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EFFECT OF RADIOTHERAPY ON ORAL MUCOSA IN SUDANESE PATIENTS WITH HEAD AND NECK CANCER ASSESSED BY NUCLEOLAR ORGANIZER REGIONS COUNT

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ABSTRACT

Background: Any tumor could be controlled by radiation therapy if sufficient dose was delivered to all tumor cells. Although technological advances in physical treatment delivery have been developed to allow more radiation dose conformity, normal tissue is invariably included in any radiation field within the tumor volume and also as part of the exit and entrance doses relevant for particle therapy. The aim of this study was to evaluate normal tissue response following radiation therapy by argyrophilic nucleolar organizer region (AgNOR) score in the normal buccal mucosa form Sudanese patients with head and neck cancer. **Methods:** The study comprised a total of 80 cytological smears, of whom fifty (50) patients with head and neck cancer that exposed to radiotherapy versus thirty (30) clinically healthy volunteers. The cytological smear was taken from each subject by cytological brush. The smear was then wet fixed in 95% ethyl alcohol and stained with argyrophilic sliver technique to assess the (AgNOR) argyrophilic nucleolar organizer region (AgNOR) score **Statistical analysis:** All results were analyzed by (SPSS) Statistical Packages for Social Sciences version 16.0 software for statistical analysis. The means were obtained and one sample T-test and other variables frequencies were calculated for comparison and presented in form of figures and tables. **Results:** the proliferation activity indicated by AgNORs score was highly increase in patients exposed to radiotherapy when compared with clinically healthy volunteers, the mean AgNORs count was found to be statistically significant increase with radiation dose and fraction. **Conclusion:** AgNOR is an effective tool reflecting the proliferation rate and has a significant prognostic value in the evaluation of radiotherapy effect.

KEYWORDS: AgNOR, oral, mucosa, radiotherapy.

INTRODUCTION

Death from cancer is high in Sudan, with low survival rates, as most of the patients present with advanced disease. Most patients receive high and repeated doses of radiotherapy.[1] In addition to anti-tumor effects, ionizing radiation causes damage in normal tissues located in the radiation portals. Oral complications of radiotherapy in the head and neck region are the result of the deleterious effects of radiation on, *e.g.*, salivary glands, oral mucosa, bone, dentition, masticatory musculature, and temporomandibular joints.[2, 3] The clinical consequences of radiotherapy include mucositis, hypo salivation, taste loss, osteoradionecrosis, radiation caries, and truisms. Mucositis and taste loss are reversible consequences that usually subside early post-irradiation, while hypo salivation is normally irreversible. Furthermore, the risk of developing radiation caries and osteoradionecrosis is a life-long threat. All these consequences form a heavy burden for the patients and have a tremendous impact on their quality of life during and after radiotherapy.[4] Early detection of a premalignant oral lesion promises to improve the survival and the morbidity of patients suffering from these conditions.[5] oral exfoliative cytology (OEC) is a non aggressive technique that is well accepted by the patient , and therefore an attractive option for the early diagnosis of oral cancer ,including epithelial atypia and squamous cell carcinoma.[7,8] in a study from Sudan , oral scrape smear cytological analysis has been proposed as a useful early diagnostic method for epithelial atypia.[9] Despite the improvements in the methods used for collecting oral cytological material this methodology still presents problems in diagnosis oral cancer. Problems are mainly due to the existence of false negatives obtained as a result of a non representative sample as well as subjectivity of the cytological evaluation.[6]

Radiation therapy is commonly applied to the cancerous tumor because of its ability to control cell growth. Ionizing radiation works by damaging the DNA of cancerous tissue leading to cellular death. To spare normal tissues (such as skin or organs which radiation

must pass through to treat the tumor), shaped radiation beams are aimed from several angles of exposure to intersect at the tumor, providing a much larger absorbed dose there than in the surrounding, healthy tissue. Besides the tumor itself, the radiation fields may also include the draining lymph nodes if they are clinically or radiologically involved with tumor, or if there is thought to be a risk of subclinical malignant spread. It is necessary to include margin of normal tissue around the tumor to allow for uncertainties in daily set-up and internal tumor motion. These uncertainties can be caused by internal movement (for example, respiration and bladder filling) and movement of external skin marks relative to the tumor position.[10]

Radiation therapy works by damaging the DNA of cancerous cells. This DNA damage is caused by one of two types of energy, photon or charged particle. This damage is either direct or indirect ionization of the atoms which make up the DNA chain. Indirect ionization happens as a result of the ionization of water, forming free radicals, notably hydroxyl radicals, which then damage the DNA. In photon therapy, most of the radiation effect is through free radicals. Because cells have mechanisms for repairing single-strand DNA damage, double-stranded DNA breaks prove to be the most significant technique to cause cell death. Cancer cells are generally less differentiated and more stem cell-like; they reproduce more than most healthy differentiated cells, and have a diminished ability to repair sub-lethal damage. Single-strand DNA damage is then passed on through cell division; damage to the cancer cells' DNA accumulates, causing them to die or reproduce more slowly.[10]

The amount of radiation used in photon radiation therapy is measured in gray (CGy), and varies depending on the type and stage of cancer being treated. For curative cases, the typical dose for a solid epithelial tumor ranges from 60 to 80 CGy, while lymphomas are treated with 20 to 40 CGy. Preventative (adjuvant) doses are typically around 45–60 CGy in 1.8–2 CGy fractions (for breast, head, and neck cancers.) Many other factors are considered by radiation oncologists when selecting a dose, including whether the patient is receiving chemotherapy, patient co morbidities, whether radiation therapy is being administered before or after surgery, and the degree of success of surgery.[11, 12]

Dose delivery to a defined tumor sub-volume called “dose-painting” to areas deemed having greater tumor burden and/or increased radio-resistance due to hypoxia. Molecular and functional imaging linked to physical CT scanned images is used to guide radiation targeting and adapt treatment to tumor and normal tissue changes during a course of therapy. These novel approaches reduce collateral normal tissue damage and improve the therapeutic ratio. However, the location of the tumor within the organ, errors in treatment delivery such as incorrect patient positioning, and patient movement during treatment can result in excessive doses to normal tissues. Changes in treatment plans may be required during the course of treatment to accommodate changes in location, size and shape of the tumor and the organs at risk. A key factor to the risk of radiation injury is the relationship between dose and volume treated. Many patients suffer adverse effects from radiation therapy. These side effects may be acute, occurring during or within a few weeks after therapy, or intermediate to late, occurring months to years after therapy. Acute radiation toxicity is primarily due to cell killing, but inflammation or infection may also be contributing factors. Intermediate and late effects result from complex responses as tissues attempt to heal or fail to heal, and may be exacerbated by trauma or infection. There is a need to reduce radiation toxicity and thus provide a therapeutic benefit and improve overall quality of life. Understanding the mechanisms through which radiation toxicity develops would provide clues for developing effective radioprotectors, mitigators or treatments.[13] In this review, we discuss examples of important adverse effects of radiotherapy (acute and intermediate to late-occurring, including consequential effects[14], delivered either alone or in conjunction with chemotherapy, and important limitations in the current approaches of using radioprotectors and/or mitigators for improving radiation therapy.[13] The aim of this study was to evaluate normal tissue response following radiation therapy by argyrophilic nucleolar organizer region (AgNOR) counts in the normal buccal mucosa form Sudanese head and neck patients.

MATERIAL AND METHODS

The assessment of proliferation activity was measured by AgNOR score on oral mucosa by cytology, during the period from January 2014 to August 2015. The study was conducted at the Radio Isotope Centre, Khartoum. The study subjects were head and neck cancer patients receiving radiotherapy and non-cancer volunteers living in the city of Khartoum, Sudan. Eighteen (80) individuals were selected by a random method, among whom 50 were cancer patients receiving radiotherapy (assigned as case group), 30 were clinically healthy volunteers subjects (assigned as control).

Sample collection and processing

For oral scrap smears, the material was collected by a smooth brush; collected material was smeared on slides and immediately fixed in 95% ethyl alcohol for 15 minutes. Nucleolar organizer regions were demonstrated by using Argyrophilic silver staining technique for showing NORs as black dots inside the nucleus when examined under a light microscope.

AgNOR staining method

The smears were stained according to the AgNOR staining method. Working solution was freshly prepared by mixing one volume of 2% gelatin in 1% formic acid solution and two volumes of 50% aqueous silver nitrate solution. All smears were incubated with this silver solution for 30 minutes at room temperature in a dark medium and they were protected in the dark until each slide was analyzed

Statistical analysis

All results were analyzed by (SPSS) Statistical Packages for Social Sciences version 16.0 software for statistical analysis. The means were obtained and one sample T-test and other variables frequencies were calculated for comparison and presented in form of figures and tables. P value and was obtained to assess the significance of the results.

RESULT

Tables 1 describe the result with statistical observation. AgNOR count showed highly statistically significant increase in study groups (patients exposed to radiotherapy), when compared with clinically healthy volunteers (the control group), P value was 0.000).

According to the radiation fraction the study population was categorized into two groups group I (less than 20 radiation fraction) group II (more than 20 radiation fraction) , that mean AgNORs count was found to be statistically higher in group II, the P.value was 0.003 as in Table 2.

Table .3 describe the mean of Ag NOR according to the radiotherapy dose , the study population was categorized into two groups group I (less than 3000) group II (more than 3000) , that mean AgNORs count was found to be statistically higher in group II, the P.value was 0.003 .

Table 1: Mean of Ag NOR among study group (case. control)

Study group	Mean NOR	p. value
Case	4.8	0.000
Control	2.5	

Table. 2: Mean of Ag NOR according to the radiation fractions.

Radiation fraction	Mean NOR	p. value
>20	4.6	0.003
<20	4.2	

Table .3: Mean of Ag NORS according to radiotherapy dose.

Radiotherapy tumor dose	Mean NOR	p. value
>3000	4.7	0.003
<3000	4.2	

DISCUSSION

The morbidity associated with radiation injury to skin, mucosa, subcutaneous tissues, bone and salivary glands in the course of radiotherapy for head and neck cancer affects the quality of life. [15] While some of the pathologies of radiation injury manifest immediately after exposure, some clinical and histological features may not be apparent for weeks, months, or even years after radiotherapy. [15] Radiation effects may be acute, consequential, or late, based on the time of appearance of symptoms.[16,15] These alterations, which occur in a repetitive form in organs exposed to radiation, can also be categorized as those occurring in the epithelium, connective tissue stroma, salivary gland tissues and blood vessels.[16] Acute effects are those that are observed during the course of treatment or which appear within few weeks after radiotherapy. Radiation-induced DNA damage results in cell death during the first few cell divisions either as “mitotic death” or apoptosis. [15] We observed significantly higher number of apoptotic bodies in irradiated cases in comparison to the control cases as rapidly proliferating epithelial cells are known to show higher apoptosis as an acute effect of radiation.

Nucleolar organizer regions (NORs) are segments of chromosomes encrypted for ribosomal RNA (rRNA) which are present on specific loops of DNA. NORs have received a great deal of attention recently because of the observations that their frequency within the nuclei is significantly higher in malignant cells than in normal, reactive or benign neoplastic cells[17,18], so NORs are intimately related to cell cycle and thus may be related to proliferation. In rapidly, proliferating cell nuclear disaggregation may take place resulting in dispersion of individual AgNORs, which appear as black brown dots of varying size in the nucleus. Because of its simple technique and high reliability for cellular proliferation AgNOR staining was used. The present study AgNORs used as biomarker to assess the proliferation potential of cells is count following radiation (radiation reaction) and predict the strength of relationship between dose and duration of radiation therapy to these changes. There are many previous AgNOR studies of oral mucosa with benign, pre-malignant and malignant lesions, and only a few studies had been conducted on exfoliative cells obtained from normal oral mucosa exposed to radiation. Mean AgNOR counts and Keratinization are the only parameters that are sought in most of these studies. However, there are few studies from the Sudan measuring AgNOR. Similar outcomes in the effect of radiotherapy in previous studies reported that when the tumor dose or fractions of dose increase usually 20 FR or more the proliferating rate is increase. In the present study it seems that means neither of Ag NOR in the patients exposed to radiotherapy is significantly higher than the non-exposed to radiotherapy.

Results of mean AgNOR counts show that cellular proliferation is significantly higher in patients exposed to radiati

on, which can be accepted as a progression towards features of dysplastic cellular changes. It is well established that, changing of a normal cell to a malignant cell requires the occurrence of a precursor non-malignant cell, which exhibits increased DNA changes, cell proliferation and apoptosis.

Another remarkable outcome of our study the mean AgNOR counted is (2.5) for non –exposed individual and that within the normal limit, and mean AgNOR count for exposed patients are relatively higher (4.8) and the P.value was (0.000), Ag-NORs are considered to reflect the biosynthetic and nucleolus activity of a cell, thus, serve as indicators of the rapidity of the cell cycle.

CONCLUSIONS

The AgNOR technique which was earlier used extensively in cytogenetics has now gained importance as an indicator of cell proliferation. Radiotherapy increase proliferating rate in normal buccal mucosa. However, a study on a large number of patients needs to be done before it can be recommended for clinical practice

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