Acta Scientific Cancer Biology

Volume 2 Issue 5 July 2018

Research Article

Evaluation of Micronuclei Frequency in Peripheral Blood Lymphocytes of Patients with Oral Squamous Cell Carcinoma

Bhaskaran Sathyapriya[1,2]*, KR Srinivasan[3] and Purushothaman Lakshmanan[4]

[1]Research Scholar, Department of Anatomy, Vinayaka Missions Aarupadai Veedu Medical College and Hospital, Vinayaka Missions University, Salem, Tamilnadu, India

[2]Reader, Department of Anatomy, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research, Chennai, Tamilnadu, India

[3]Professor, Department of Anatomy, Vinayaka Missions Aarupadai Veedu Medical College and Hospital, Vinayaka Missions University, Salem, Tamilnadu, India

[4]Consultant Orthodontist, Apollo Hospitals and Apollo White Dental, Chennai, Tamilnadu, India

***Corresponding Author:** Bhaskaran Sathyapriya, Research Scholar, Department of Anatomy, Vinayaka Missions Aarupadai Veedu Medical College and Hospital, Vinayaka Missions University and Reader, Department of Anatomy, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research, Tamilnadu, India.

Received: June 12, 2018; **Published:** June 28, 2018

Abstract

Aim: To assess DNA/chromosome damage in peripheral blood lymphocytes (PBL) of patients with oral carcinoma by micronucleus assay.

Materials and Methods: DNA damage was assessed in PBL of 10 oral cancer patients using micronucleus assay. Samples of venous blood (3 ml) were collected in heparinized vials under sterile conditions on day 1 of the first radiotherapy cycle 2 hours before irradiation (sample 1), 2 hours after the application of the first dose (sample 2), during the middle of the radiotherapy (sample 3) and after 6 months (sample 4) after radiotherapy.

Results: The frequency of structural chromosome aberrations in lymphocytes gradually increased during the radiation cycle. In pretherapy sample 1, the mean total number of micronuclei of 23.00 ± 3.559. After administration of the first fraction of radiotherapy (sample 2), the mean total number of micronuclei was 29.60 ± 3.239. In most participants, the highest level micronuclei frequency was recorded in blood samples collected during the middle of the radiotherapy (138.50 ± 20.22). Later on, the levels of primary DNA damage slightly diminished (53.30 ± 13.881).

Conclusions: In conclusion, introduction of genotoxicological methods in medical surveillance of cancer patients before and after radiotherapy could be important in evaluating secondary cancer risk and, in case of cancers such as oral carcinoma which may have initial genome burden, could be an important factor for individual therapy adjustment.

Keywords: Micronuclei; Lymphocytes; Radiotherapy; Oral Carcinoma; Chromosome Damage

Introduction

Oral cancer is one of the frequently occurring head and neck cancers worldwide [1]. Approximately 90% of oral cancers are squamous cell carcinoma (OSCC), which is seen typically on the lateral border of the tongue, oropharynx, and floor of the mouth, lower lip, tongue, floor of the mouth, soft palate, gingival/alveolar ridge as a red (erythroplakia) or, white (leukoplakia), or a mixture of the two (erythroleukoplakia) with an ulcer [2]. Biopsy is mandatory for every ulcerated lesion persisting for 4 weeks or longer [3].

Older males are the common victims of OSCC, though it affects younger patients and women too [4]. OSCC is caused by several factors, the most important being tobacco, betel nut chewing, alcohol use, poor oral hygiene, nutritional deficiencies immune defects or in DNA disrepair [5,6]. The prevalence of squamous cell carcinoma of the oropharynx is about 5 - 10% [7]. Radiation therapy destroys all cancer cells which ultimately abolishes the development of tumors [8]. Intensity-modulated radiation therapy (IMRT) confers specific (computerized) radiation doses in multiple fractions to the tumor in particular, thereby preserving the adjacent normal structures from radiation hazards [9]. Therefore, the adverse effects are less compared with conventional radiotherapy. The major drawback in IMRT is that the treatment duration is time-consuming because of its complex techniques than the conventional radiotherapy. The number of IMRT sessions depends on the site and extent of the growth size. Usually it is five days a week for five to eight weeks lasting between 15 and 30 minutes [8,10].

Although radiation is the mainstay of current therapy for oral cancer, the variability in intrinsic radiosensitivity significantly contributes to the outcome of the disease control. Apart from the beneficial effect of radiotherapy, adverse consequences on normal tissue are almost always present [11]. Following γ- irradiation, different types of lesions can be detected in the nuclear DNA. Although unrepaired DNA damage is useful in killing cancerous cells, it can be detrimental to normal cells, leading to the onset of secondary cancer [12]. The most extensively genotoxic and carcinogenic risks in humans involve cytogenetic endpoints such as chromosomal aberrations, sister chromatid exchanges, and micronuclei in mitogen-stimulated peripheral blood lymphocytes. Micronuclei (MN) are eccentric chromosome section or whole chromosome which failed to segregate during the anaphase of cell division resulting in the formation of one normal daughter cell and the other abnormal daughter cell with smaller nuclei. The number of micronuclei depends on the extent of genetic damage [13]. Micronucleus assay is an important biomarker for assessing the chromosomal damage [14,15]. It is easier to conduct than the other cytogenetic assays, chromosomal aberration test in terms of cost, reliability, proce-

dures and evaluation [14]. In this study, the micronucleus assay in peripheral blood lymphocytes is used as a biomarker for the assessment of DNA/chromosome damage in patients with oral cancer.

Materials and Methods

The participants of the present study included 10 males with squamous cell carcinoma of the oral mucosa with age ranging from 30 - 60 years who had undergone excision of the primary oral tumor. Patients smoking more than 20 packets of cigarettes per day with long term alcohol consumption were included in the study. Patients under chemotherapy and other treatment known to cause DNA damage were excluded from the study. Before planning treatment, a complete medical history review, physical examination, complete blood count, CT/MRI and Tumor nodes metastasis (TNM) staging was done. All patients were irradiated with photon beams from a 60 Co source in the area of the primary tumor. Total tumor dose applied after surgery was 60Gy in 30 daily fractions.

DNA damage was evaluated in peripheral blood lymphocytes of 10 oral cancer patients using micronucleus assay. Samples of venous blood (3 ml) were collected in heparinized vials under sterile conditions 2 hours before irradiation on day 1 of the first radiotherapy cycle (sample 1), 2 hours after the application of the first dose (sample 2), during the middle radiotherapy (sample 3) and after 6 months (sample 4) after radiotherapy. The blood samples were then transported to the laboratory in an ice bath. These samples were brought back to room temperature prior to setting up of the culture.

To 0.5 ml of the blood sample, 5 ml culture medium supplemented with 7.5% NaHCO , 20% fetal calf serum, 200 mM L-glutamine, penicillin 100 units/ml and streptomycin 100 mg/ml, was added. A total of 200 ml of phytohaemagglutinin-M PHA-M (to initiate cell division) at a final concentration of 3 mgr 5 ml culture was added at 44h. The cells were incubated for another 28h at 370C. The harvested cells were then treated hypotonically. Three drops of cell suspension were allowed to fall on to a clear cooled slide from a height of 1 cm, air dried and stained with Giemsa. Scoring of micronuclei in 1000 binucleated cells was performed at 1000×magnification under oil immersion. Total number of micronuclei and their distribution were determined.

Statistical Analysis

The collected data were thoroughly screened and entered into MS – Excel spread sheet and analysis was carried out using statistical package for social science software version 16 (SPSS). Descriptive statistics such as mean and standard deviation (SD) were reported for micronuclei frequencies. Comparison of mean number of micronuclei among 4 samples was done by ANOVA. A value of p ≤0.05 conclude that a significant difference does exist.

Results

In pre-therapy sample 1 the total number of micronuclei were in range from 18 to 30 micronuclei per 1000 binuclear cells, with the mean total number of micronuclei of 23.00 ± 3.559. After administration of the first fraction of radiotherapy (sample 2) the total number of micronuclei was in range from 24 to 36 micronuclei per 1000 binuclear cells, with the mean total number of 29.60 ± 3.239 micronuclei. In blood samples analyzed in the middle of radiotherapy cycle (sample 3) the total number of micronuclei was in range from 114 to 176 micronuclei per 1000 binuclear cells, with the mean total number of micronuclei of 138.50 ± 20.22. In the blood samples taken six months following radiotherapy (sample 4) the total number of micronuclei were in range from 36 to 78 micronuclei per 1000 binuclear cells, with the mean total number of micronuclei 53.30 ± 13.881 (Table 1 and Figure 1). One way ANOVA revealed significant differences between four blood samplings for the total number of micronuclei ($P < 0.001$) (Table 1 and Figure 2).

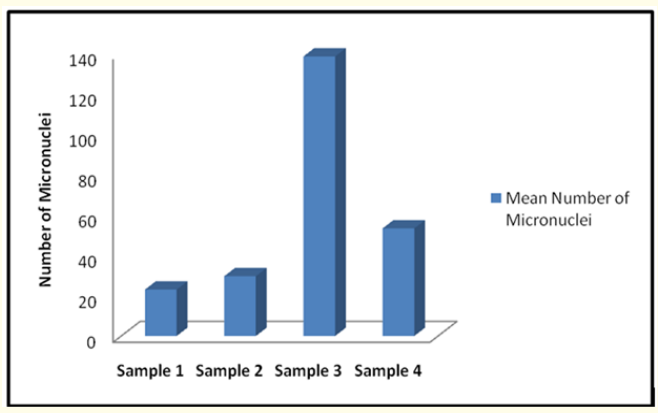

Figure 1: Distribution of the mean number of micronuclei (MN) ± Standard Deviation recorded in peripheral blood lymphocytes of patients with oral carcinoma before and after radiotherapy.

S. No.	Blood Samples	Mean Number of Micronuclei ± Standard Deviation
1	Sample 1	23.00 ± 3.559
2	Sample 2	29.60 ± 3.239
3	Sample 3	138.50 ± 20.22
4	Sample 4	53.30 ± 13.881

Table 1: Distribution of the mean number of micronuclei (MN) ± Standard Deviation recorded in peripheral blood lymphocytes of patients with oral carcinoma before and after radiotherapy.

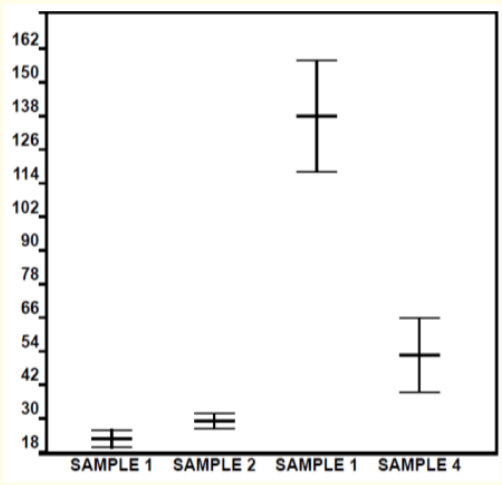

Figure 2: Box plot distribution of the mean number of micronuclei (MN) ± Standard Deviation recorded in peripheral blood lymphocytes of patients with oral carcinoma before and after radiotherapy

Discussion

The prevalence of squamous cell carcinoma of the oral mucosa is about 5 - 10%. The development of oral cancer is strongly associated with tobacco use and alcohol consumption, as well as with the exposure to several occupational carcinogens, vitamin deficiencies, and poor oral hygiene. Although radiation is the mainstay of current therapy for oral cancer, the variability in intrinsic radiosensitivity significantly contributes to the outcome of the disease control. Apart from the beneficial effect of radiotherapy, adverse consequences on normal tissue are almost always present. Oral cancer patients had relatively higher levels of micronuclei in their peripheral blood lymphocytes even before therapy. The frequency of complex structural chromosome aberrations and the frequency of micronuclei increased with the progression of the radiation cycle and the doses delivered. In this study, peripheral blood lymphocytes were chosen because of their easy availability, synchronous population, low frequency of spontaneous chromosomal aberrations, convenient culture methods, and ease of sample collection. Different human health problems are associated with chromosomal instability in lymphocytes [16]. Micronuclei originate from chromosome fragments or whole chromosomes that fail to engage with the mitotic spindle and, therefore, lag behind when the cell divides [16]. The micronucleus assay showed a time-dependent occurrence and elimination of DNA and chromosomal damage before, during the course and six months year after radiotherapy. The decrease in the frequency of genetic damage could probably be due to the elimination of damaged cells, dilution into the blood stream, or the instability of these cells to complete mitotic division. As the frequency of chromosomal aberrations did not return to pre-therapy values, six months after radiotherapy, it represents an important risk factor related to the onset of secondary cancer. Inter-individual differences in response to radiotherapy were obviously influenced by different mutagen sensitivity and DNA repair capacity in the participants. Similar was also reported in previous investigations on cancer patients [17]. The results obtained by the micronucleus assay correlated well with the levels of chromosomal damage. This study shows, the micronuclei assay can be used as an important biomarker of genomic instability and a good cancer risk predictor for untreated and therapeutically exposed cancer patients [18].

Conclusion

In wrapping up, prologue of genotoxicological methods in medical supervision of cancer patients before and after radiotherapy might be imperative in evaluating secondary cancer risk and, in case of cancers such as oral carcinoma which may have preliminary genome lumber, could be a significant factor for individual treatment modification.

Bibliography

1. AK Dutta. "Essentials of Human Anatomy: Head and Neck". In: Mouth, Palate, Tongue And Teeth: 5th Edition Current Books International (2010): 272.

2. Squier CA., *et al*. "Regional differences of the oral mucosa". In: Human Oral Mucosa: Development, Structure and Function. Blackwell, Oxford (1976): 73-85.

3. Koss LG and Melamed MR. "Koss' Diagnostic Cytology and its Histopathologic Basis". In: Epithelial Lesions of Oral Cavity, Larynx, Trachea, Nasopharynx And Paranasal Sinus. Chapter 21, 5th Edition, Volume 1, Section 2 Lippincott and Wilkins, Philadelphia, USA (2006): 720-725.

4. Shillitoe EJ. "The role of viruses in squamous cell carcinoma of the oropharyngeal mucosa". *Oral Oncology* 45.4-5 (2009): 351-355.

5. Boffetta P., *et al*. "Carcinogenic effect of tobacco smoking and alcohol drinking on anatomic sites of the oral cavity and oropharynx". *International Journal of Cancer* 52.4 (1992): 530-533.

6. CJ Piyathilake., *et al*. "Cigarette smoking, intracellular vitamin deficiency, and occurrence of micronuclei in epithelial cells of the buccal mucosa". *Cancer Epidemiology, Biomarkers and Prevention* 4.7 (1995): 751-758.

7. Bradley PJ. "Management of squamous cell carcinoma of the oropharynx. Head and neck oncology". *Current Opinion in Otolaryngology and Head and Neck Surgery* 8 (2000): 80-86.

8. Hendry JH. "Genomic instability: potential contributions to tumour and normal tissue response, and second tumours, after radiotherapy". *Radiotherapy and Oncology* 59.2 (2001): 117-126.

9. Purdy JA. "Intensity modulated radiation therapy (editorial)". *International Journal of Radiation Oncology * Biology * Physics* 35 (1996): 845-846.

10. Butler EB., *et al*. "SMART (Simultaneous Modulated Radiation Therapy) boost-a new accelerated fractionation schedule for the treatment of head and neck cancer with intensity modulated radiotherapy". *International Journal of Radiation Oncology * Biology * Physics* 45.1 (1999): 21-32.

11. de Zwart LL., *et al*. "Biomarkers of free radical damage applications in experimental animals and in humans". *Free Radical Biology and Medicine* 26.1-2 (1999): 202-226.

12. Tolbert PE., *et al*. "Micronuclei and other nuclear anomalies in buccal smears: Method development". *Mutation Research* 271.1 (1992): 69-77.

13. Singh NP., *et al*. "A simple technique for quantitation of low levels of DNA damage in individual cells". *Experimental Cell Research* 175.1 (1988): 184-191.

14. Albertini RJ., *et al*. "IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. International Programme on Chemical Safety". *Mutation Research* 463.2 (2000): 111-172.

15. Desai SS., *et al*. "Cytogenetic damage in exfoliated oral mucosal cells and circulating lymphocytes of patients suffering from precancerous oral lesions". *Cancer Letters* 109.1-2 (1996): 9-14.

16. Fenech M., *et al*. "HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures". *Mutation Research* 534.1-2 (2003): 65-75.

17. Mariano Ruiz de Almodovar J., *et al*. "Individualization of radiotherapy in breast cancer patients: possible usefulness of a DNA damage assay to measure normal cell radiosensitivity". *Radiotherapy and Oncology* 62.3 (2002): 327-333.

18. El-Zein RA., *et al*. "Cytokinesis-blocked micronucleus assay as a novel biomarker for lung cancer risk". *Cancer Research* 66.12 (2006): 6449-6456.

Volume 2 Issue 5 July 2018