Karyoanomalic frequency during radiation therapy

ABSTRACT

Aim: To identify the relationship between the radiosensitivity of oral cancers and to evaluate the dose-dependent relationship of nuclear abnormalities by serial cytology during fractionated radiotherapy in head and neck cancer patients.

Materials and Methods: 30 patients with histologically proven cases of squamous cell carcinoma were included in the study. Serial scrape smear were taken from the tumor before and during radiotherapy (0 to 24 Gy), and stained with Giemsa and May Grunwald’s stain and frequency of micronucleated, binucleated and multinucleated cells were evaluated with the help of light microscope. The counts were expressed per 1000 uninucleated cells.

Results: Each parameter showed a statistical increase with increase dose. Before treatment, the mean values of micronucleated cells, binucleated cells and multinucleated cells were 3.5, 10.1 and 4.2. At 4 Gy these were 7.7, 12.0 and 6.2 which further increased with radiation dose; and the mean values were 8.8, 16.2 and 14.9 at 14 Gy and 15.1 at 24 Gy. After analysis of p-value, all such abnormal cells showed significant difference (p < 0.0001) with respect to normal subjects.

Conclusion: Our study results that micronucleus assay is a very useful tool in the assessment of biological damage that can help to identify tumor radiosensitivity.

Key words: Radiosensitivity, Oral cancer, Micronucleated cells, Binucleated cells

INTRODUCTION

The term oral cancer generally refers to squamous cell carcinoma (SCC) of oral mucosal origin, which accounts for, more than 90% of all malignancies of this location.[1] The mainstay of current therapy for oral cancer is surgery or radiation. Variability in intrinsic radiosensitivity is an important factor that determines the control of cancers with radiotherapy.[2-4]

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.[5] Cytologic evaluation of irradiation effects on oral mucosa was first reported in 1957[6] and on oral cancer in 1959.[7] 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. Both malignant and benign cells were known to show the same changes, although cancer cells showed significant hyperchromasia.[8] Even though micronucleation had been reported as a radiation-related change[9] 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.

Presence of micronucleated and multinucleated cell in untreated tumors have been reported by many,[10-12] but they have been used rather as markers of radiosensitivity or chemosensitivity. As both micronucleation need cell division or attempted cell division for their induction. Hence they may be considered as markers of proliferation too, though no serious attempt has been made to evaluate them from that aspect. Cell structure studies have shown that both micronucleated and multinucleated cells are nonclonogenic. Yet, they are not physically dead, but continues to divide a few times.

The micronucleus assay is a widely used technique for monitoring and evaluation of the clastogenic effect of chemical, radiological and...
other damaging agents in various types of cells.\textsuperscript{[13-17]} The ability of this method to evaluate the response of individual cells has led to an increase in its application in recent years. The micronucleus assay is routinely used as a biological dosimeter\textsuperscript{[13,18-20]} and can be a useful tool in the evaluation of cell sensitivity to ionizing radiation with implications for tumor therapy.\textsuperscript{[21,22]} The aim of the present study was to find out whether there is any relationship with the frequency of nuclear anomalies in the smears of oral cancer patients with the disease and whether the such frequency could serve as an indicator for the treatment outcome.

**MATERIALS AND METHODS**

The present study has been carried out on 30 patients with biopsy proven malignancies of head and neck cancer stage II, III, IV. Patients of head and neck cancers were classified according to UICC classification. Patients were treated by external telecobalt beam radiotherapy. Pretreatment scrape smears were collected from the tumor of each patient. Subsequently, 3-4 smears were collected from each patient after delivery of various fractions (2 Gy/fraction), usually after 2, 7 and 12 fractions. Care was taken from the tumor, avoiding adjacent normal areas (smears were usually not collected after 12 fractions because radiation mucositis made it difficult to distinguish tumor from normal tissue). Scraping was done with previously wetted spatula and material spread on slide, then material was fixed with the help of methanol and stained with Giemsa and May-Grunwald’s stain. After dehydration, the slides were mounted in DPX and were evaluated under light microscope.

The following abnormally nucleated cells were evaluated –

1. Micronucleation: intracytoplasmic, DNA staining bodies found in the same plane as the main nucleus with the same or slightly lesser intensity, one third to one fifth the size of the main nucleus, placed within two nuclear diameters from the main nucleus but distinctly separate from it.

2. Binucleation: two nuclei in a single cell with no micronucleus or budding of the nucleus.

3. Multinucleation: more than two nuclei in a single cell.

Cells were counted separately for the number of micronuclei, multinuclei and binucleated cells. Around 500-1000 cells were evaluated from the samples collected on each fraction and the results were expressed in terms of 1,000 tumor cells with normal nucleated cells. In each group, the mean of micronucleated cells, binucleated cells and multinucleated cells were calculated at each dose. Variance was analyzed within the group and p-value was calculated. Patient’s characteristic is given in Table 1.

**RESULTS**

All the parameters analyzed showed increase with cumulative doses of radiation. The details, presented in Table 2, contains mean values, variance and p-value of each parameter at each dose interval. Figure 1 is showing the change in nuclear abnormalities with different doses of radiation. Before treatment, the mean values of micronucleated cells, binucleated cells and multinucleated cells were 3.5, 10.1 and 4.2. At 4 Gy, these were 7.7, 12.0 and 6.2 which increased at 14 and 24 Gy; and the mean values were 8.8, 16.2 and 14.9 at 14 Gy and 12.8, 18.5 and 15.1 at 24 Gy.

After 24 Gy, the micronucleated cells were 3.7 times, binucleated cells were about twice and multinucleated cells were 3.6 times than the pretreatment values. After analy-

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<th>Table 2: Micronucleated, binucleated and multinucleated cells in head and neck cancer patients during fractionated radiotherapy</th>
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<td><strong>Dose (Gy)</strong></td>
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sis of p-value, all such abnormal cells showed significant difference (p < 0.0001) with respect to normal subjects.

DISCUSSION

Detailed studies of tumors in the past few years have revealed that even tumors which are classified into the same category by the pathologist are very heterogenous in their biological attributes.\(^{23-28}\) As success of tumor and normal tissue, rapid assay systems for determination of radiation response of various tissues are urgently required.

Results from the present study showed a noticeable nuclear change occurred during the radiation treatment of oral cancer cells. On analysis, all of them showed significant dose-dependent increase. Micronuclei are acentric, chromatid or chromosome fragments that lag behind during mitosis and fail to be incorporated into the main nucleus of the daughter cells after cell division. In fact it is an accepted test for monitoring toxicity of chemicals\(^ {39}\) and effectiveness of chemopreventive agents and radiotherapy against cancer\(^ {30}\) since ionizing radiation causes dose dependent mitotic delay in exposed cells. Micronuclei are due to damage to the chromosome leading to loss of genetic material. Micronucleation assay in cultured lymphocytes by the cytokinesis-blocked method is recommended as a biologic dosimetre for radiation exposure.\(^ {31}\)

In the present study, the frequency of micronucleated cells showed a statistically significant increase with radiation, the count of 24 Gy was more than 3.7 times the pretreatment count. The high variance in the counts at each dose point suggests the presence of a high degree of intertumoral heterogenicity in micronucleus induction. This suggests that it may have the potential for use as a predictive test for radiosensitivity.

At least with regard to proliferating tissues, the micronucleus assay seems to be helpful when it comes to the determination of radiation sensitivity. This is mainly due to the long known and often confirmed observation that micronuclei are indicators of the extent of cell death.\(^ {32-34}\)

Micronucleation can occur if there is damage to and interference with the functioning of the centrioles, pericentriolar matrix or cell membrane. Radiation-induced micronucleation have been noted in animal expriments.\(^ {35}\) Experiments with proton microbeams showed that irradiation of cytoplasm (with the nucleus shielded) results in damage to the centrioles leading to micronucleation.\(^ {34}\) Damage to pericentriolar matrix can lead to multipolar mitosis and micronucleation.\(^ {37}\)

The frequency of micronuclei in the present study increased with the increase in radiation dose. Zamboglou et al.\(^ {39}\) observed a pronounced increase in the number of micronuclei in cells of recurrent heads and neck carcinoma after combined radiotherapy and intratumoral instillation of mitoxantrone.\(^ {39}\) This increase was clearly greater than that expected from the addition of the single effects. The marked response with regard to micronucleus formation was accompanied by an improved tumor remission.

Similarly, a dose-dependent increase in the frequency of micronuclei \textit{in vivo} has been reported in polychromatic erythrocytes\(^ {38,39}\) and in bone marrow cells\(^ {40,41}\) of mice after exposure to various doses of X or g-radiation. Mitchell and Norman (1987) and Ramalho et al. (1988) have reported a dose-dependent increase in the counts of micronuclei in the lymphocytes of human peripheral blood exposed to low doses of X-rays.\(^ {42,43}\)

Radiation-induced peroxidation of lipids in the cell membrane can cause structural and functional alterations to it. If the damage is severe, there will be cytokinesis block resulting in the formation of binucleated cells and further division of one or both nuclei leads to multinucleation. The present study shows a significant increase in multinucleation during fractionated radiotherapy as compared to pretreatment values. Multinucleated cells are also incapable of giving rise to new colonies\(^ {37}\) and therefore can be considered as dead cells. Thus, evaluation of multinucleated cells also suggest that it is likely to provide information about radiosensitivity.

CONCLUSION

The present study concludes that micronucleus assay is a very useful tool in the assessment of biological damage that can help to identify tumor radiosensitivity. This may be useful in the development of the \textit{in vivo} models of cell killing in human cancer radiotherapy. The results of micronucleus assay may be helpful for the decision on the appropriate method of treatment, they allow to monitor the response to the treatment, and they may be useful in giving prognosis of the final outcome of the therapy.

REFERENCES

Kumari R, et al.: Karyoanomalous frequency during radiation therapy


