BUCCAL MUCOSAL DNA DAMAGE FOLLOWING RADIOTHERAPY IN PATIENTS WITH HEAD AND NECK CANCER

Vinay Kumar V ¹, Sachin KS ^{*2}, Vishak R ³.

^{*1} Professor, Department of Anatomy, K S Hegde Medical Academy, Mangaluru, Karnataka, India.

² Assistant Professor, Department of Anatomy, K S Hegde Medical Academy, Mangaluru, Karnataka, India.

³ Department of Anatomy, K S Hegde Medical Academy, Deralakatte, Mangaluru, Karnataka, India.

ABSTRACT

Patients with head and neck cancer receiving radiotherapy in addition to antitumour effects also are exposed to ionizing radiation which may also damage normal tissue located in the field of radiation. The study was done on buccal scrapings in patients with head and neck cancer patients receiving radiotherapy at the hospital. The present study aims at determining the changes in MN in the buccal smears in pre and post RT in patients with head and neck cancer patients. The mean number of MN was significantly high in buccal scrapings of patients post RT suggesting DNA damage following exposure to radiations during RT.

KEY WORDS: Head and neck cancer, Radiotherapy, Micronucleus assay, Buccal mucosa.

Address for Correspondence: Dr. Sachin KS, Assistant Professor, Department of Anatomy, K S Hegde Medical Academy, Deralakatte, Mangaluru- 575018. Karnataka, India. PH 7795767676.

E-Mail: dr7795767676@gmail.com

Access this Article online	Journal Information
Quick Response code DOI: 10.16965/ijar.2018.220	International Journal of Anatomy and Research ICV for 2016 90.30 ISSN (E) 2321-4287 ISSN (P) 2321-8967 https://www.ijmhr.org/ijar.htm DOI-Prefix: https://dx.doi.org/10.16965/ijar
Article Information	
Received: 11 Apr 2018 Peer Review: 12 Apr 2018 Revised: None	Accepted: 30 May 2018 Published (O): 05 Sep 2018 Published (P): 05 Sep 2018

INTRODUCTION

Head and neck cancer (HNC) is the sixth most common human malignancy worldwide representing 3% of all types of malignant tumors [1]. Approximately 48% of the cases are located in the oral cavity, and 90% of these cases are squamous cell carcinoma (SCC), which affects the lips, mouth, tongue, nasopharynx, oropharynx, hypopharynx, larynx and paranasal sinuses [2,3]. Annually, more than 5,00,000 new cases of SCC are diagnosed worldwide [4]. High rates of morbidity and mortality are observed, mainly because of the advanced clinical stage at the time of diagnosis. However, the use of concurrent chemotherapy (CT) and radiotherapy (RT) demonstrates that survival has substantially improved over the past decades for patients with most of the forms of HNC [5].

RT is usually recommended as the primary treatment or as an adjunct to surgery, in combination with or without chemotherapy, or as a palliative measure in the management of HNC. The dose of radiation necessary for cancer treatment depends upon the location, the type of malignancy and whether or not RT is the sole treatment or is to be given in combination with other modalities. Majority of patients treated with curative intent receive a total dose between 50 and 70 Gy, usually given over a 5 to 7 week period~ treatment being given once a day, five days a week, with 2 Gy per fraction [6].

In addition to the antitumor effects of ionizing radiation it may also damage normal tissue located in the field of radiation. The oral cavity has many complex areas with dissimilar structures that respond differently on exposure to

ionizing radiation, e.g., mucosal lining, skin covering, submucosal connective tissue, salivary gland tissue, teeth, and bone/cartilage. The RT produces early changes in the oral mucosa (oral mucositis), skin (erythema, desquamation), salivary glands (hyposalivation), taste buds (decrease acuity) and teeth (radiation caries) [7-11]. Changes is also observed in all tissues at a later stage [12,13], especially of gingival and periodontal changes, including loss of attachment at the radiation sites are also observed [14,15].

The ionizing radiation damages DNA, including single & double-strand breaks, base damage, and DNA-protein cross links. As a consequence, a second tumor may develop immediately or years after the primary tumor treatment [16-18]. Attempts have been made to evaluate the genotoxicity of ionizing radiation in patients undergoing RT. Gamma rays have been reported as inducing linear increase of micronucleated buccal mucosa cells in oral cancer patients undergoing RT [19]. The effects of radiation on the simultaneous MNA in cytokinesis-blocked peripheral blood lymphocytes, as developed by Fenech and Morley [20] and exfoliated cells are increasingly being applied for monitoring human exposure to mutagens. The present study aims at determining the changes in antioxidants status of pre and post RT saliva samples of HNC patients.

MATERIALS AND METHODS

The study population included a group of HNC patients without any prior treatment (RT and/or CT) scheduled to receive RT at the department of radiotherapy. The study was conducted after approval from institutional ethical committee. A signed informed consent was obtained from each subject. The details about the age, gender, occupation & the history regarding tobacco and alcohol consumption were collected from each subject.

The Inclusion criteria were

1. Patient above the age of 18 years.
2. Patient having HNC of stage II to stage IV, according to TNM classification.
3. The radiation dose received by patient should be greater than or equal to 60 Gy, delivered in

30 fractions, over a period of 7 weeks.

4. At least one third of the oral cavity mucosa should be included in the RT field.

The Exclusion criteria were

1. Patients having open mouth sores before RT.
2. Patients who had undergone prior RT or CT.
3. Patients with HIV infections, diabetes mellitus or hyperthyroidism.
4. Those patients who died during the treatment period or moved to another RT centre.

Collection of buccal mucosal scrapings and Saliva sample for analysis:

The subjects were advised to rinse their mouth with normal saline, then about 5ml of saliva and buccal mucosal scrapings were collected from each patient. The buccal mucosa cells were collected by using a cytobrush moistened with water by gently scraping the mucosa of the inner lining of cheeks. The buccal smear were collected in the sterile container and labeled.

The Pre Radiotherapy (RT) samples were collected before initiation of RT. All the patients received a megavoltage therapy (4 MeV) using a source to skin distance of 100 cm. The doses ranged from 45 to 70.4Gy, delivered in daily fractions of 2.0Gy, 5 days per week for 5–7 weeks. At the end of 7th week a Post RT sample was collected.

The smears collected from buccal mucosal scrapings were subjected to Micronucleus Assay (MNA).

Micronucleus Assay: The buccal scrape were shaken in a centrifuge tube containing phosphate buffered saline (pH 7.2) to release the cells and the tube is then centrifuged to wash the cells in a buffer solution. This washing procedure helps to remove bacteria and cell debris, which confound the scoring. Buccal cell smears are prepared by spreading the cells on a clean slide. Then slides are fixed 80% methanol. The slides were stained by Giemsa stain (10%) prepared in Sorensen's buffer (pH 6.8). Slides were kept in Giemsa working stain solution for 20 minutes. The stained slides were scanned under 1000 fold magnification (100x using oil immersion. About 1,000 epithelial cells were scored to determine the frequency of micronucleus cells (MNC). Only cells free from

clumping or overlapping and those containing intact cytoplasmic boundaries were included in the scoring. Micronuclei were scored in the buccal cells according to the criteria defined by Tolbert et al., [21].

Tolbert et al. criteria parameters for identifying micronucleus are as follows:

- Rounded smooth perimeter suggestive of a membrane.
- Less than a third the diameter of associated nucleus, but large enough to discern shape and color.
- Staining intensity similar to nucleus.
- Texture similar to nucleus.
- Same focal plane as nucleus.
- Absence of overlap with or bridge to nucleus.

The mean MN in pre RT and post RT buccal scrapings were calculated and were statistically analysed. Statistical analysis was done using student t test. P value < 0.05 was considered significant.

RESULTS

The mean number of MN was higher in buccal mucosa following radiotherapy. The number of micronucleus in pre RT was 9.96 ± 1.47 and 22.72 ± 4.86 in post RT samples. On comparing the pre and post RT samples it was found that the p value was <0.0001 and was statistically highly significant (Table 1).

Table 1:

		Minimum	Maximum	Mean	SD	P Value
1	MNA	7	13.4	9.96	1.47	<0.0001 Highly significant
	Post Radiotherapy	13.1	30	22.72	4.86	

Fig. 1: Showing the micronucleus.

DISCUSSION

The micronucleus (MN) represents chromosome fragments or whole chromosomes which fail to

get incorporated into main nuclei at mitosis. The MN consequently appear only in cells that undergo nuclear division [22].

The MNA in buccal mucosal cells is an innovative genotoxicity technique, which holds promise for the study of epithelial carcinogens. MN are suitable internal dosimeters for revealing tissue specific genotoxic damage in individuals exposed to carcinogenic mixtures.

Casartelli et al. observed the frequency of MN in buccal mucosal cells of normal mucosa, precancerous lesions and squamous cell carcinoma. They observed a gradual increase in MN counts from normal mucosal to precancerous lesions to carcinoma suggested a link of this biomarker with neoplastic progression [23].

RT plays an important role in the treatment of many cancers, but it also produces genetic damage. Many studies have been done on MN in buccal cells of patients undergoing RT for HNC treatment. The most striking increase in cytogenetic damage (150-300 MN/1000 cells) was observed in an early study of three patients exposed to a cumulative dose of 3400-4000cGy [24].

Some authors reported 68 MN/1000 cells after 2000 cGy and 16 MN/1000 cells after treatment with 1000 cGy for 3 weeks [19,25].

Moore et al., [26] observed more than 16 fold increase in MN frequency shortly after the initiation of RT, followed by return to baseline 12 weeks later and 3 weeks after cessation of the RT.

Many studies have shown that there is a statistically significant increase in the MN frequency in buccal mucosal cells after exposure to genotoxic agents. The MN frequency decreases after micronutrient supplementation or chemoprevention, but the magnitude of changes is usually relatively small [27].

In the present study, the frequency of MN increased from 9.96 in pre RT samples to 22.72 MN/100cells in post RT samples which was statistically significant. The extent of DNA damage evaluation by the comet assay in peripheral blood cells is perfectly reflected by the MNA on oral exfoliated epithelial cells, and MN frequency can be used with the same effectiveness and greater efficiency in early detection of

oral premalignant conditions.

Scope for Future work: The DNA damage study can be carried out by Comet assay to attain confirmed results. Buccal cell damage can be assessed by using DNA fragmentation assay. Further studies on patients with different stages of head and neck cancer and assessment of the effect of antioxidant supplementation before and during the RT regimen are needed. The main limitation of the present study is the small sample size. Further studies with larger sample size should continue this line of research.

CONCLUSION

The mean number of MN was significantly high in buccal scrapings of patients post RT suggesting DNA damage following exposure to radiations during RT. However the profile can also be influenced by the patients by their history of smoking, drinking and others factors. Further investigation is necessary to confirm and expand these findings.

Conflicts of Interests: None

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