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# QUANTITATIVE CYTOMORPHOMETRY AS A TOOL TO ASSESS THE CELLULAR ALTERATIONS OF ORAL MUCOSAL CELLS FOLLOWING ORTHO-PANTOMOGRAPHY

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## ABSTRACT

**Background:** X-rays forms an integral part of management of diseases that are used commonly in dentistry to arrive at an appropriate diagnosis. During such procedures the rays emitted in the form of ionizing radiations are capable of inducing cytotoxic alterations in exposed oral buccal mucosa cells.

**Aim:** To monitor the cytomorphometric alterations taken place within the exposed buccal mucosal cells before and after orthopantomography.

**Method:** The present study is a cross-sectional study undertaken in the Depts of Oral Medicine and Oral Pathology, Dhulikhel hospital, Dhulikhel, Kavre, Nepal. Apparently healthy individuals requiring Orthopantomography, who met the inclusion criteria were involved in this study. Informed consent was taken, after which the pre-post buccal mucosal samples were obtained and stained using Papanicolaou stain. These stained slides were observed using light microscope for recording the cytomorphometric changes.

**Results:** A statistically significant change was observed in the nuclear area (p- 0.03) from the samples obtained from the buccal mucosal smears following exposure to orthopantomography.

**Conclusion:** Buccal mucosal cells exposed to ionizing radiation did show some cytomorphometric alterations following orthopantomography.

**KEYWORDS :** buccal mucosa, cytomorphometry, orthopantomography.

## INTRODUCTION:

Recent decades have seen an increase among the population seeking dental care for which radiographs play an important role in framing the diagnosis and treating the pathology. Many of these procedures require the use of different types of radiographs depending upon the pathology which ranges from two to three dimensional projections. During these procedures the oral cells both keratinized as well as non-keratinized mucosa are continuously exposed to the ionizing radiations (Little, 2003).

It is well documented in the literature that ionizing radiations are capable of causing genotoxic and cytotoxic changes in exposed oral buccal mucosa cells and are dose dependent. Higher doses of radiation tend to cause mutations either directly or indirectly in the exposed epithelial cells by the formation of reactive compounds (Ogden,1989).

Carcinogenesis is the process which involves many stages where alteration of the cell takes place at cytogenetic level causing mutations of important genes which are involved in cells signalling, renewal, division and cell death all of which possess the survival characteristics of a cell (Arora, 2014). The biological effects of radiation are well determined by the genetic alterations seen in the form of chromosomal aberrations and formation of micronuclei which are assessed by simple technique of exfoliative oral cytology (Fenech, 1985) (Muller, 1996). In the year 1951 Miller et., al., were the first to study the exfoliative cytology of the normal oral epithelial cells. The rationale of this technique depends upon the process of desquamation of the superficial cells of the oral epithelium and any alterations in these cells serve as reliable indicators of dysplastic changes (Kazanowska, 2014).

A sensitive and a specific approach is required to detect these effects of radiation at lower dosage levels hence, the present study is designed to evaluate the cytotoxic effects in oral buccal mucosal cells in patients subjected to orthopantomography using quantitative cytomorphometry as a tool.

## MATERIALS AND METHODS:

### Study Setting

This is a cross sectional study undertaken in the Department of Oral and Maxillofacial Pathology and Department of Oral Medicine and Radiology, Dhulikhel Hospital, Dhulikhel, Kavre, Nepal. Apparently healthy patients requiring Orthopantomography (OPG) who had no oral lesions, debilitating oral diseases, adverse oral habits and systemic conditions affecting oral health, were randomly selected. A written informed consent was taken from the participants who were willing to participate in this study. The present study was approved by the Institutional Review Committee of Kathmandu University School of Medical Sciences, Dhulikhel, Kavre, Nepal.

Pre and post radiation, buccal mucosal samples were collected of which the pre exposure samples were taken as controls. Post exposure samples were collected 10 days after exposure to radiation. The buccal mucosal samples were collected using a wooden tongue depressor and were smeared onto a clean glass slide, which was immediately fixed using Biofix fixative and then stained using Rapid Papanicolaou (PAP) stain (BIOLABS, India). Olympus light microscope (CX-22) was used to view the stained slides to determine the adequacy of the smear. For cytomorphometric analysis, digital images of 50 cells were taken randomly Images were recorded using a digital camera and were transferred onto a computer for image analyses as shown in figure 1 & 2.

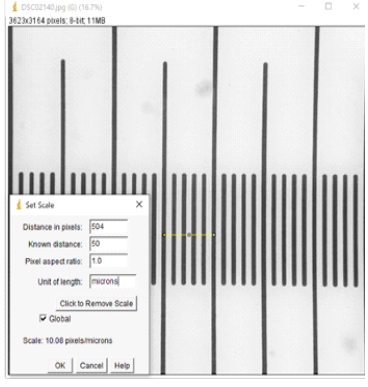


**Figure 1- Showing calibration of the stage micrometer using image 'J' software.**

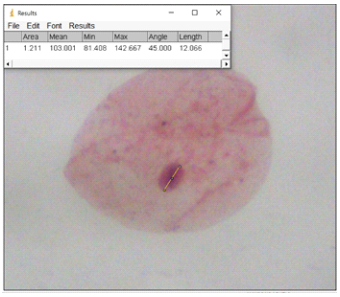


**Figure 2- Showing measurement of nuclear diameter using image 'J' software.**

Quantitative cytomorphometric analysis included parameters like nuclear diameter (ND), cell diameter (CD), nuclear diameter: cell diameter ratio (ND:CD), nuclear area (NA), cell area (CA), and nuclear area: cell area ratio (NA:CA) and were analyzed using Image 'J' (version 1.48) software. Statistical analysis was performed using SPSS software version 20.0. Normal distribution of data was checked using Shapiro - Wilk test, later parametric and non-parametric tests were performed depending upon the values obtained from Shapiro - Wilk test.

## RESULTS

Pre - post samples taken from buccal mucosa showed a normal distribution curve for variable CD and NA hence paired 't' test was used to compare the data as shown in table-1. Analysis of remaining variables like ND, ND:CD, CA & NA:CA were compared using Wilcoxon test as shown in table-2. A value of $p \leq 0.05$ was considered significant. The cell parameters like NA (p-0.03) showed statistically significant differences in values.

**Table 1: Displaying results of (OPG) in buccal mucosa using paired 't' test.**

| Parameter | | Mean | Std. Dev. | Mean diff. | p-value |
|---|---|---|---|---|---|
| CD | pre | 51.59 | 8.71 | -4.16 | 0.07 |
| | post | 55.75 | 5.56 | | |
| NA | pre | 59.70 | 12.95 | -7.62 | 0.03 |
| | post | 66.42 | 9.61 | | |

**Table 2: Displaying results of OPG in buccal mucosa using Wilcoxon test.**

| Parameter | | Mean | p-value |
|---|---|---|---|
| ND | pre | 8.72 | 0.16 |
| | post | 8.93 | |
| ND:CD | pre | 0.17 | 0.36 |
| | post | 0.17 | |
| CA | pre | 2095.12 | 0.06 |
| | post | 2437.21 | |
| NA:CA ratio | pre | 0.03 | 0.19 |
| | post | 0.03 | |

## DISCUSSION

Quantitative cytomorphometric analysis was undertaken in the present study to analyse the alterations happened within the oral mucosal cells that are exposed to ionizing radiation. Most of the studies in the past were undertaken to observe the genotoxic changes in the form of micronuclei and nuclear morphologic alterations like multi-nuclei, karyorrhexis, pyknosis and karyolysis. There are limited studies in the literature who have evaluated the alterations happened in the cell exposed to ionizing radiation using quantitative cytomorphometry including parameters like NA, CA and NA:CA ratio. Hence the present study high-lightens the use of quantitative cytomorphometry including parameters like nuclear diameter (ND), cell diameter (CD), nuclear diameter to cell diameter ratio (ND:CD) along with other parameters like Nuclear area (NA), cell area (CA), nuclear area to cell area ratio (NA:CA) to evaluate the changes happening in the cell that is exposed to ionizing radiation.

Due to limited availability of studies in the literature that uses quantitative cytomorphometric technique to assess the effects of ionizing radiation on oral mucosa the results of the present study are compared with that of the other studies done on patients having oral potentially malignant disorders, oral cancers (oral squamous cell carcinoma) and systemic diseases (diabetes and blood disorders).

Gender segregation was not done in the present study as there wasn't any significant changes observed in the normal oral mucosal squames between the genders according to a study done by Montgomery and Von Haam (Montgomery, 1951).

In the present study buccal mucosal smears showed a significant increase in the NA (mean diff.: -7.62) and a slight increase in the ND (mean diff.: -0.21) which is in accordance with the study done by Shah et., al., where a significant increase was found in NA in patients associated with oral habits. It was also observed that there was a slight decrease in CA in diabetic patients when compared to that of the normal subjects (Shah, 2017) which is in contrast to the present study where an increase was observed in the CA (mean diff.: -342.09) in patients exposed to orthopantomography. This could be due to the radiation exposure levels and initial reaction due to radiation that could produce increase in the cell area. Similar studies were conducted by Alberti et., al., where they found a significant increase in NA but no significant changes in the CA in Type II diabetic patients when compared with the samples obtained from normal subjects (Alberti, 2003). Similarly, Paraizo et., al., also observed an increase in NA and CA and a decrease in the NA/CA ratio in children with sickle cell anemia when compared with normal children and concluding that sickle cell anemia could produce some alterations in the NA in the cells of the oral mucosa (Paraizo, 2013).

In the present study it was noted that there was an increase in the CD (mean diff. = -4.16) which is similar to a study done by Prasad et., al., as their results revealed that the CD showed only a marginal decrease when compared between the mean values obtained from the control subjects and the diabetic patients (mean: 54.97vs 54.32). But when compared among the different groups of diabetic patients it was noted that the marginal increase in poorly controlled diabetic patients (mean= 55.19) compared to controls (Prasad, 2010).

These results are also in accordance to the data obtained from the study done by Ogden et., al., which revealed an increase in the ND and CD in buccal mucosal samples following radiotherapy. These nuclear and cellular alterations may be due to increased frequency and radiation dosage administered in patients following radiotherapy (Ogden, 1989).

## CONCLUSION

Based upon the above results it could be concluded that ionizing radiations may have the potential to induce cellular and nuclear alterations in oral mucosal cells in patients who are subjected to diagnostic radiation hence, it should be used judiciously in the field of medicine. Though using quantitative cytomorphometric technique there are few short-comings of the present study, which should include a larger sample size with longer follow-up patient appointments to study the effects of these ionizing radiations more precisely.

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