in subjects before and after exposure to OPG.

**Pre exposure:** The total number of nuclear buds counted was 170(0.24%) and the number of nuclear buds counted per subject were between 0 to 10 with 6(17.14%) had 0-2, 11(31.43%) had 3-4, 10(28.57%) had 5-6, 4 (11.43%) had 7-8 and 4(11.43%) had 9-10.

**Post exposure:** The total number of nuclear buds counted was 193(0.28%) and the number of nuclear buds counted per subject were between 0 to 10 with 8(22.86%) had 0-2, 3(8.57%) had 3-4, 8(22.86%) had 5-6, 14 (40.00%) had 7-8 and 2(5.71%) had 9-10.

Since the p- value was 0.31, the nuclear bud count is not statistically significant after exposure.

**Table 11-Graph 11: Prevalence of nuclear projections in subjects:**

Table 11 shows the prevalence of nuclear projections in subjects before and after exposure to OPG.

**Pre exposure:** The total number of nuclear projections counted was 207(0.24%) and the number of nuclear projections counted per subject were between 0 to 10 and above 10 with 5 (14.29%) were between 0-2, 7(20.00%) were between 3-4, 8 (22.86%) were between 5-6, 10 (28.57%) were between 7-8, 2 (5.71%) were between 9-10 and 3 (8.57%) were above 10.

**Post exposure:** The total number of nuclear projections counted was 260(0.37%) and the number of nuclear buds counted per subject were between 0 to 10 and above 10 with 5 (14.29%) were between 0-2, 4(11.43%) were between 3-4, 6 (17.14%) were between 5-6, 8(22.86%) were between 7-8, 7 (20.00%) were between 9-10 and 5 (14.29%) were above 10.

Since the p- value was 0.15, the nuclear projections count is not statistically significant after exposure.

**Table 12-Graph 12: Comparison between pre radiation exposure and post radiation exposure values among males:**

Table 12 shows the comparison between pre radiation exposure and post radiation exposure values among males.

**Pre exposure:** The number of micronuclei counted was 19(0.03%) and the mean and SD was 1.06 and 0.64.The number of karyorrhexis counted was 1153(1.65%) and the mean and SD was 64.06 and 33.65. The number of condensed chromatin counted was 859(1.23%) and the mean and SD was 47.72 and 25.42.The number of pyknosis counted was 254(0.36%) and the mean and SD was 10.67 and 4.61.The number of karyolysis counted was 18(0.03%) and the mean and SD was 0.94 and 0.64. The number of broken eggs counted was 20(0.03%) and the mean and SD was 1.11 and 0.96.The number of nuclear buds counted was 85(0.12%) and the mean and SD was 4.72 and 2.19.The number of nuclear projections counted was 105(0.15%) and the mean and SD was 5.83 and 2.75.

**Post exposure:**The number of micronuclei counted was 22(0.03%) and the mean and SD was 1.11 and 0.90. The number of karyorrhexis counted was 1661(2.37%) and the mean and SD was 92.28 and 55.78. The number of condensed chromatin counted was 1356(1.94%) and the mean and SD was 75.33 and 45.68.The number of pyknosis counted was 237(0.42%) and the mean and SD was 13.17 and 7.06.The number of karyolysis counted was 25(0.04%) and the mean and SD was 1.44 and 0.98. The number of broken eggs counted was 38(0.06%) and the mean and SD was 2.11 and 1.37.The number of nuclear buds counted was 96(0.14%) and the mean and SD was 5.44 and 2.33.The number of nuclear projections counted was 134(0.18%) and the mean and SD was 7.22 and 3.08.

The p value for karryorhexis was 0.003; condensed chromatin p=0.001; pyknosis p=0.05, karyolysis p=0.02 and broken eggs p=0.002 these values are statistically significant after exposure.

The p value for micronuclei was 0.74, nuclear buds p=0.39 and nuclear projections p=0.14 these values are statistically not significant after exposure.

**Table 13-Graph 13: Comparison between pre radiation exposure and post radiation exposure values among females:**

Table 13 shows the comparison between pre radiation exposure and post radiation exposure values among females.

**Pre exposure:** The number of micronuclei counted was 20(0.03%) and the mean and SD was 1.18 and 0.88.The number of karyorrhexis counted was 972(1.39%) and the mean and SD was 57.18 and 27.65. The number of condensed chromatin counted was 791(1.13%) and the mean and SD was 46.53 and 24.06.The number of pyknosis counted was 175(0.25%) and the mean and SD was 13.94 and 8.55.The number of karyolysis counted was 13(0.02%) and the mean and SD was 0.82 and 0.64. The number of broken eggs counted was 17(0.02%) and the mean and SD was 1.00 and 0.79.The number of nuclear buds counted was 85(0.12%) and the mean and SD was 5.00 and 2.87.The number of nuclear projections counted was 102(0.15%) and the mean and SD was 6.00 and 3.22.

**Post exposure:** The number of micronuclei counted was 21(0.03%) and the mean and SD was 1.24 and 1.03. The number of karyorrhexis counted was 1254(1.80%) and the mean and SD was 73.76 and 33.35. The number of condensed chromatin

counted was 1079(1.54%) and the mean and SD was 63.47 and 31.03.The number of pyknosis counted was 279(0.40%) and the mean and SD was 16.41 and 10.06.The number of karyolysis counted was 20(0.03%) and the mean and SD was 1.18 and 0.88. The number of broken eggs counted was 29(0.04%) and the mean and SD was 1.71and 0.85.The number of nuclear buds counted was 97(0.14%) and the mean and SD was 5.59 and 2.90.The number of nuclear projections counted was 126(0.18%) and the mean and SD was 6.53 and 3.22.

The p value for karyorrhexis was 0.001, condensed chromatin p=0.001 pyknosis p=0.01, and broken eggs p=0.006 these values are statistically significant after exposure.

The p value for micronuclei was 0.33, karyolysis p= 0.16,nuclear buds p=0.57 and nuclear projections p=0.59 these values are statistically not significant after exposure.

**Table 14-Graph 14: Comparison of pre radiation exposure values between males and females.**

Table 14 shows the comparison of pre radiation exposure values between males and females.

**Males:** The number of micronuclei counted was 19(0.03%) and the mean and SD was 1.06 and 0.64.The number of karyorrhexis counted was 1153(1.65%) and the mean and SD was 64.06 and 33.65. The number of condensed chromatin counted was 859(1.23%) and the mean and SD was 47.72 and 25.42.The number of pyknosis counted was 254(0.36%) and the mean and SD was 10.67 and 4.61.The number of

karyolysis counted was 18(0.03%) and the mean and SD was 0.94 and 0.64. The number of broken eggs counted was 20(0.03%) and the mean and SD was 1.11 and 0.96.The number of nuclear buds counted was 85(0.12%) and the mean and SD was 4.72 and 2.19.The number of nuclear projections counted was 105(0.15%) and the mean and SD was 5.83 and 2.75.

**Females:** The number of micronuclei counted was 20(0.03%) and the mean and SD was 1.18 and 0.88.The number of karyorrhexis counted was 972(1.39%) and the mean and SD was 57.18 and 27.65. The number of condensed chromatin counted was 791(1.13%) and the mean and SD was 46.53 and 24.06.The number of pyknosis counted was 175(0.25%) and the mean and SD was 13.94 and 8.55.The number of karyolysis counted was 13(0.02%) and the mean and SD was 0.82 and 0.64. The number of broken eggs counted was 17(0.02%) and the mean and SD was 1.00 and 0.79.The number of nuclear buds counted was 85(0.12%) and the mean and SD was 5.00 and 2.87.The number of nuclear projections counted was 102(0.15%) and the mean and SD was 6.00 and 3.22.

The p value for micronuclei was 0.64, karyolysis p=0.51, condensed chromatin p=0.81, pyknosis p=0.16, karyolysis p= 0.16, broken eggs p=0.58, nuclear buds p=0.71 and nuclear projections p=0.81 these values are statistically not significant between males and females.

**Table 15-figure 15: Comparison of post radiation exposure values between males and females:**

Table 15 shows the comparison of post radiation exposure values between males and females.

**Males:** The number of micronuclei counted was 22(0.03%) and the mean and SD was 1.11 and 0.90. The number of karyorrhexis counted was 1661(2.37%) and the mean and SD was 92.28 and 55.78. The number of condensed chromatin counted was 1356(1.94%) and the mean and SD was 75.33 and 45.68.The number of pyknosis counted was 237(0.42%) and the mean and SD was 13.17 and 7.06.The number of karyolysis counted was 25(0.04%) and the mean and SD was 1.44 and 0.98. The number of broken eggs counted was 38(0.06%) and the mean and SD was 2.11 and 1.37.The number of nuclear buds counted was 96(0.14%) and the mean and SD was 5.44 and 2.33.The number of nuclear projections counted was 134(0.18%) and the mean and SD was 7.22 and 3.08.

**Females:** The number of micronuclei counted was 21(0.03%) and the mean and SD was 1.24 and 1.03. The number of karyorrhexis counted was 1254(1.80%) and the mean and SD was 73.76 and 33.35. The number of condensed chromatin counted was 1079(1.54%) and the mean and SD was 63.47 and 31.03.The number of pyknosis counted was 279(0.40%) and the mean and SD was 16.41 and 10.06.The number of karyolysis counted was 20(0.03%) and the mean and SD was 1.18 and 0.88. The number of broken eggs counted was 29(0.04%) and the mean and SD was 1.71and 0.85.The number of nuclear buds counted was 97(0.14%) and the mean and SD was 5.59 and 2.90.The number of nuclear projections counted was 126(0.18%) and the mean and SD was 6.53 and 3.22.

The p value for micronuclei was 0.71, karyolysis p=0.25, condensed chromatin p=0.38, pyknosis p=0.27, karyolysis p= 0.40, broken eggs p=0.30, nuclear buds p=0.40 and nuclear projections p=0.30 these values are statistically not significant between males and females.

**Comparison of study variables between Pre- exposure and Post-**

**Exposure in subjects:**

**TABLE-1**

**GENDER WISE DISTRIBUTION OF SUBJECTS**

| SEX | NO | % |
|---|---|---|
| MALE | 18 | 51.43% |
| FEMALE | 17 | 48.57% |
| TOTAL | 35 | 100.0% |

`

**TABLE-2**

**AGE WISE DISTRIBUTION OF SUBJECTS**

| AGE | NO | % |
|---|---|---|
| 16-20 yrs | 11 | 31.43% |
| 21-24 yrs | 6 | 17.14% |
| 25-28 yrs | 9 | 25.71% |
| 29-32 yrs | 6 | 17.14% |
| >33 yrs | 3 | 08.57% |
| **TOTAL** | 35 | 100.0% |

**TABLE-2A**

**AGE WISE DISTRIBUTION OF SUBJECTS WITH DISTRIBUTION OF MALE AND FEMALE**

| AGE | MALES | | FEMALES | |
|---|---|---|---|---|
| | NO | % | NO | % |
| 16-20 yrs | 4 | 22.22% | 7 | 41.17% |
| 21-24 yrs | 1 | 5.56% | 5 | 29.41% |
| 25-28 yrs | 6 | 33.33% | 3 | 17.65% |
| 29-32 yrs | 5 | 27.78% | 1 | 5.88% |
| >33 yrs | 2 | 11.11% | 1 | 5.88% |
| TOTAL | 18 | 100.0% | 17 | 100.0% |

**TABLE-3**

**OCCUPATION WISE DITRIBUTION OF SUBJECTS**

| OCCUPATION | NO | % |
|---|---|---|
| Unemployed | 3 | 08.57% |
| Employed | 10 | 28.57% |
| Business | 5 | 14.29% |
| Student | 17 | 48.57% |
| **TOTAL** | 35 | 100.0% |

**TABLE-3A**

**OCCUPATION WISE DISTRIBUTION OF SUBJECTS WITH DISTRIBUTION OF MALE AND FEMALE**

| OCCUPATION | MALES | | FEMALES | |
|---|---|---|---|---|
| | NO | % | NO | % |
| Unemployed | 0 | 0.00% | 3 | 17.65% |
| Employed | 8 | 44.44% | 2 | 11.76% |
| Business | 5 | 27.78% | 0 | 0.00% |
| Student | 5 | 27.78% | 12 | 70.59% |
| TOTAL | 18 | 100.0% | 17 | 100.0% |

**TABLE-4**

**PREVALANCE OF MICRONUCLEI IN SUBJECTS**

| MICRONUCLEI | PRE EXPOSURE Cells with micronuclei = 39 ( 0.06%) | | POST EXPOSURE Cells with micronuclei = 41 ( 0.06%) | | t-value | p-value |
|---|---|---|---|---|---|---|
| | NOS | % | NOS | % | | |
| 0 | 7 | 20.00% | 9 | 25.71% | 0.627 | 0.54 |
| 1 | 18 | 51.43% | 14 | 40.00% | | |
| 2 | 9 | 25.71% | 10 | 28.57% | | |
| 3 | 1 | 02.86% | 1 | 02.86% | | |
| 4 | 0 | 00.00% | 1 | 02.86% | | |
| TOTAL | 35 | 100.0% | 35 | 100.0% | | |

**TABLE-5**

**5. PREVALANCE OF KARYORRHEXIS IN SUBJECTS**

| **KARYORRHEXIS** | **PRE EXPOSURE** Cells with karyorrhexis = 2125 (3.04%) | | **POST EXPOSURE** Cells with karyorrhexis = 2915 (4.16%) | | **t-value** | **p-value** |
|---|---|---|---|---|---|---|
| | **NOS** | **%** | **NOS** | **%** | | |
| 0-20 | 4 | 11.43% | 0 | 00.00% | | |
| 21-40 | 6 | 17.14% | 6 | 17.14% | | |
| 41-60 | 8 | 22.86% | 6 | 17.14% | | |
| 61-80 | 9 | 25.71% | 6 | 17.14% | 5.129 | 0.001 |
| 81-100 | 6 | 17.14% | 8 | 22.86% | | |
| >100 | 2 | 05.71% | 9 | 25.71% | | |
| TOTAL | 35 | 100.0% | 35 | 100.0% | | |

**TABLE-6**

**6. PREVALANCE OF CONDENSED CHROMATIN IN SUBJECTS**

| **CONDENSED CHROMATIN** | **PRE EXPOSURE** Cells with condensed chromatin = 1650 (2.36%) | | **POST EXPOSURE** Cells with condensed chromatin = 2435 (3.48%) | | **t-value** | **p-value** |
|---|---|---|---|---|---|---|
| | **NOS** | **%** | **NOS** | **%** | | |
| 0-20 | 7 | 20.00% | 4 | 11.43% | | |
| 21-40 | 8 | 22.86% | 5 | 14.29% | | |
| 41-60 | 9 | 25.71% | 7 | 20.00% | 5.713 | 0.001 |
| 61-80 | 9 | 25.71% | 6 | 17.14% | | |
| 81-100 | 1 | 02.86% | 8 | 22.86% | | |
| >100 | 1 | 02.86% | 5 | 14.29% | | |
| TOTAL | 35 | 100.00% | 35 | 100.0% | | |

**TABLE-7**

**PREVALANCE OF PYKNOSIS IN SUBJECTS**

| **PYKNOSIS** | **PRE EXPOSURE** Cells with pyknosis= 429 (0.61%) | | **POST EXPOSURE** Cells with pyknosis= 516 (0.74%) | | **t-value** | **p-value** |
|---|---|---|---|---|---|---|
| | **NOS** | **%** | **NOS** | **%** | | |
| 1-10 | 17 | 48.57% | 10 | 28.57% | | |
| 11-20 | 15 | 42.86% | 20 | 57.14% | | |
| 21-30 | 2 | 05.71% | 4 | 11.43% | 3.351 | 0.001 |
| 31-40 | 1 | 02.86% | 0 | 00.00% | | |
| 41-50 | 0 | 00.00% | 0 | 00.00% | | |
| >50 | 0 | 00.00% | 1 | 02.86% | | |
| TOTAL | 35 | 100.00% | 35 | 100.0% | | |

**TABLE-8**

**PREVALANCE OF KARYOLYSIS IN SUBJECTS**

| KARYOLYSIS | PRE EXPOSURE Cells with karyolysis= 31 (0.04%) | | POST EXPOSURE Cells with karyolysis= 46 (0.07%) | | t-value | p-value |
|---|---|---|---|---|---|---|
| | NOS | % | NOS | % | | |
| 0 | 9 | 25.71% | 7 | 20.00% | 2.766 | 0.009 |
| 1 | 21 | 60.00% | 14 | 40.00% | | |
| 2 | 5 | 14.29% | 10 | 28.57% | | |
| 3 | 0 | 00.00% | 4 | 11.43% | | |
| TOTAL | 35 | 100.0% | 30 | 100.0% | | |

**TABLE-9**

**PREVALANCE OF BROKEN EGGS IN SUBJECTS**

| BROKEN EGGS | PRE EXPOSURE Cells with broken eggs= 37 (0.05%) | | POST EXPOSURE Cells with broken eggs= 67(0.1%) | | t-value | p-value |
|---|---|---|---|---|---|---|
| | NOS | % | NOS | % | | |
| 0 | 10 | 28.57% | 3 | 08.57% | 4.779 | 0.001 |
| 1 | 15 | 42.86% | 10 | 28.57% | | |
| 2 | 8 | 22.86% | 13 | 37.14% | | |
| 3 | 2 | 05.71% | 6 | 17.14% | | |
| 4 | 0 | 00.00% | 3 | 08.57% | | |
| TOTAL | 35 | 100.0% | 30 | 100.0% | | |

**TABLE-10: PREVALANCE OF NUCLEAR BUDS IN SUBJECTS**

| NUCLEAR BUDS | PRE EXPOSURE Cells with nuclear buds= 170 (0.24%) | | POST EXPOSURE Cells with nuclear buds= 193 (0.28%) | | t-value | p-value |
|---|---|---|---|---|---|---|
| | NOS | % | NOS | % | | |
| 0-2 | 6 | 17.14% | 8 | 22.86% | 1.026 | 0.31 |
| 3-4 | 11 | 31.43% | 3 | 08.57% | | |
| 5-6 | 10 | 28.57% | 8 | 22.86% | | |
| 7-8 | 4 | 11.43% | 14 | 40.00% | | |
| 9-10 | 4 | 11.43% | 2 | 05.71% | | |
| TOTAL | 35 | 100.00% | 35 | 100.0% | | |

**TABLE-11: PREVALANCE OF NUCLEAR PROJECTIONS IN SUBJECTS**

| NUCLEAR PROJECTION | PRE EXPOSURE Cells with nuclear projections= 207 (0.24%) | | POST EXPOSURE Cells with nuclear projections= 260 (0.37%) | | t-value | p-value |
|---|---|---|---|---|---|---|
| | NO | % | NO | % | | |
| 0-2 | 5 | 14.29% | 5 | 14.29% | 1.488 | 0.15 |
| 3-4 | 7 | 20.00% | 4 | 11.43% | | |
| 5-6 | 8 | 22.86% | 6 | 17.14% | | |
| 7-8 | 10 | 28.57% | 8 | 22.86% | | |
| 9-10 | 2 | 05.71% | 7 | 20.00% | | |
| >10 | 3 | 08.57% | 5 | 14.29% | | |
| TOTAL | 35 | 100.00% | 35 | 100.00% | | |

**TABLE-12**

**COMPARISON BETWEEN PRE RADIATION EXPOSURE AND POST RADIATION EXPOSURE VALUES AMONG MALES**

| | Pre exposure(n=18) | | | Post exposure(n=18) | | | Student paired t-test | |
|---|---|---|---|---|---|---|---|---|
| | **No(%)** | **Mean** | **SD** | **No (%)** | **Mean** | **SD** | **T value** | **P value** |
| **MN** | 19 (0.03%) | 1.06 | 0.64 | 22(0.03%) | 1.11 | 0.90 | 0.33 | 0.74 |
| **KR** | 1153(1.65%) | 64.06 | 33.65 | 1661(2.37 %) | 92.28 | 55.78 | 3.53 | 0.003 |
| **CC** | 859(1.23 %) | 47.72 | 25.42 | 1356(1.94 %) | 75.33 | 45.68 | 3.94 | 0.001 |
| **PK** | 254(0.36 %) | 10.67 | 4.61 | 237(0.42 %) | 13.17 | 7.06 | 2.05 | 0.05 |
| **KL** | 18(0.03 %) | 0.94 | 0.64 | 25(0.04 %) | 1.44 | 0.98 | 2.47 | 0.02 |
| **BE** | 20(0.03 %) | 1.11 | 0.96 | 38(0.06 %) | 2.11 | 1.37 | 3.57 | 0.002 |
| **NB** | 85(0.12 %) | 4.72 | 2.19 | 96(0.14 %) | 5.44 | 2.23 | 0.89 | 0.39 |
| **NP** | 105(0.15 %) | 5.83 | 2.75 | 134(0.18 %) | 7.22 | 3.08 | 1.54 | 0.14 |

**TABLE-13**

**COMPARISON BETWEEN PRE RADIATION EXPOSURE AND POST RADIATION EXPOSURE VALUES AMONG FEMALES**

| | Pre exposure (n=18) | | | Post exposure (n=18) | | | Student paired t-test | |
|---|---|---|---|---|---|---|---|---|
| | **No (%)** | **Mean** | **SD** | **No (%)** | **Mean** | **SD** | **T value** | **P value** |
| **MN** | 20(0.03 %) | 1.18 | 0.88 | 21(0.03 %) | 1.24 | 1.03 | 1.00 | 0.33 |
| **KR** | 972(1.39 %) | 57.18 | 27.65 | 1254(1.80 %) | 73.76 | 33.35 | 5.63 | 0.001 |
| **CC** | 791(1.13 %) | 46.53 | 24.06 | 1079(1.54 %) | 63.47 | 31.03 | 5.78 | 0.001 |
| **PK** | 175(0.25 %) | 13.94 | 8.55 | 279(0.40 %) | 16.41 | 10.06 | 2.88 | 0.01 |
| **KL** | 13(0.02 %) | 0.82 | 0.64 | 20(0.03 %) | 1.18 | 0.88 | 1.46 | 0.16 |
| **BE** | 17(0.02 %) | 1.00 | 0.79 | 29(0.04 %) | 1.71 | 0.85 | 3.16 | 0.006 |
| **NB** | 85(0.12 %) | 5.00 | 2.87 | 97(0.14 %) | 5.59 | 2.90 | 0.57 | 0.57 |
| **NP** | 102(0.15 %) | 6.00 | 3.22 | 126(0.18 %) | 6.53 | 3.22 | 1.55 | 0.59 |

**TABLE-14**

**COMPARISON OF PRE RADIATION EXPOSURE VALUES**

**BETWEEN MALES AND FEMALES**

| | **Males** | | | **Females** | | | **Student paired t-test** | |
|---|---|---|---|---|---|---|---|---|
| | **No (%)** | **Mean** | **SD** | **No (%)** | **Mean** | **SD** | **T value** | **P value** |
| **MN** | 19 (0.03%) | 1.06 | 0.64 | 20(0.03 %) | 1.18 | 0.88 | 0.46 | 0.64 |
| **KR** | 1153(1.65%) | 64.06 | 33.65 | 972(1.39 %) | 57.18 | 27.65 | 0.66 | 0.51 |
| **CC** | 859(1.23 %) | 47.72 | 25.42 | 791(1.13 %) | 46.53 | 24.06 | 0.14 | 0.81 |
| **PK** | 254(0.36 %) | 10.67 | 4.61 | 175(0.25 %) | 13.94 | 8.55 | 1.42 | 0.16 |
| **KL** | 18(0.03 %) | 0.94 | 0.64 | 13(0.02 %) | 0.82 | 0.64 | 1.46 | 0.16 |
| **BE** | 20(0.03 %) | 1.11 | 0.96 | 17(0.02 %) | 1.00 | 0.79 | 0.56 | 0.58 |
| **NB** | 85(0.12 %) | 4.72 | 2.19 | 85(0.12 %) | 5.00 | 2.87 | 0.37 | 0.71 |
| **NP** | 105(0.15 %) | 5.83 | 2.75 | 102(0.15 %) | 6.00 | 3.22 | 0.17 | 0.81 |

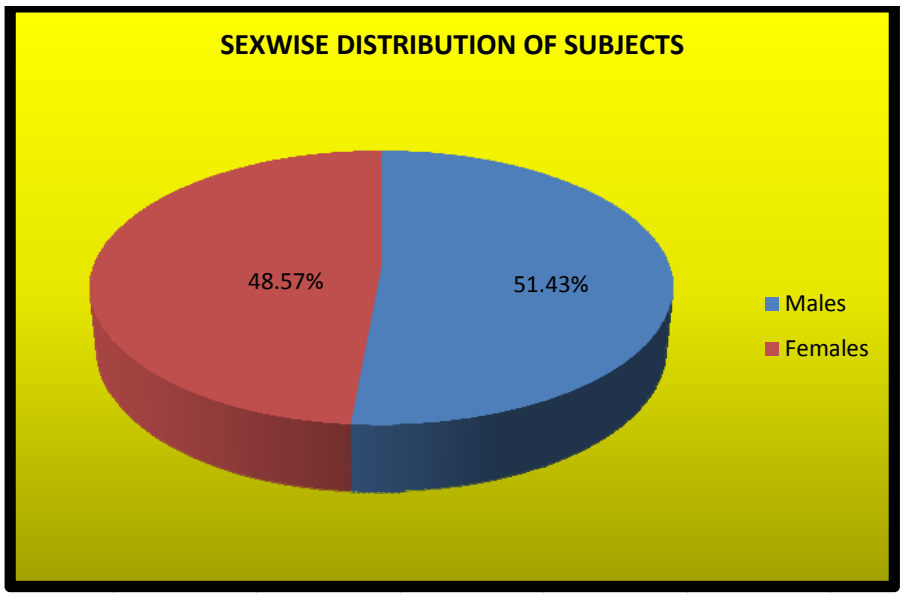
**TABLE-15**

**COMPARISON OF POST RADIATION EXPOSURE VALUES BETWEEN MALES AND FEMALES**

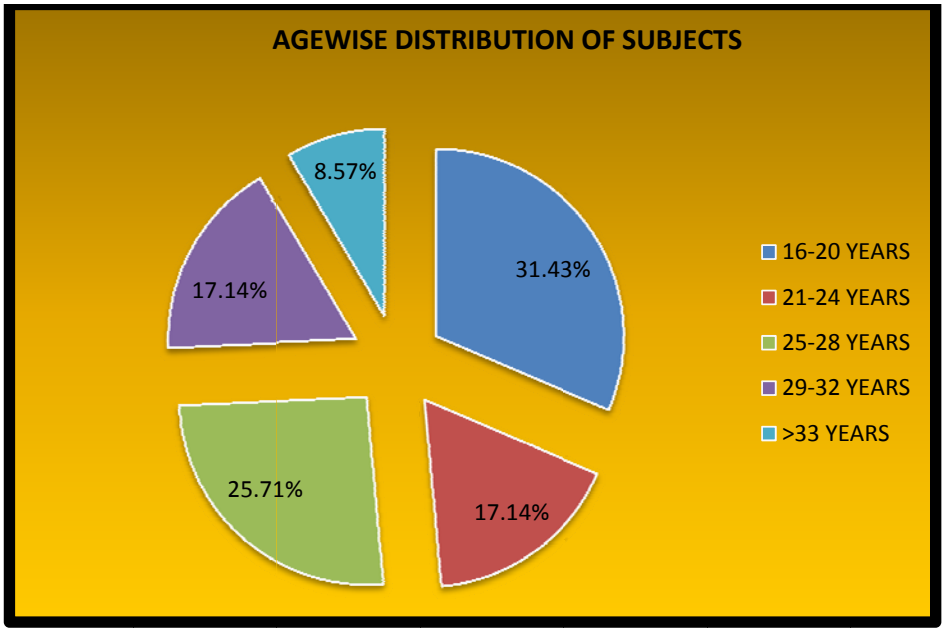
| | Males | | | Females | | | Student paired t-test | |
|---|---|---|---|---|---|---|---|---|
| | **No(%)** | **Mean** | **SD** | **No(%)** | **Mean** | **SD** | **T value** | **P value** |
| **MN** | 22(0.03%) | 1.11 | 0.90 | 21(0.03 %) | 1.24 | 1.03 | 0.38 | 0.71 |
| **KR** | 1661(2.37 %) | 92.28 | 55.78 | 1254(1.80 %) | 73.76 | 33.35 | 1.18 | 0.25 |
| **CC** | 1356(1.94 %) | 75.33 | 45.68 | 1079(1.54 %) | 63.47 | 31.03 | 0.89 | 0.38 |
| **PK** | 237(0.42 %) | 13.17 | 7.06 | 279(0.40 %) | 16.41 | 10.06 | 1.11 | 0.27 |
| **KL** | 25(0.04 %) | 1.44 | 0.98 | 20(0.03 %) | 1.18 | 0.88 | 0.84 | 0.40 |
| **BE** | 38(0.06 %) | 2.11 | 1.37 | 29(0.04 %) | 1.71 | 0.85 | 1.04 | 0.30 |
| **NB** | 96(0.14 %) | 5.44 | 2.23 | 97(0.14 %) | 5.59 | 2.90 | 0.16 | 0.40 |
| **NP** | 134(0.18 %) | 7.22 | 3.08 | 126(0.18 %) | 6.53 | 3.22 | 0.65 | 0.40 |

# Graphs

**GRAPH: 1**

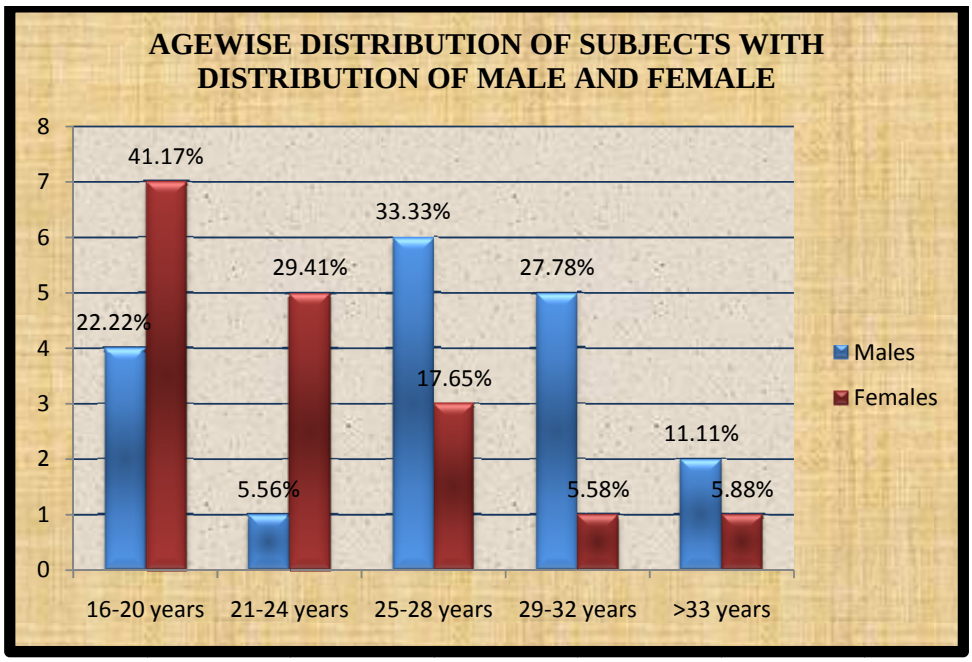



**GRAPH: 2**

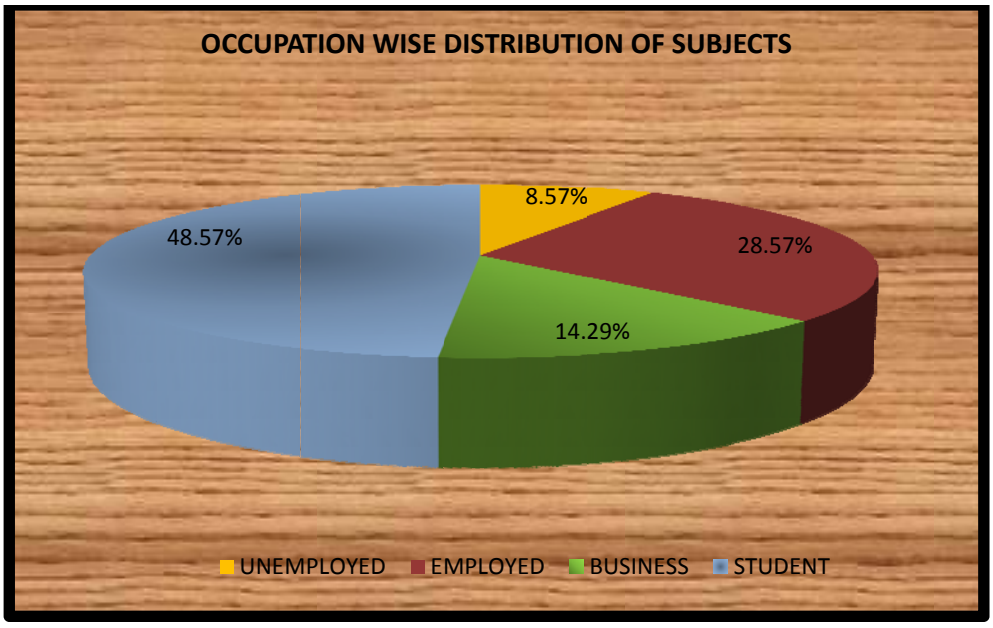


# Graphs

**GRAPH-2A**

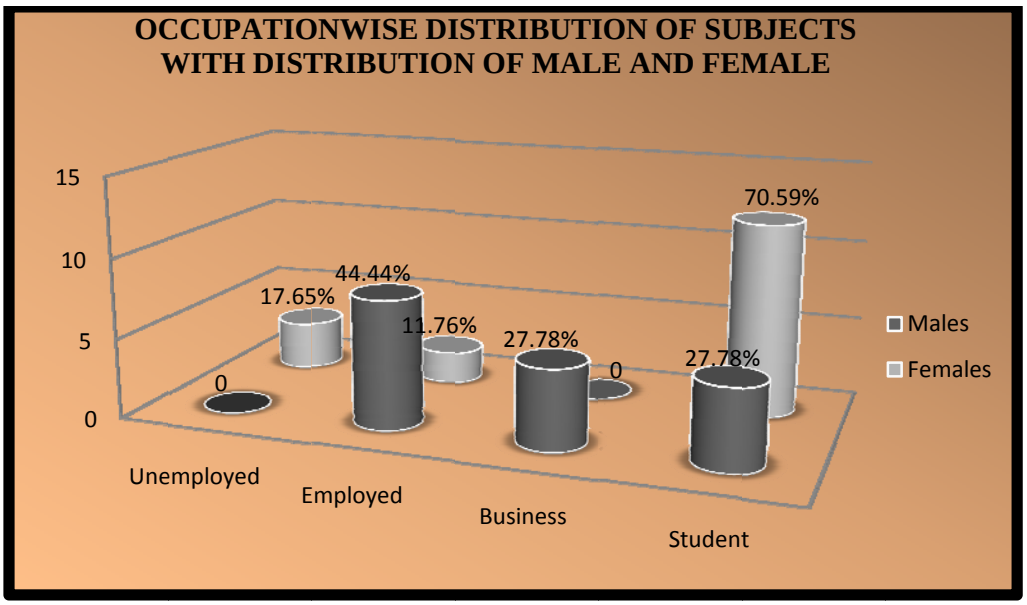



**GRAPH-3**

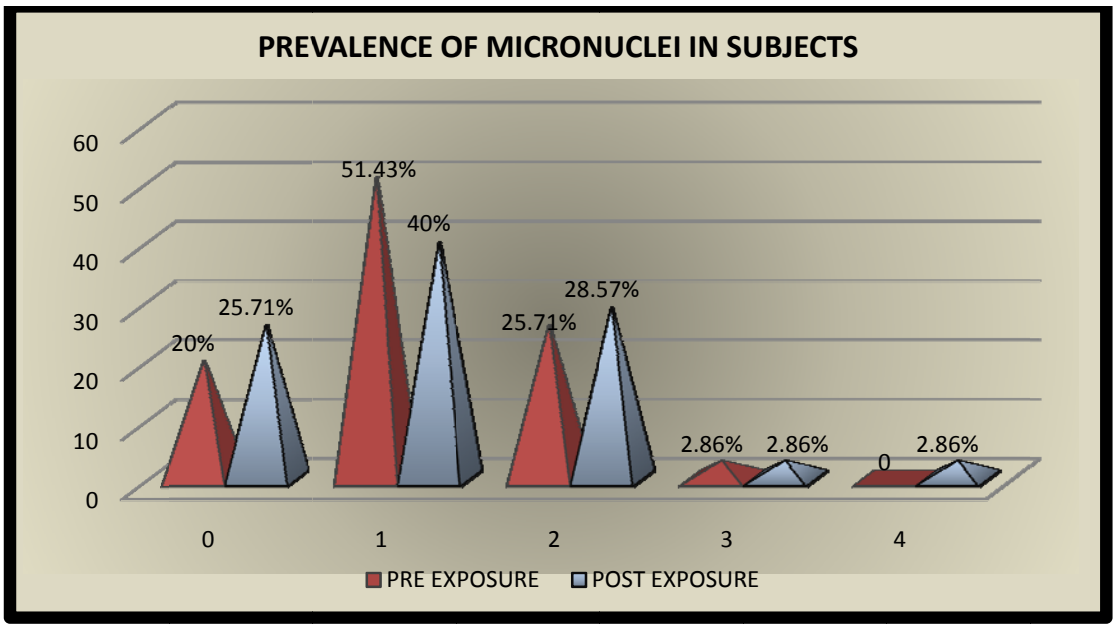


# Graphs

**GRAPH-3A**

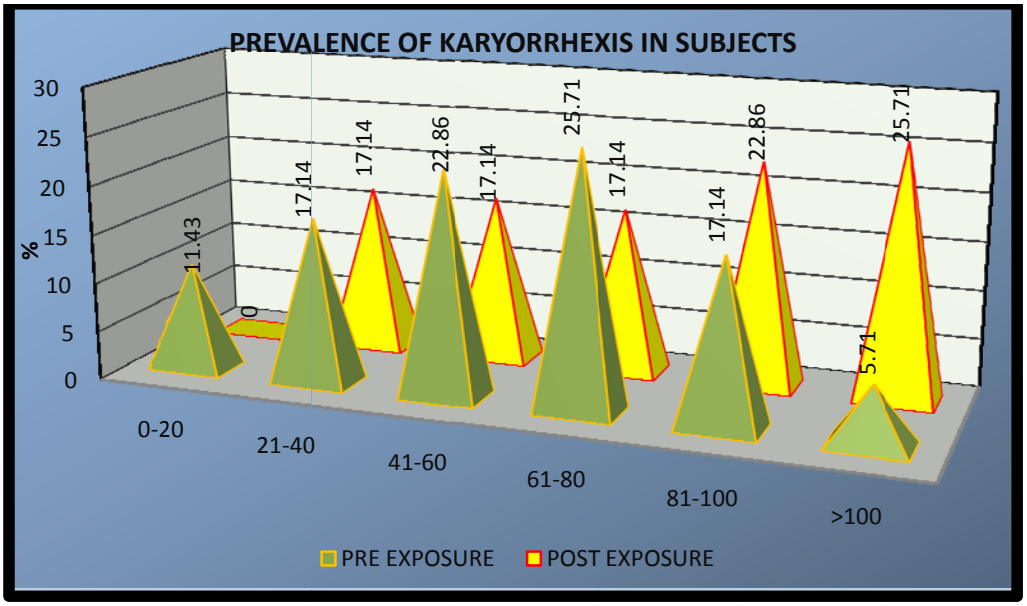



**GRAPH-4**

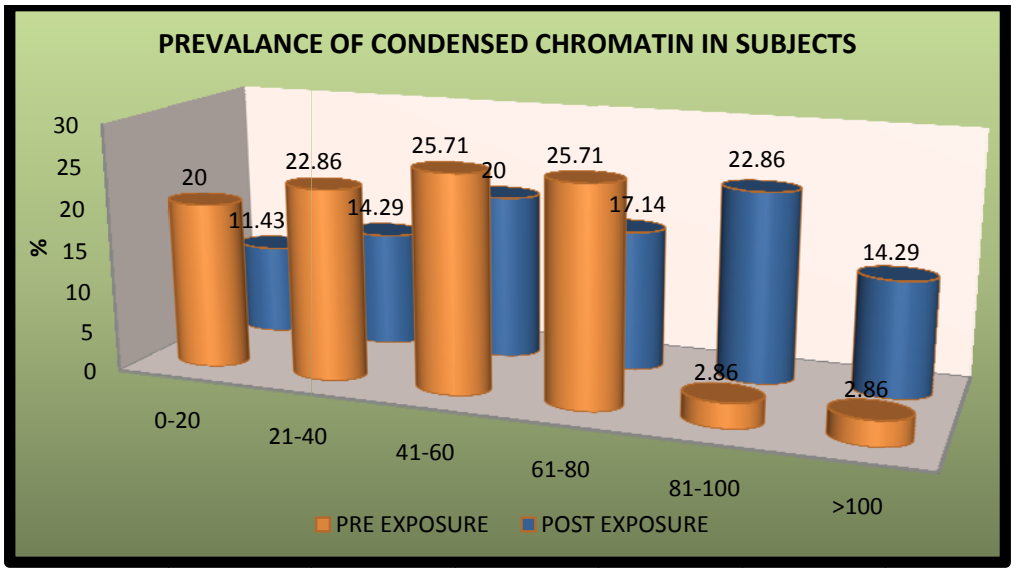


# Graphs

**GRAPH-5**

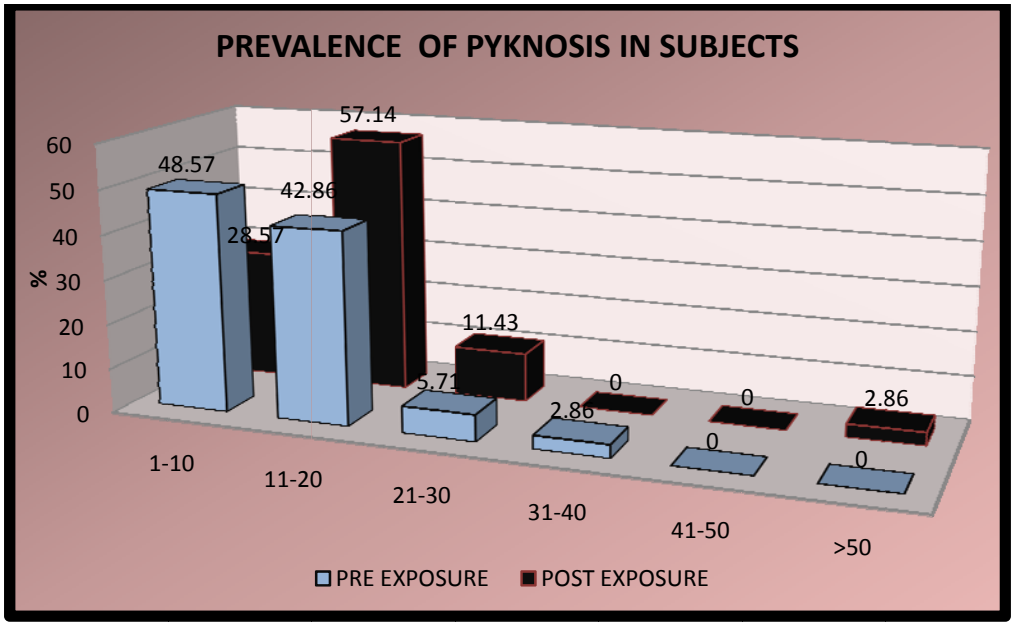



**GRAPH-6**



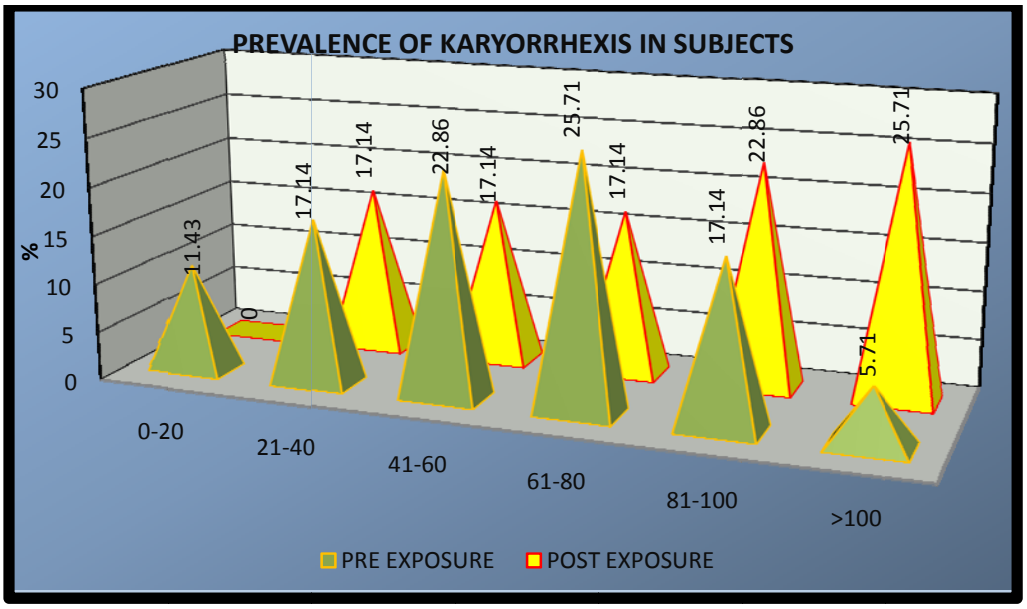
# Graphs

**GRAPH-7**

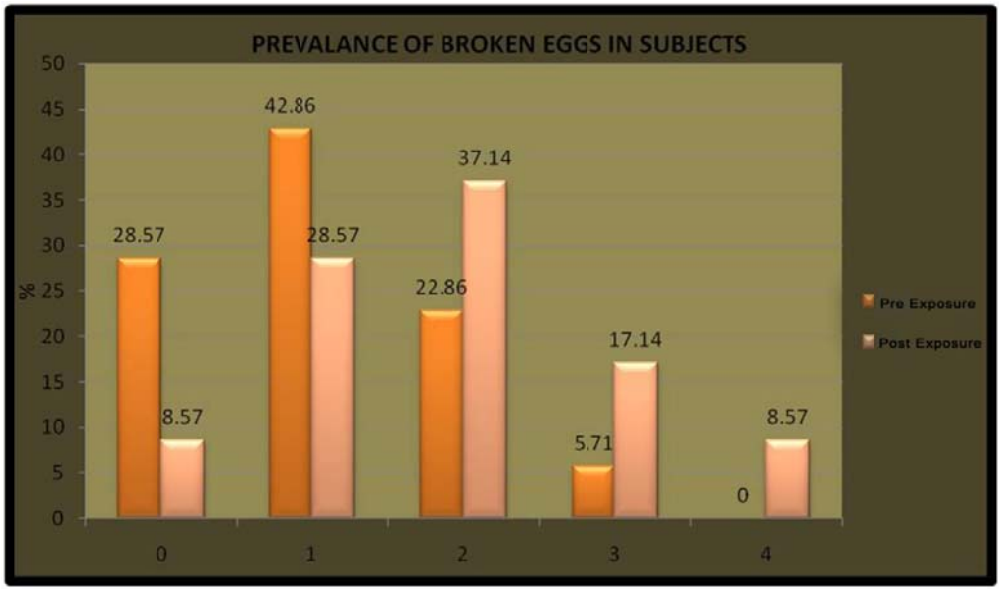



**GRAPH-8**

# Graphs

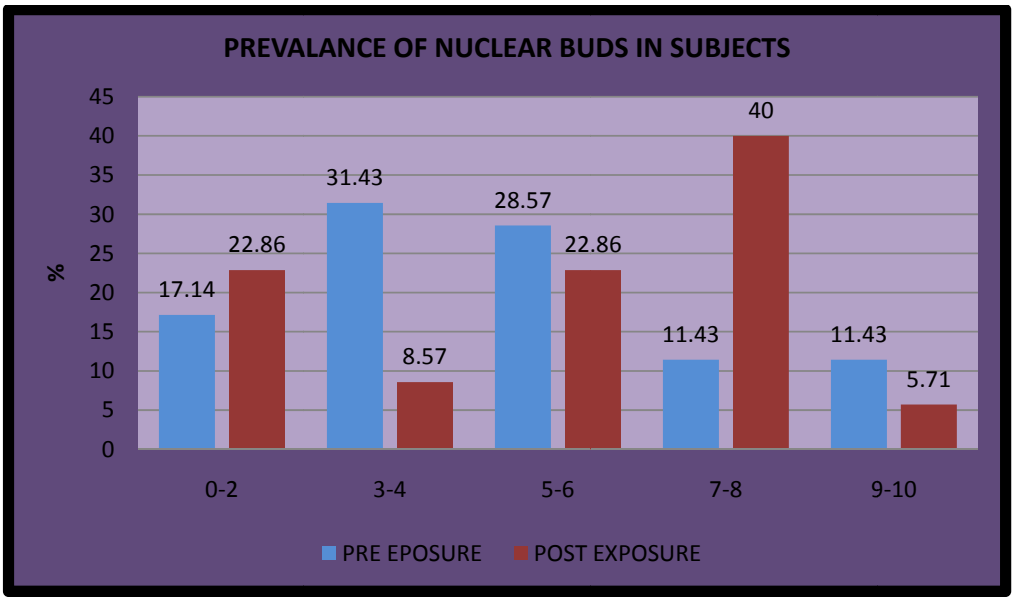



**GRAPH-9**

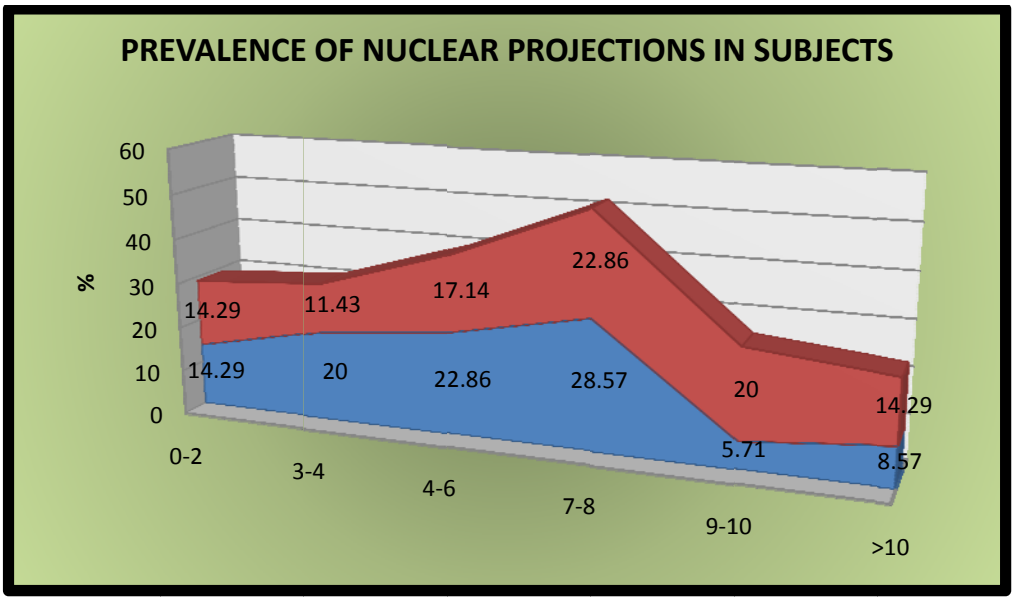



**GRAPH-10**

# Graphs

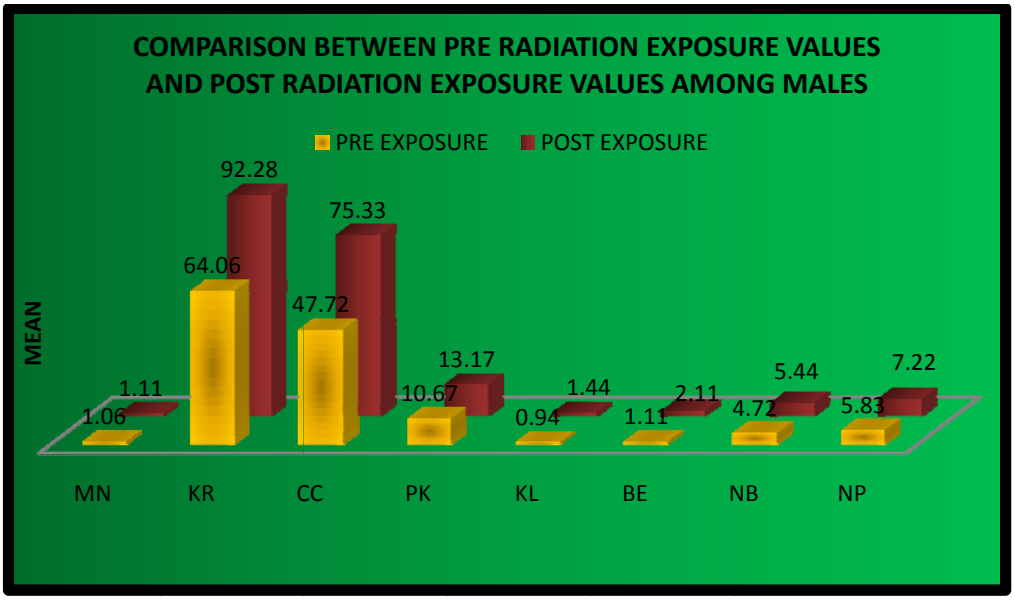



**GRAPH-11**

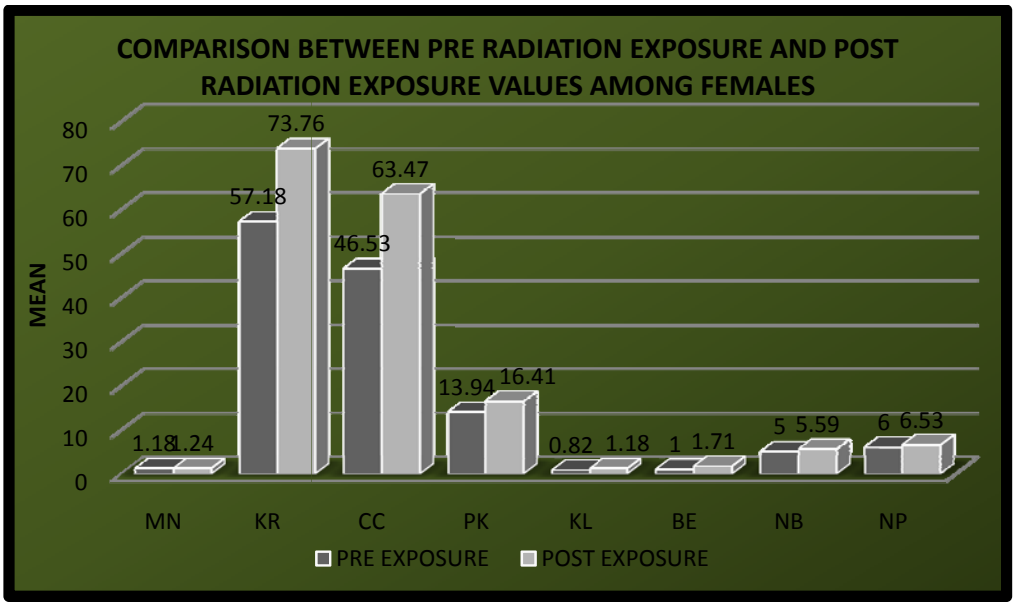


# Graphs

**GRAPH-12**

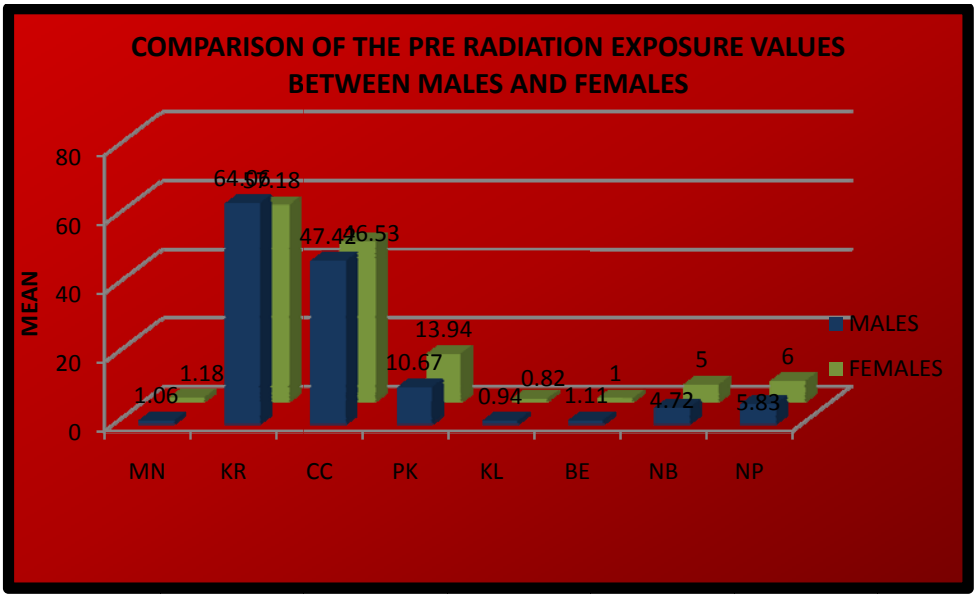



**GRAPH-13**



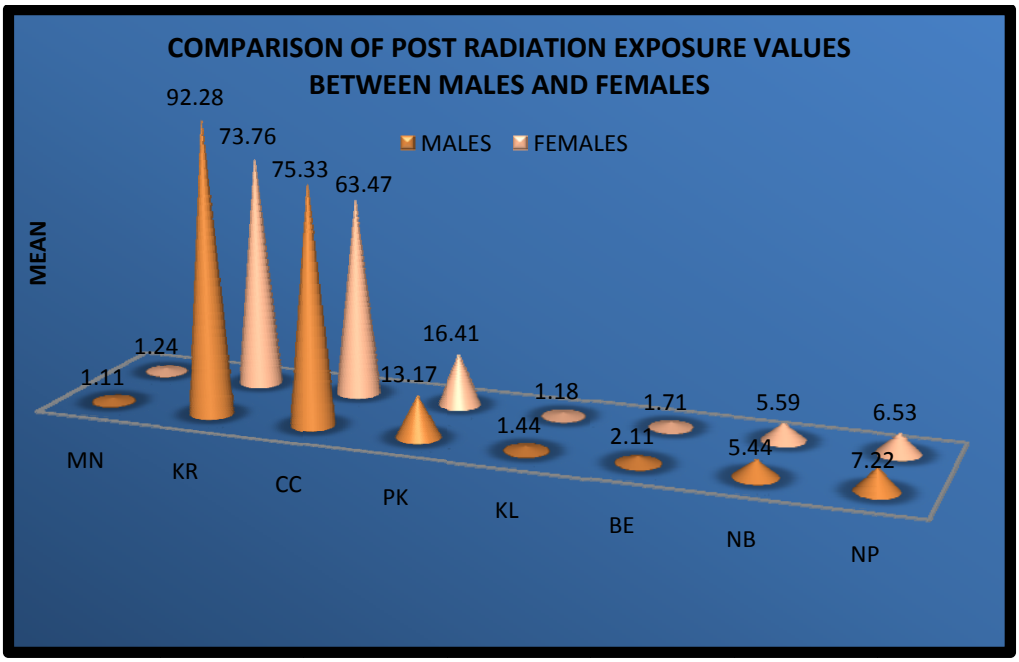
# Graphs

**GRAPH-14**




**GRAPH-15**

# Graphs


COMPARISON OF POST RADIATION EXPOSURE VALUES BETWEEN MALES AND FEMALES
MALES
FEMALES
MEAN
92.28
73.76
75.33
63.47
16.41
13.17
1.24
1.11
1.18
1.44
1.71
2.11
5.59
5.44
6.53
7.22
MN
KR
CC
PK
KL
BE
NB
NP

Panoramic dental radiographs are widely used in dentistry for diagnostic purposes. In this study, the genotoxic effects of X-ray exposure during OPG were evaluated immediately before and on the tenth day after exposure. A 10 day period following exposure is enough time to detect micronucleus formation. Chromosomal damage leading to micronucleus formation occurs during the division of cells from the basal layer of the oral epithelium, but it is only observed later in exfoliated cells, between 1 week and 3 weeks after exposure to a genotoxic agent.

Day 10 was chosen on the basis of the fast turnover in epithelial cell kinetics (from 7–16 days).Adoption of an expanded protocol including not only the micronucleus frequencies, but also evaluations of degenerative nuclear phenomena and nuclear projections, as proposed by **Tolbert[73] et al 1991,** increased the sensitivity of the test because this made it possible to deduce the occurrence of apoptosis or necrosis.

The following discussion explains the relevance of various factors like micronuclei, karyorrhexis, condensed chromatin, pyknosis, karyolysis, broken eggs, nuclear buds and nuclear projections before and after exposure to panoramic dental radiography.

**1. PREVALANCE OF MICRONUCLEI BEFORE AND AFTER EXPOSURE TO OPG IN SUBJECTS:**

In our study the number of MN before exposure was 39 (0.06%) and post exposure was 41(0.06%) with a p- value of 0.54 which is statistically insignificant which

indicates that low dose radiation from OPG doesn't produce any chromosomal aberrations in the targeted buccal epithelial cells.

**Tolbert[73] et al. 1991** stated that micronuclei are regarded as markers of abnormal mitoses involving chromosomal breakage and misintegreted chromatin. Different laboratories have reported variable normal background MN frequency in human oral epithelial cells: 0.16% (**Tolbert[73] et al. 1991**), 0.04% (**Karahalil[47] et al. 1999**), 0.1-0.3% (**Fenech[28] et al. 1999**) and 0.08% (**Burgaz[9] et al. 1999**). In this study it was found that the presence of MN both in pre and post exposure was 0.06%, which is in general agreement with the published reports.

The occurrence of micronuclei before OPG exposure was 39(0.06%) and post exposure was 41 (0.06%) with a p value of 0.54 which is not statistically significant. In a similar study by **Cerqueira[11] et al 2004**($p>0.90$), **Angelieri[1] et al 2007**($p>0.05$), **Popova[60] et al 2007**($p>0.05$), and **Ribeiro[64] et al 2008**($p>0.05$) did not detect any statistically significant difference in micronucleus occurrence between the two times, although the number of these structures were greater after exposure which was similar to our study.

The MN index may reflect genomic instability. The detection of an elevated frequency of micronuclei in a given population indicates an increased risk of cancer. The micronucleus frequencies were not significantly different before and after X-ray exposure in this trial which may be due to low dose of radiation although such findings are fully in line with other authors.

Conversely, some authors have reported higher rates of cytogenetic damage induced by X-rays**. Cerqueira[12] et al 2008**($p<0.05$), in their study found higher frequency of micronuclei from exfoliated gingival cells after exposure ($p=0.05$) to panoramic radiography. The higher micronucleus frequency in epithelial cells obtained from the gingiva observed in their study after exposure can be explained by the direct exposure of gingival epithelium to X-rays, since the radiation from panoramic radiography is directly absorbed by gingival cells.

**2. PREVALANCE OF KARYORRHEXIS BEFORE AND AFTER EXPOSURE TO OPG IN SUBJECTS:**

Karyorrhexis is the destructive fragmentation of the nucleus of dying cell whereby its chromatin is distributed irregularly throughout cytoplasm. In this study the number of karyorrhexis before exposure were 2125 (3.04%) and post exposure were 2915 (4.16%) with a p- value of 0.001 which is statistically significant indicative of apoptosis.

This is similar to the results obtained by **Cerqueira[11] et al 2004** ($p<0.001$), **Angelieri[1] et al 2007, Da silva[14] et al 2007**($p=0.01$), **Cerqueira[12] et al 2008**($p<0.01$), **Ribeiro,Angelieri[64] 2008**, and **Ribeiro[63] et al 2008**($p<0.05$) in a similar study.

**3. PREVALANCE OF CONDENSED CHROMATIN BEFORE AND AFTER EXPOSURE TO OPG IN SUBJECTS:**

In this study the number of condensed chromatin found before exposure were 1650 (2.36%) and post exposure were 2915 (3.48%) with a p- value of 0.001 which is statistically significant which indicates nuclear alterations indicative of apoptosis. This is similar to the results obtained by **Cerqueira[11] et al 2004**($p<0.001$), **Angelieri[1] et al 2007**, **Da Silva[14] et al 2007** ($p=0.01$), **Cerqueira[12] et al 2008** ($p<0.001$), **Ribeiro,Angelieri[64] 2008**, and **Ribeiro[63] et al 2008**($p<0.05$).

**4. PREVALANCE OF PYKNOSIS BEFORE AND AFTER EXPOSURE TO OPG IN SUBJECTS:**

Pyknosis is the irreversible condensation of the chromatin in the nucleus of cells undergoing programmed cell death or apoptosis and in this study the number of pyknosis found before exposure were 429 (0.61%) and post exposure were 516 (0.74%) with a p- value of 0.001 which is statistically significant. This is similar to the results obtained by **Angelieri[1] et al 2007** and **Ribeiro[63] et al 2008** ($p<0.05$).

**Cerqueira[11] et al 2004** ($p>0.90$), **Cerqueira[12] et al 2008** ($p<0.01$) did not detect any increase in pyknosis in exfoliated cells from the oral mucosa. They stated that pyknosis occurs preferentially in the apoptotic process relating to cell death under normal conditions.

**5. PREVALANCE OF KARYOLYSIS BEFORE AND AFTER EXPOSURE TO OPG IN SUBJECTS:**

Karyolysis is the dissolution of the nucleus of the cell by swelling or necrosis. It is the complete dissolution of the chromatin matter of the dying cell due to the activity of DNA. In this study the number of karyolysis found before exposure were 37 (0.04%) and post exposure were 46 (0.07%) with a p value of 0.009 which is statistically significant which suggests that the cell response to x-rays does induce a mild cytotoxic effect that may lead to necrosis.

This is similar to the results obtained by **Angelieri[1] et al 2007, Ribeiro Angelieri[64] 2008 and Ribeiro[63] et al 2008** ($p<0.05$).

In a similar study, **Cerqueira[11] et al 2004**($p>0.90$**), Cerqueira[12] et al 2008**($p<0.01$) did not detect a greater occurrence of this alteration.

**6. PREVALANCE OF BROKEN EGGS BEFORE AND AFTER EXPOSURE TO OPG IN SUBJECTS:**

Broken eggs are considered as a fragmented nucleus, the little one corresponding to one third of the larger nucleus diameter and are connected with the main nucleus by means of thread or stalk like structure. Broken eggs were well described **by Tolbert[73] et al 1991**, and they have been recorded in some other studies but few studies have discussed their relevance and meaning consequent to genotoxic exposure.

In this study the number of broken eggs found before exposure were 37 (0.05%) and post exposure were 46 (0.06%) with a p- value of 0.001 which is statistically significant indicative of genotoxicity. This is similar to the results obtained by **Angelieri[1] et al 2007, Da silva[14] et al 2007**(p=0.01), **Ribeiro Angelieri[64] 2008.**

According to **Cerqueira[12] et al 2008** (p<0.01) there was no statistically significant difference between the broken egg rates before and after exposure, and they related these structures to the normal process of epithelial differentiation.

**Torres-Bugarın[75] et al 2004,** described significantly higher frequencies of broken eggs in the control group than in subjects who underwent antineoplastic chemotherapy. Similar results were described by Cerqueira11 et al 2004 (p<0.005), who observed a higher frequency of these structures in the exfoliated cells from the oral mucosa before exposure to X-rays. However, **Serrano-Garcia and Montero-Montoya[68] 2001,** suggested that broken eggs must be considered to be genotoxicity biomarkers. Therefore, the real significance of broken eggs remains to be identified.

**7. PREVALANCE OF NUCLEAR BUDS BEFORE AND AFTER EXPOSURE TO OPG IN SUBJECTS:**

The term nuclear buds refer to the abnormal shape of nucleus in which part of nucleus appears to be leaking or budding out from main nucleus. In this study the number of nuclear buds found before exposure were 170 (0.24%) and post exposure were 193 (0.28%) with a p- value of 0.31 which is statistically not significant.

In a similar study done on the lateral border of tongue, **Da silva[14] et al 2007**(p=0.01) found a greater number of nuclear buds after exposure to OPG.

**Cerqueira[11] et al 2004** (p<0.005), who observed a higher frequency of these structures in the exfoliated cells from the oral mucosa before exposure to X-rays suggesting these structures are related to normal process of tissue differentiation.

**8. PREVALANCE OF NUCLEAR PROJECTIONS BEFORE AND AFTER EXPOSURE TO OPG IN SUBJECTS**:

Some authors have considered broken eggs and nuclear buds under one definition as nuclear projections which are indicative of genotoxicity. In this study the number of nuclear projections found before exposure were 207 (0.24%) and post exposure were 260 (0.37%) with a p- value of 0.15 which is statistically not significant and is not indicative of genotoxicity.

**9. COMPARISON BETWEEN PRE RADIATION EXPOSURE AND POST RADIATION EXPOSURE VALUES AMONG MALES:**

On comparison between pre exposure and post exposure values among males the p value for karryorhexis was 0.003, condensed chromatin p=0.001, pyknosis p=0.05, karyolysis p=0.02 and broken eggs p=0.002 and these values are statistically significant before and after exposure. The p value for micronuclei was 0.74, nuclear buds p=0.39 and nuclear projections p=0.14 these values are statistically not

significant before and after exposure. This results implies that radiation from OPG produce certain cellular damage producing genotoxicity in males.

**10. COMPARISON BETWEEN PRE RADIATION EXPOSURE AND POST RADIATION EXPOSURE VALUES AMONG FEMALES:**

On comparison between the pre exposure and post exposure values among females the p value for karyorrhexis was 0.001, condensed chromatin p=0.001 pyknosis p=0.01, and broken eggs p=0.006 these values are statistically significant before and after exposure causing cytotoxicity. The p value for micronuclei was 0.33, karyolysis p= 0.16,nuclear buds p=0.57 and nuclear projections p=0.59 these values are statistically not significant before and after exposure.

In contrast to males, in females the process of karyolysis is insignificant but this cannot be confirmed due to less number of sample size.

**11. COMPARISON OF PRE RADIATION EXPOSURE VALUES BETWEEN MALES AND FEMALES:**

On comparison of the pre exposure values between males and females the p value for micronuclei was 0.64, karyorrhexis p=0.51, condensed chromatin p=0.81, pyknosis p=0.16, karyolysis p= 0.16, broken eggs p=0.58, nuclear buds p=0.71 and nuclear projections p=0.81 these values are statistically not significant between males and females which indicates that sex doesn’t influence the formation of nuclear anomalies.

**12. COMPARISON OF PRE RADIATION EXPOSURE VALUES BETWEEN MALES AND FEMALES:**

On comparison of the post exposure values between males and females the p value for micronuclei was 0.71, karyorrhexis p=0.25, condensed chromatin p=0.38, pyknosis p=0.27, karyolysis p= 0.40, broken eggs p=0.30, nuclear buds p=0.40 and nuclear projections p=0.40 these values are statistically not significant between males and females which indicates that sexually there is no difference between males and females in formation of nuclear anomalies after low dose radiation exposure.

There was also no association between gender and micronucleus induction, which is similar to other studies by **Burgaz[9] et al 1999, Cerqueira[12] et al 2008.**

According to **Sobol MV, Bezrukov VF[71] 2007**, who studied micronuclei (MN) frequencies among 266 participants of Ukrainian school biological Olympiads found significantly higher MN frequencies in females than in males at the age of sixteen. This may be due to the more number of sample size in their study. Biomonitoring studies of populations exposed to X-rays are quite difficult and rather specific because each population is exposed to different doses of radiation. This could explain why some studies find an increase of genetic damage in populations exposed to X-rays. To monitor cytotoxic effects, the frequencies of karyorrhexis, karyolysis and pyknosis were evaluated in this experimental design. Despite the lack of micronuclei formation, the results demonstrated that panoramic dental radiography was able to induce cellular death and cytotoxicity as depicted by statistically significant differences between values before and after X-ray exposure.

Also in the post exposure period, a significant higher number of nuclear alterations characterized by disruption of nuclear contour and chromatin shrinkage, which may result from cytotoxicity. If true, this will be an additional factor interfering in the micronucleus occurrence, once it is known that such frequency generally declines as the concentrations of genotoxic chemicals reach toxic levels. However, further studies are necessary to confirm these findings.

The epithelial cell kinetics is especially important in the interpretation of results obtained as a result of low dose exposure of x rays from orthopantamograph. Chromosomal alterations leading to nuclear anomalies occurs in dividing cells from basal layer of oral epithelium, but is only observed later in exfoliated cells after the differentiation.

A case control study was conducted during July 2008-April 2009 to assess the genetic damage from exfoliated cells of oral mucosa in individuals subjected to panoramic dental radiography. The study population consisted of normal healthy subjects who were attending the extra oral radiology department in Ragas Dental College and Hospital, Chennai.

The study group comprised of 35 subjects of both sexes in which, 18(51.43%) males and 17 (48.57%) females. All the subjects were adults between the age group of 16 to 38 years of age.

For each subject, two sets of cytological smears were prepared immediately before and 10 days after exposure to panoramic dental radiography. The smears were stained by using Schiff's staining and analyses were performed in a blind fashion among 2000 cells. The

following nuclear alterations were considered: micronucleus, karyorrhexis, condensed chromatin, pyknosis, karyolysis, broken eggs, nuclear buds and nuclear projections. The alterations were identified under light microscope.

**To summarize the results of the study**

- In the study the occurrence of micronucleus frequencies were not altered before and after exposure with p value of 0.54 and is statistically insignificant which states that panoramic dental radiography does not produce chromosomal alterations.
- The presence of karyorrhexis was increased after the exposure with a p value of 0.001 which is statistically significant which is indicative of apoptosis.
- The presence of condensed chromatin was increased after the exposure with a p value of 0.001 which is statistically significant which is indicative of apoptosis.
- The presence of pyknosis was increased after the exposure with a p value of 0.001 which is statistically significant which is indicative of apoptosis.
- The presence of karyolysis were increased after the exposure with a p value of 0.009 which is statistically significant suggesting that

the cellular response to x rays produce a cytotoxic effect which may lead to necrosis.

- The presence of broken eggs was increased after the exposure with a p value of 0.001 which is statistically significant and should be considered as genotoxicity bio marker.
- The presence of nuclear buds was increased after the exposure with a p value of 0.31 which is statistically not significant and is indicative of normal epithelial differentiation.
- The presence of nuclear projections was increased after the exposure with a p value of 0.15 though not statistically significant it is indicative of mild cellular damage due to radiation.
- On comparison between pre exposure and post exposure values among males the p value for karyorrhexis was 0.003, condensed chromatin p=0.001 pyknosis p=0.05, karyolysis p=0.02 and broken eggs p=0.002 these values are statistically significant before and after exposure. The p value for micronuclei was 0.74, nuclear buds p=0.39 and nuclear projections p=0.14, these values are statistically not significant before and after exposure.
- On comparison between the pre exposure and post exposure values among females the p value for karyorrhexis was 0.001,condensed chromatin p=0.001 pyknosis p=0.01, and broken eggs p=0.006 these values are statistically significant before and after exposure.

The p value for micronuclei was 0.74, karyolysis p= 0.16,nuclear buds p=0.39 and nuclear projections p=0.14 these values are statistically not significant before and after exposure.

- On comparison of the pre exposure values between males and females the p value for micronuclei was 0.64, karyorrhexis p=0.51, condensed chromatin p=0.81, pyknosis p=0.16, karyolysis p= 0.16, broken eggs p=0.58, nuclear buds p=0.71 and nuclear projections p=0.81 these values are statistically not significant between males and females which indicates that sex doesn't influence the formation of nuclear anomalies.
- On comparison of the post exposure values between males and females the p value for micronuclei was 0.71, karyorrhexis p=0.25, condensed chromatin p=0.38, pyknosis p=0.27, karyolysis p= 0.40, broken eggs p=0.30, nuclear buds p=0.40 and nuclear projections p=0.40 these values are statistically not significant between males and females which indicates that sexually there is no difference between males and females in formation of nuclear anomalies..

The present study analyzed the epithelial cells from oral mucosa because this anatomical location is centrally located when the source of radiation moves around the head of patient in the radiographic technique we adopted. Panoramic radiographs are

frequently requested by dentists and incorrect positioning of the patient may require the procedure to be repeated.

In human cytogenetic studies, it is important to consider some confounding factors. Viruses, alterations in the immune system, failures in DNA repair system and individual variations have already been associated with increased frequencies of chromosome aberrations. Moreover, the influence of tobacco smoke has usually been considered as a relevant confounding factor. Thus, all adults recruited to participate in this study were non-smokers.

Due to the cost factors in depth investigations like any DNA analysis, FISH analysis, nuclear alterations in lymphocytes was not assessed.

According to the results from this investigation, exposure to X-rays during panoramic radiography induces genotoxic effects in oral mucosal buccal epithelial cells that increase chromosomal damage and induce apoptosis. Thus panoramic dental radiography should be requested only when necessary because it cannot be considered a risk-free procedure. It is also recommended that the expanded protocol for the micronucleus test suggested by **Tolbert[71] et al 1991** should be

adopted, including not only micronuclei but also other types of nuclear abnormalities that are in themselves cell damage markers.

The frequencies of nuclear alterations indicative of apoptosis (karyorrhexis and condensed chromatin) were significantly higher after the exposure in contrast to micronuclei results. Apoptosis is a fundamental biological process, which is genetically controlled and required for normal development and tissue homeostasis. The results from the study showed that panoramic dental radiography induced the apoptotic response, which probably interfered with the micronucleus induction.

In some cases the cells with nuclear anomalies were greater before x-rays suggesting that they may be associated with normal process of cell differentiation.

Taken as a whole, such results support the notion that X-rays are a cytotoxic agent. It is important to stress that cytotoxicity interferes with micronucleus induction since some MN are inevitably lost after cytotoxic insult, therefore confirming the lack of mutagenic effect induced by X-rays.

Nevertheless, it has been postulated that repeated exposure to cytotoxicants can result in chronic cell injury, compensatory cell proliferation, hyperplasia and, ultimately, tumor development.

In fact, a correlation between cell proliferation and induction of cancer is assumed. Proliferation probably increases the risk of mutations within target cells, and may also be important in selective clonal expansion of (exogenously or endogenously) initiated cells from preneoplastic foci and eventually tumors. Our results demonstrated that the micronucleus frequency did not increase following exposure to ionizing radiation.

In conclusion, the results of the present study indicate that high levels of genotoxicity and cytotoxicity in exposed tissues, expressed respectively by increased apoptotic or necrotic responses may be a factor in the low micronucleus frequencies observed after x- ray exposure suggesting that X-rays can induce cytotoxic effects in oral mucosal cells. The risks associated with dental radiographs are small but should not be overlooked.

Since cellular death is considered to be a prime mechanism in non-genotoxic mechanisms of carcinogenesis, dental X-rays should be used only when necessary. More frequent, both as substitute for and as a complement to intra oral radiographs, their indication should always follow the concept of maximum benefit with minimum risk. Panoramic radiography should be carefully performed in order to avoid to retakes and increase in radiation doses.

**RAGAS DENTAL COLLEGE & HOSPITAL**

2/102, East Coast Road, Uthandi, Chennai - 600119

**DEPARTMENT OF ORAL MEDICINE & RADIOLOGY**

## Case Sheet Performa

Serial no: O.P No: Date:

1. **Name** :
2. **Age** :
3. **Sex** :
4. **Address** :

**5. Occupation :**

i. Unemployed ☐
ii. Employed ☐
iii. Business ☐
iv. Student ☐

**6. Income :**

i. < Rs. 1000 /-month ☐
ii. > Rs.1000-5000 /-month ☐
iii. > Rs. 5000 /-month ☐

**7. Religion :**

**8. Chief complaint:**

**9. History of presenting illness:**

**10. Past medical history:**

a. Presence of any systemic disease

(i)Yes ☐

(ii)No ☐

If yes specify

b. History of medication

(i)Yes ☐

(ii)No ☐

If yes specify

**11. Past surgical history:**

**12. Personal history:**

a. Food habits -

b. Brushing habits -

c. Use of oral mouth rinses-

**13. Previous exposure to X-rays:**

(i)Yes ☐

(ii)No ☐

If yes specify

**14. Indication for OPG exposure :**

**15. Investigation**:

Cytological analysis (pre-exposure)

[Scraping taken from right & left buccal mucosa before OPG radiograph]

i. Micronuclei (%) :
ii. Apoptosis (%) :
iii. Pycnosis (%) :
iv. Broken eggs (%) :
v. Nuclear bud (%) :

Cytological analysis (post-exposure)

[Scraping taken from right & left buccal mucosa after OPG radiograph]

i. Micronuclei (%) :
ii. Apoptosis (%) :
iii. Pyknosis (%) :
iv. Broken eggs (%) :
v. Nuclear bud (%) :

## CONSENT LETTER

I _____ the undersigned hereby give my consent for the performance of diagnostic test on myself for **'Genetic damage in exfoliated cells from oral mucosa of individuals exposed to x-rays after panoramic radiograph'** being conducted by **Dr. M.Ramalakshmi** under guidance of **Dr.Capt. S.Elangovan MDS,** Professor, Department of **ORAL MEDICINE AND RADIOLOGY, RAGAS DENTAL COLLEGE & HOSPITAL.** I have been informed and explained the status of my disorder, investigation procedure, risk involved and likelihood of success. I also understand and accept this as a part of study protocol, there by voluntarily, unconditionally, freely give my consent without any fear or pressure in mentally sound and conscious state to participate in the study.

Witness/Representative — Patient signature

(If any) — Date

## ஒப்புதல் படிவம்

..... ஆகிய நான் மரு. ம.ராமலஷ்மி, அவர்களால், மருத்துவர் கேப்டன் இளங்கோவன், பேராசிரியர் வாய் மருத்துவம் (ம) கதிர் வீச்சு துறை ராகாஸ் மருத்துவ மனை, அவர்களின் மேற்பார்வையில் நடத்தப்படும் பல் பனோரமா நுண்கதிர் படத்திற்கு உட்படுத்தப்படுவோருக்கு அதன் ஊடுகதிரினால் ஏற்படும். மரபியல் மாற்றங்களை வாயின் உட்புறத்தில் இருந்து எடுக்கப் பட்ட உதிர்ந்த அணுக்களில் இருந்து அளவெடுத்து அறிவதற்கான பரிசோதனையை பற்றி அறிந்து கொண்டேன். என் வாயில் மேற்கொள்ளப்படும் பரிசோதனைகளையும், அதனால் ஏற்படக்கூடிய பக்க விளைவுகளையும், அறிந்து கொண்டேன். நான் எந்தவித கட்டாயமும் இன்றி தன்னிச்சையாக முழு மனதுடனும் சுய நினைவுடனும் இந்த ஆய்வில் பங்கு கொள்ள ஒப்புதல் அளிக்கிறேன்.

தேதி :

இடம் : கையொப்பம்

# Annexure

## MASTER CHART

**Pre Exposure**

| Pt no | Age | Sex | Micronuclei | Karyorrhexis | Condensed chromatin | Pyknosis | Karyolysis | Broken eggs | Nuclear buds | Nuclear projections |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 21 | female | 1 | 18 | 8 | 26 | 4 | 1 | 0 | 8 |
| 2 | 28 | male | 2 | 36 | 12 | 48 | 12 | 2 | 2 | 12 |
| 3 | 26 | male | 0 | 19 | 14 | 33 | 10 | 0 | 3 | 14 |
| 4 | 30 | male | 0 | 25 | 22 | 47 | 8 | 1 | 1 | 22 |
| 5 | 37 | male | 1 | 34 | 28 | 62 | 7 | 1 | 0 | 28 |
| 6 | 18 | female | 3 | 40 | 22 | 62 | 10 | 2 | 2 | 22 |
| 7 | 17 | female | 0 | 28 | 19 | 47 | 12 | 0 | 1 | 19 |
| 8 | 29 | male | 1 | 70 | 40 | 110 | 13 | 1 | 2 | 40 |
| 9 | 17 | male | 1 | 58 | 42 | 100 | 25 | 1 | 1 | 42 |
| 10 | 20 | female | 0 | 123 | 80 | 203 | 40 | 1 | 0 | 80 |
| 11 | 28 | male | 1 | 15 | 10 | 25 | 7 | 1 | 0 | 10 |
| 12 | 33 | male | 2 | 97 | 78 | 175 | 13 | 2 | 1 | 78 |
| 13 | 22 | female | 0 | 86 | 74 | 160 | 11 | 1 | 1 | 74 |

# Annexure

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 14 | 29 | female | 2 | 48 | 37 | 85 | 28 | 1 | 1 | 37 |
| 15 | 19 | male | 2 | 68 | 52 | 120 | 11 | 1 | 1 | 52 |
| 16 | 32 | female | 1 | 38 | 35 | 73 | 13 | 0 | 0 | 35 |
| 17 | 21 | male | 1 | 47 | 39 | 85 | 18 | 0 | 2 | 39 |
| 18 | 23 | female | 0 | 18 | 12 | 90 | 8 | 0 | 0 | 12 |
| 19 | 26 | male | 1 | 52 | 48 | 100 | 9 | 0 | 1 | 48 |
| 20 | 19 | male | 1 | 151 | 101 | 252 | 8 | 1 | 0 | 101 |
| 21 | 24 | female | 2 | 71 | 66 | 137 | 14 | 1 | 2 | 66 |
| 22 | 30 | male | 2 | 63 | 60 | 123 | 10 | 1 | 1 | 60 |
| 23 | 31 | male | 1 | 98 | 42 | 140 | 8 | 1 | 0 | 42 |
| 24 | 27 | female | 1 | 48 | 36 | 84 | 11 | 0 | 1 | 36 |
| 25 | 18 | female | 1 | 89 | 77 | 166 | 9 | 1 | 2 | 77 |
| 26 | 19 | male | 1 | 76 | 63 | 139 | 7 | 1 | 1 | 63 |
| 27 | 17 | female | 2 | 54 | 42 | 96 | 9 | 0 | 1 | 42 |
| 28 | 29 | male | 1 | 85 | 68 | 153 | 6 | 2 | 1 | 68 |
| 29 | 27 | male | 0 | 90 | 83 | 173 | 9 | 1 | 3 | 83 |

# Annexure

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 30 | 25 | male | 1 | 69 | 57 | 126 | 11 | 0 | 0 | 57 |
| 31 | 19 | female | 2 | 79 | 75 | 154 | 13 | 1 | 2 | 75 |
| 32 | 17 | female | 1 | 38 | 29 | 67 | 11 | 1 | 1 | 29 |
| 33 | 22 | female | 2 | 59 | 55 | 114 | 9 | 1 | 2 | 55 |
| 34 | 25 | female | 1 | 63 | 59 | 122 | 20 | 2 | 1 | 59 |
| 35 | 34 | female | 1 | 72 | 65 | 137 | 15 | 1 | 0 | 65 |

**Post Exposure**

| **Pt no** | **Age** | **Sex** | **Micronuclei** | **Karyorrhexis** | **Condensed chromatin** | **Pyknosis** | **Karyolysis** | **Broken eggs** | **Nuclear buds** | **Nuclear projections** |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 21 | female | 1 | 38 | 18 | 8 | 1 | 1 | 1 | 2 |
| 2 | 28 | male | 0 | 31 | 17 | 9 | 0 | 1 | 6 | 7 |
| 3 | 26 | male | 0 | 29 | 17 | 5 | 0 | 3 | 6 | 9 |
| 4 | 30 | male | 0 | 30 | 30 | 11 | 2 | 1 | 5 | 6 |
| 5 | 37 | male | 1 | 50 | 30 | 6 | 1 | 1 | 4 | 5 |
| 6 | 18 | female | 4 | 49 | 33 | 12 | 1 | 2 | 6 | 8 |

# Annexure

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 7 | 17 | female | 0 | 38 | 27 | 13 | 1 | 2 | 8 | 10 |
| 8 | 29 | male | 1 | 90 | 79 | 15 | 2 | 3 | 7 | 10 |
| 9 | 17 | male | 2 | 68 | 59 | 25 | 1 | 1 | 7 | 8 |
| 10 | 20 | female | 0 | 142 | 97 | 52 | 1 | 2 | 3 | 5 |
| 11 | 28 | male | 0 | 66 | 40 | 10 | 1 | 0 | 2 | 2 |
| 12 | 33 | male | 3 | 138 | 112 | 15 | 3 | 2 | 6 | 2 |
| 13 | 22 | female | 0 | 136 | 125 | 15 | 0 | 2 | 5 | 2 |
| 14 | 29 | female | 2 | 75 | 68 | 24 | 0 | 2 | 7 | 2 |
| 15 | 19 | male | 2 | 52 | 48 | 29 | 2 | 3 | 7 | 10 |
| 16 | 32 | female | 1 | 47 | 44 | 17 | 1 | 0 | 8 | 8 |
| 17 | 21 | male | 1 | 63 | 47 | 26 | 1 | 4 | 7 | 11 |
| 18 | 23 | female | 0 | 29 | 17 | 13 | 0 | 1 | 6 | 7 |
| 19 | 26 | male | 1 | 163 | 152 | 7 | 1 | 1 | 4 | 5 |
| 20 | 19 | male | 2 | 249 | 182 | 16 | 2 | 4 | 8 | 12 |
| 21 | 24 | female | 2 | 101 | 98 | 17 | 2 | 2 | 7 | 9 |
| 22 | 30 | male | 2 | 120 | 118 | 15 | 2 | 3 | 8 | 11 |
| 23 | 31 | male | 1 | 147 | 97 | 11 | 3 | 2 | 7 | 9 |

# Annexure

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 24 | 27 | female | 1 | 57 | 49 | 15 | 2 | 3 | 0 | 3 |
| 25 | 18 | female | 1 | 97 | 89 | 11 | 3 | 2 | 9 | 10 |
| 26 | 19 | male | 1 | 87 | 85 | 9 | 1 | 2 | 1 | 3 |
| 27 | 17 | female | 2 | 63 | 59 | 12 | 1 | 2 | 9 | 11 |
| 28 | 29 | male | 1 | 90 | 75 | 8 | 3 | 1 | 5 | 6 |
| 29 | 27 | male | 0 | 105 | 97 | 7 | 1 | 5 | 1 | 6 |
| 30 | 25 | male | 2 | 83 | 71 | 13 | 0 | 1 | 7 | 8 |
| 31 | 19 | female | 2 | 84 | 81 | 15 | 1 | 3 | 8 | 11 |
| 32 | 17 | female | 1 | 45 | 43 | 11 | 2 | 2 | 2 | 4 |
| 33 | 22 | female | 2 | 68 | 65 | 7 | 2 | 1 | 7 | 8 |
| 34 | 25 | female | 1 | 96 | 88 | 18 | 2 | 0 | 7 | 7 |
| 35 | 34 | female | 1 | 89 | 78 | 19 | 0 | 2 | 2 | 4 |

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