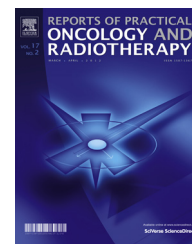




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Original research article

Dependence of micronuclei assay on the depth of absorbed dose



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ABSTRACT

Aim: The purpose of the present study is to investigate the dependence of micronuclei response on the depth of absorbed dose.

Background: One of the most common cytogenetic methods used for radiation dosimetry is micronuclei (MN). Being less complex and faster than other methods are two remarkable advantages of the MN method which make it suitable for monitoring of population. In biological dosimetry based on the micronuclei method, the investigation into the dependence of response on the depth in which dose is absorbed is significant, though has received less attention so far.

Materials and methods: Blood samples were poured in separate vials to be irradiated at different depths using a linear accelerator system.

Results: According to the results, MN, as a function of the absorbed dose, had the best fitness with the linear–quadratic model at all depths. Furthermore, the results showed the dependence of MN response on the depth of absorbed dose. For doses up to 2 Gy, the maximum difference from the reference depth of 1.5 cm was related to the depth of 10 cm; however, by increasing the absorbed dose, the response associated with the depth of 20 cm showed the maximum deviation from the reference depth.

Conclusions: Consequently, it is necessary to apply a correction factor to the biological dosimetry. The correction factor is dependent on the depth and the absorbed dose.

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1. Background

There is considerable evidence that ionizing radiation affects cells directly or indirectly.¹ In general, these effects increase the frequency of apoptosis, micronucleation, DNA strand breaks and mutations, altered levels or activity of regulatory proteins and enzymes, reduced clonogenic efficiency, and oncogenic transformation.² Cytogenetic biodosimetry is a methodology based on the measurement of radiation induced biological effects visible at the cytogenetic level in order to correlate them with the dose of radiation.³ Since the mid-1960s, biological dosimetry using chromosome damage biomarkers has been a valuable dose assessment method, especially when there are difficulties in interpreting data given by physical dosimetry or in cases of radiation overexposure with or without physical dosimetry data.⁴ Nowadays, chromosomal aberrations in Peripheral Blood Lymphocytes (PBLs) is being studied for the dosimetry of ionizing radiations.⁵ Metaphase analysis,⁶⁻⁸ G-banding technique,⁹ Premature Chromosome Condensation (PCC),¹⁰ FISH (fluorescence in situ hybridization)¹¹ and micronuclei are amongst the conventional cytogenetic methods used for the dosimetry of radiation. Cytokinesis-block micronucleus (CBMN) assay was developed by Fenech and Morely in 1985.¹² MN method is less complex and faster than other methods which makes it suitable for monitoring large populations.^{13,14}

Micronucleus incorporates in cytoplasm in the form of a small nucleus along with the main nucleus. This small nucleus originates from centromere-free chromatin elements (acentric fragment) or lagging chromosomes, which are not transferred to the daughter nucleus during mitosis, around which a nuclear coating is formed in telophase.¹² In this method, the cytokinesis of the cells that have accomplished a mitosis through Cytochalasin B stops; therefore, they are easily identified based on their bi-nucleate appearance.¹⁵ Micronuclei are usually counted in peripheral blood lymphocytes which have accomplished a mitosis due to the stimulation of phytohemagglutinin (PHA).¹⁶ With regard to the fact that irradiation is a strong clastogenic factor and, hence, a strong induction of micronuclei, it has been proven that CBMN is a reliable, valid and standard method to determine occupational, medical or accidental exposure to ray in the field of radiation biology. For instance, a study on chromosomal damage to people who were occupationally exposed to low-levels of ionizing radiations showed that the frequency of MN in employees was higher than in the control group.¹⁷ In another study on the applicability of the micronuclei method to estimate the radiation dose in patients undergoing radiotherapy, the results indicated that MN assay, up to 2 Gy, was in a favorable agreement with the results of *in vitro*. Nevertheless, the frequency of MN in patients undergoing radiotherapy was lower than the *in vitro* conditions in higher doses.¹⁸

The direct and indirect effect of irradiations on the Song et al.¹⁹ studied the extent of damage caused by radiation to lymphocytes in *in vivo* conditions after irradiation with alpha particles. They found that micronuclei assay can be used as a biological dosimeter for Internal Alpha Immunotherapy. In a survey on 10 workers who had been irradiated with a ⁶⁰Co source, the estimated dose by MN method was in an

agreement with the measured dose by the dicentric method.²⁰ The accuracy of the MN method is also to the extent that illustrates the irradiation effect on patients undergoing radiotherapy at different stages of treatment.²¹ They also found that the frequency of MN in the patients with cancer before radiotherapy is higher than healthy control groups. During the middle stages of radiotherapy, the frequency of MN was more than twice as much as the frequency of the pre-treatment stage while at the post-treatment stage, MN increased so that it was completely different from the pre-treatment and in-treatment stages.²¹ Taghavi-Dehaghani et al. studied 26 patients with breast cancer to assess their sensitivity to gamma rays. The results showed that the frequency of MN, induced by radiation, was significantly higher in patients with early reactions than in patients with late reactions after being radiated with the dose of 4 Gy.²²

2. Aim

The investigation into the dependence of biological dosimetry response on the depth of the absorbed dose is of utmost importance, though has received little attention so far. In most cases of MN application including estimation of the absorbed dose in nuclear accidents and chromosomal damages to patients undergoing radiotherapy,²³⁻²⁶ the receiving point of the absorbed dose is located at different depths of the body. Nevertheless, the calibration curve is obtained at a specific depth (maximum depth); therefore, it is necessary to investigate the dependence of MN response on the depth of the absorbed dose. The purpose of the present study is to investigate the dependence of micronuclei response on the depth of the absorbed dose.

3. Materials and methods

3.1. Sampling

For the purpose of blood sampling, 25cc of fresh blood was obtained by venipuncture from a healthy non-smoker 29-year-old female. However, it has been proved that smoking had no significant effect on the micronucleus yields.²⁷ In order to neutralize the effect of individual differences in the results, all samples were collected from one individual.²⁸⁻³¹ The blood samples were poured in separate heparin vials, 1 cc each, under laminar hood in sterilized conditions. One of the vials was used as a non-irradiated/control and the other 20 vials were divided into four pentamerous groups. In order to investigate the effect of depth on the same dose, 4 separate vials were considered.

3.2. Irradiation

The pentamerous groups of the blood-containing vials, in four similar containers, were placed at the depths of 1.5 cm, 5 cm, 10 cm and 20 cm in water. The dimensions of the containers were chosen big enough to consider the effect of radiation scattering (length: 35 cm, width: 23 cm, height: 24 cm).

The source surface distance (SSD) was 100 cm for each four groups. Also, the Field size was 10 cm × 10 cm for each four

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