Discussion

“All substances are poisons; there is none which is not a poison.

The right dose differentiates a poison from a remedy.”

Paracelsus (1493–1541)

Radiation is indispensable in modern medicine. All fields of medical and dental services use radiographic examination as one of the principal diagnostic methods. Imaging science is getting advanced day by day, leading to improvements in the field of oral care. Today radiography plays an important role in diagnosis of orofacial diseases.

Radiological methods like full mouth IOPA radiographs, panoramic radiographs, CT scans and CBCT scans are used for the assessment of the maxillary and mandibular dentoalveolar component. There are merits and demerits of all these four techniques. There is low risk associated with low-level diagnostic exposures but it is greater than zero. The comparison of radiation risk assessment and the merits of all these techniques can be useful in selecting the correct diagnostic method.

The X-ray radiation has special characteristic of ionizing radiation to consider it as universal potential carcinogen. The damage to the cells caused by X-ray radiation is different from chemical toxic agents or other physical carcinogens. The damage done by chemical carcinogens is usually tissue specific in action while radiation is unaffected by the cellular barriers which protect from hazardous effects of chemical and other toxic agents, further radiation shows its ability to penetrate cells and to
deposit energy within them in a random fashion. Thus, ionizing radiation can cause damage to all the cells in the body and the amount of damage is related to the physical parameters that determine the radiation dose received by the particular cells or tissue.\(^8\)

The reliable and relevant minimally invasive biomarkers are essential to improve the implementation of biomonitoring, diagnostics and treatment of diseases caused by, or associated with, genetic damage. The micronucleus assay in exfoliated cells is potentially an excellent method to serve as such a biomarker.\(^62\)

X-ray induced micronucleus formation is a well-known phenomenon that has been used to investigate the biological effects of radiation exposure.\(^63\) Micronuclei frequencies in exfoliated human cells has potential to become a beneficial 'internal dosimeter' for estimation of exposure to genotoxic agents and carcinogenic agents. It is noninvasive, simple, easy, rapid clinical chair side technique, risk free that is well-accepted by the patient with no contraindications and special experts are not required with this method.\(^11\)

Dr. George N. Papanicolaou was the first to introduce Pap smear in 1928 in cervical tissues and since then this technique has helped to reduce cervical cancer incidence and mortality rates by 75%. This is an easy technique and can be replicated in the oral cavity for analysis of the changes caused by smoking. Palaskar and Jindal have shown that Pap is better stain for counting MN due to the fact that MN were easily seen in clear cytoplasm in regard to other stains like Giemsa stain.\(^11\)

Several markers have been used to detect early cell changes in carcinogenesis. An increase in the micronuclei number in the epithelial cells of the oral mucosa is
observed after exposed to carcinogens. The other methods are also reported to evaluate the changes in epithelial cells. The investigation for these markers is costly hence they should be indicated only after Papanicolaou screening.62

Grover et al. 2012 conducted study on comparison of MNi frequencies with three different stains i.e., Feulgen stain, Papanicolaou stain (Pap) and hemotoxylin and eosin stain (H and E). Oral exfoliated cells from 45 cases of potentially malignant disorders (15 oral submucous fibrosis, 15 lichen planus and 15 leukoplakia) and 15 controls with healthy mucosa, were taken and MNi frequencies (No. of MNi/1000 cells) were compared using these three different stains. They found statistically significant results from the comparison of MNi frequency between cases and controls with all the three stains.60

Studies are needed to determine whether some MNi and nuclei may lose DNA through karyolysis while maintaining the protein structure of chromatin and the nuclear envelope, so that they would still be detectable by stains that are not DNA-specific (like FFG stain). Therefore, a systematic study investigating the performance characteristics and the extent of correlations among the MN frequencies determined with various staining methods currently in use is urgently required so that an optimal staining protocol can be determined.35

Holland et al.35, evaluated the MN frequency in human lymphocytes with irradiation from gamma rays by 51 scorers from 34 laboratories in 21 countries. The study evaluated factors affecting variability when scoring MN in human lymphocytes. The study demonstrated that the staining method contributed to only
0.5% variation in the MN frequency while major variation was contributed from dose (65.3%) and covariance (26.3%).

The three main advantages of PAP staining procedure are: (1) Good definition of nuclear detail. (2) Cytoplasmic transparency. (3) Indication of cellular differentiation of squamous epithelium. The Papanicolaou technique makes it an ideal tool for screening patients exposed to risk factors for oral cancer because of the low cost. It is a rapid quantitative analysis of cells that takes about 20 minutes per slide and the maturation pattern of cells also can also be assessed.

Considering all the above factors, in the present study MNi and other cytotoxic abnormalities were evaluated with Pap stain.

Micronuclei assay in exfoliated cells holds promise as, one of the biomarkers of exposure to genetic toxins and can provide as screening prognostic and educational tool in community centers. The MN test is a promising extensively used in molecular epidemiology and cytogenetics as a biomarker to study and evaluate the presence and the extent of damage to chromosomes in human populations exposed to genotoxic agents or having a susceptible genetic profile and genomic stability.

The most frequently used cells for cytogenetic biomonitoring; human peripheral blood lymphocytes appear to be an inappropriate cell system for monitoring the cytotoxic and genotoxic effects in oral cells after radiographic examinations. Buccal epithelium cells which are under direct radiation exposure and by default are primary target for radiation-induced damage during radiographic examination of oral cavity provide an alternative source of tissue for human monitoring to
occupational and environmental toxic exposures. The advantage for using this tissue is rapid and easy sampling of these cells by brushing the buccal mucosa.\textsuperscript{56}

We propose a hypothesis that as gingival cells are closer to the alveolar bone and likely to get more scattered radiations from alveolar bone while radiographic examination and as result of this; there are more chances of genotoxic and cytotoxic effects in the gingival cells than buccal cells.

The present study proposes that the gingival cells rather than buccal mucosa cells should be primary target to study biological effects of X-rays radiation of orofacial region.

For these reasons in the present study, both gingival and buccal exfoliative cells were considered to assess and compare biological effects of X-rays radiation.

The genotoxic and cytotoxic effects of panoramic radiography on epithelial gingival cells are assessed only once in published literature (Cerqueira EMM \textit{et al} 2008).\textsuperscript{10} Our study claims to be first in assessing and comparing genotoxic and cytotoxic effects on both buccal and gingival cells. At present no study with comparison of genotoxic and cytotoxic effects of four main radiographic techniques (full mouth IOPA, panoramic radiology, CBCT and CT) used in the dental radiology is reported in the literature. This approach has not been addressed in the literature so far to the best of our knowledge.

Micronuclei are formed because of damage to the basal layer of epithelial cells where cell mitosis occurs. The cells which exfoliate are moved to the surface by rapid turnover of epithelial cells. Thus, the maximal rate of micronuclei formation
in exfoliated cells is seen between 1 and 3 weeks after exposure to the genotoxic agent. Superficial buccal cells are pulled off continuously and replaced by cell division of the basal stem cells. A period 10 days is required for basal cells from the squamous epithelium to exfoliate. When the basal stem cells divide, damaged and fragmented chromosomes can lag during mitotic division and appear in the cytoplasm of the daughter cells as a small nuclear particle, termed as micronucleus. This was the reason to adopt a period of 10 days after radiographic exposure in the present study to evaluate the genotoxic and cytotoxic effects on oral epithelial cells.

Viruses, alterations in the immune system, failures in the DNA repair system and inter individual variations are some of the confounding factors in human cytogenetic studies which have already been associated with increased frequencies of chromosome aberrations. Furthermore, an age-related increase in micronuclei count has been postulated in some of the studies.

For the above reason, study groups with gender and age matched individuals are recruited in our study. The mean age of all the four study groups is in the range of 34 to 37 years. There is no significant difference in mean age as well as male and female genders of all four our study groups. Furthermore, one of the relevant confounding factors in evaluation of cytogenetic effect is tobacco smoke and hence all the participants recruited in the present study are non-smokers and non-tobacco users.

Micronuclei are considered as markers of abnormal mitoses involving chromosomal breakage and missegregated chromatin. Different laboratory studies have reported
variable normal background MNC frequency in human oral epithelial cells: 0.04% (Karahalil et al. 1999) 0.16% (Tolbert et al. 1991), 0.1-0.3% (Fenech et al. 1999) and 0.33% (Burgaz et al. 2002).

The pre exposure MN frequencies among buccal and gingival cells of all the four groups of our study are in the range of 0.22 % to 0.27 % which is similar to previous studies in published literature.

Pre exposure mean frequency of MNC in the gingival cells of IOPA group is 2.42 as compared to 2.97 after post exposure to full mouth IOPA radiographs (Table 7). p value <0.05, so it is concluded that there is significant increase in between pre and post exposure MNC. Pre exposure mean MNC frequency in the buccal mucosa cells of IOPA group is 2.28 as compared to 2.80 after post exposure to full mouth IOPA radiographs (Table 7). p value <0.001, so it is concluded that there is significant increase in between pre and post exposure micronuclei. Similar results were seen in other cytotoxic abnormalities (Table 8): gingival cells- pre exposure 8.30 and post exposure 8.89, p < 0.05 and buccal mucosa cells- pre exposure 8.36 and post exposure 8.83, p < 0.05). Hence, it is concluded that exposure to full mouth IOPA radiographs induce genotoxic effects in the oral mucosal buccal and gingival epithelial cells that includes chromosomal damage and cytotoxic effects. There are no studies published in literature to compare these results.

In our study the occurrence of MNC frequency in the gingival mucosal cells are not significantly increased after exposure to panoramic radiographs with p-value of 0.16 which is statistically insignificant and similar results were seen in the buccal mucosa cells with p-value of 0.86 (Table 9). The occurrence of other cytotoxic
abnormalities frequencies is also not significantly increased in the gingival and buccal mucosa cells after exposure to panoramic radiograph with respective p-values of 0.23 and 0.47, both being statistically insignificant (Table 10). The reason for these negative results could be the low radiation doses of panoramic radiology.

The results of our study for MNC frequency in buccal mucosal cells in individuals exposed to panoramic radiograph are in agreement with previous studies by Papova et al 2007\textsuperscript{56} and Waingade et al 2012\textsuperscript{67}.

Study done by Riberio et al \textsuperscript{57} and Agarwal et al 2015\textsuperscript{62} concluded that dental panoramic radiography may not be a factor that induces chromosomal damage, but it is able to promote cytotoxicity. The cytotoxic abnormalities are not increased significantly after panoramic radiographs in our study.

In contrast to our results, study done by Madhavan et al 2012\textsuperscript{68} and Vidya KB et al 2014\textsuperscript{69} concludes that panoramic radiographs are able to induce DNA damage and cytotoxicity in buccal oral mucosal cells.

The MNC frequency in gingival cells is assessed in one previous study done by Cerqueria et al 2008\textsuperscript{10}. The results of this study indicate that panoramic X-ray radiation induces a genotoxic effect on epithelial gingival cells that increases the frequency of chromosomal damage and nuclear alterations indicative of apoptosis. This study results differ from our study results.

Our study also shows highly significant increased frequency of MNC after CT scan exposure than pre exposure MNC in both gingival and buccal epithelial cells with p value <0.0001 (Table 11). Similar results are also seen with other cytotoxic abnormalities with p value <0.0001 (Table 12). Our study appears to be first among
the published literature to assess and compare genotoxic & cytotoxic effects of CT scan on epithelial oral mucosal cells hence there are no published studies available in literature to compare these findings.

As per results of our study there is significant increased frequency of MNC after CBCT scan exposure than pre exposure MNC in both gingival and buccal epithelial cells with p value <0.05 (Table 13). Similar results are seen with other cytotoxic abnormalities (Table14).

Carlin et al 2010\textsuperscript{66} evaluated DNA damage in the form of micronucleus and cellular death in the form of pyknosis, karyolysis, karyorrhexis in 19 individuals exposed to cone beam computed tomography. The results showed no significant statistical differences in micronucleus frequency pre and post exposure to cone beam computed tomography (CBCT) whereas cytotoxicity such as karyorrhexis, pyknosis and karyolysis was increased after CBCT exposure. The study concluded that cone beam computed tomography may promote cytotoxicity.

As per the study done by Lorenzoni et al \textsuperscript{70}, genotoxicity was not induced by the CBCT or the conventional radiographs, but increased cytotoxicity was seen after exposure to these radiographic examinations, especially after CBCT. Our study results vary from these studies for post CBCT exposure MN assessment but similar results are seen with other cytotoxic abnormalities after CBCT exposure.

Biomonitoring studies of genotoxicity and cytotoxicity of populations exposed to X-rays can be difficult and rather specific because of the different doses of radiation each population is exposed to. This is the reason why some studies have found increased genetic damage in populations exposed to X-rays.\textsuperscript{50} The variations between studies with respect to MN are difficult to interpret clinically due to
complex interactions between the environment and the genotype within the matrix of growth dynamics, development and adaptation. In such studies, some confounding factors like viruses, alterations in the immune system, failures in the DNA repair system, etc. are important to take into consideration.\textsuperscript{64}

The frequencies of MNC in the gingival and buccal cells after CT scan exposure is significantly increased when compared with other radiographic techniques (Full mouth IOPA, panoramic radiograph and CBCT scan) while comparisons of other radiographic techniques with each other (other than CT Scan) are insignificant (Table 15 and Table 16). Similar results are seen with other cytotoxic abnormalities (Table 17 and Table 18). This suggests that CT scan induces highest genotoxic and cytotoxic effects on oral epithelial cells. These results may be because of highest radiation doses of CT scan. To the best of our knowledge, this kind of comparison is never addressed in the literature so far. Further large sample studies are necessary to confirm these findings.

Here we want to focus on one of the results in the present study that the comparisons of frequencies of MNC and other cytotoxic abnormalities after full mouth IOPA radiographs, panoramic radiographs and CBCT scans (intergroup comparison) are statistically insignificant. There is no significant difference in the frequencies of MNC and other cytotoxic abnormalities after exposure in between Panoramic (OPG) group and CBCT group.

This suggests that genotoxic and cytotoxic effects induced by CBCT scans of gingival and buccal cells are not significantly high as compared with panoramic radiographs. Hence, we propose that by ALARA (as low as reasonably achievable) principle, panoramic radiology is the best technique but when added information is
required, CBCT scan can be preferred on panoramic radiography by overlooking genotoxic and cytotoxic effects, as CBCT is known to provide accurate three-dimensional information of orofacial structures. We also suggest that CBCT can be used as screening radiographic method instead of panoramic radiography.

As a general observation of our study, there is increase of difference in pre and post exposure MNC and other cytotoxic abnormalities in the gingival cells than buccal cells (Table NO.19), but this difference is statistically not significant in other than CT group-Group III. There is no significant difference in the MNC frequencies and other cytotoxic abnormalities (p > 0.05) when comparison was done in between the gingival and buccal mucosa cells of three study groups (full mouth IOPA radiographs, panoramic radiography and CBCT scan) while only CT scan group shows significant increase (p < 0.05) in MNC and other cytotoxic abnormalities in gingival cells than buccal cells.

This increase in MNC and other cytotoxic abnormalities may be due to higher exposure doses to the gingival cells than buccal cells due to more amount of scattered radiation from alveolar bone. This was one of the proposed hypotheses and it is partially fulfilled by our results, or this hypothesis holds true only with higher radiation exposure like CT scan. Further studies need to be done on this aspect with larger sample sizes and varied populations.

Cells that divide frequently or undergo many divisions over time are more sensitive to radiation. Immature cells, which are not highly specialized are more sensitive to radiation. Hence, stem cells are more sensitive to radiation hazards.
Tomar G et al\textsuperscript{71} concluded that human gingiva is a better source of mesenchymal stem cells (MSCs) than bone marrow, and large number of functionally competent clinical grade MSCs can be generated in short duration from the human gingiva. This can be an additional factor for the increase in MNC and other cytotoxic abnormalities in gingival cells than buccal mucosa cells after exposure to CT scan.

The MNC index may be indicative of genomic instability and the detection of an elevated micronuclei frequency in a given population indicates an increased risk of cancer.\textsuperscript{66} The cellular death is considered to be an important and prime mechanism in non-genotoxic mechanisms of carcinogenesis.\textsuperscript{57}

In the end our study results indicate that exposure to X-rays during full mouth IOPA radiographs, CT scans and CBCT scans induce genotoxic effects in oral mucosal cells that increase chromosomal damage and induce apoptosis. All these radiographic techniques requested only when necessary. The comparison of merits and demerits of all the radiographic techniques can help in deciding better radiographic methods for the patients.