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Original article

Investigating micronucleus assay applicability for prediction of normal tissue intrinsic radiosensitivity in gynecological cancer patients

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ABSTRACT

Background: Pelvic organs morbidity after irradiation of cancer patients remains a major problem although new technologies have been developed and implemented. A relatively simple and suitable method for routine clinical practice is needed for preliminary assessment of normal tissue intrinsic radiosensitivity. The micronucleus test (MNT) determines the frequency of the radiation induced micronuclei (MN) in peripheral blood lymphocytes, which could serve as an indicator of intrinsic cell radiosensitivity.

Aim: To investigate a possible use of the micronucleus test (MNT) for acute radiation morbidity prediction in gynecological cancer patients.

Materials and methods: Forty gynecological cancer patients received 50 Gy conventional external pelvic irradiation after radical surgery. A four-field "box" technique was applied with 2D planning. The control group included 10 healthy females.

Acute normal tissue reactions were graded according to NCI CTCAE v.3.0. From all reaction scores, the highest score named "summarized clinical radiosensitivity" was selected for a statistical analysis.

MNT was performed before and after in vitro irradiation with 1.5 Gy. The mean radiation induced frequency of micronuclei per 1000 binucleated cells (MN/1000) and lymphocytes containing micronuclei per 1000 binucleated cells (cells with MN/1000) were evaluated for both patients and controls.

An arbitrary cut off value was created to pick up a radiosensitive individual: the mean value of spontaneous frequency of cells with MN/1000 ± 2SD, found in the control group.

Both mean spontaneous frequency of cells with MN/1000 and MN/1000 were registered to be significantly higher in cancer patients compared to the control group ($t = 2.46$, $p = 0.02$ and $t = 2.51$, $p = 0.02$). No statistical difference was registered when comparing radiation induced MN frequencies between those groups.

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Eighty percent (32) of patients developed grade 2 summarized clinical radiosensitivity, with great variations in MNT parameters. Only three patients with grade 2 "summarized clinical radiosensitivity" had values of cells with MN/1000 above the chosen radiosensitivity threshold.

Conclusion: The present study was not able to confirm in vitro MNT applicability for radiosensitivity prediction in pelvic irradiation.

1. Background

The contemporary radiotherapy aims at achieving local tumor control with contribution to the overall survival with a good quality of life.

The effects in early responding tissues are a very important issue in radiotherapy.[1,2]

Normal tissue intrinsic radiosensitivity is the main limiting factor for total irradiation dose in clinical practice defining a normal tissue tolerance to irradiation.[3–5]

Pelvic organs morbidity after irradiation of cancer patients remains a major problem, although new technologies have been developed and implemented.

A relatively simple and suitable method for routine clinical practice is needed for preliminary assessment of normal tissue intrinsic radiosensitivity. It would allow individualization of radiotherapy dose applying higher dose in radioresistant patients to achieve a potentially better local tumor control of up to 20%.[1,6]

Multiple studies have shown that normal tissue morbidity after irradiation in cancer patients correlates with radiosensitivity of skin fibroblasts and peripheral blood lymphocytes of these patients. This finding stimulates the search of prognostic criteria for evaluating the intrinsic individual cellular radiosensitivity.[1,3,7]

The micronucleus test (MNT) determines the frequency of the radiation induced micronuclei (MN) in peripheral blood lymphocytes, which could serve as an indicator of intrinsic cell radiosensitivity.

After mutagen attack, MN in interphase cells are formed by mitotic loss of acentric fragments or chromosomes which are not incorporated in the daughter cell nuclei.[8] Because of its reliability and easy performance, MNT could be a promising method for evaluating normal tissue morbidity in cancer patients during radiotherapy[1] with results yielded in less than 2 weeks.

The in vitro radiation response of the peripheral blood lymphocytes correlates with the in vivo response.[7,9] Human lymphocytes from peripheral blood could be easier to collect with results faster to obtain. Besides, the method is easily reproducible, which makes it a preferred modality in assessing the normal tissue radiosensitivity.[1,8,10] Some studies on cancer patients show the association of increased radiosensitivity with side effects.[1,10] Study of prostate cancer patients showed association between the frequencies of ex vivo induced MN before the beginning of radiotherapy and gastrointestinal and genitourinary side effects.[11] So, there are convincing and logical evidence for using radiation changes in the peripheral blood lymphocytes in the Go phase as a quick prognostic biomarker for normal tissue morbidity.

In the search for the best model for clinical radiosensitivity testing, cervical and endometrial cancers were found to be very appropriate cancer localizations.

2. Aim

In an effort to find a prognostic method for normal tissue intrinsic radiosensitivity for patients receiving pelvic irradiation, we used the micronucleus test (MNT).

We investigated if patients with high frequency of in vitro radiation induced micronuclei in peripheral blood lymphocytes are at high risk for developing more severe early normal tissue adverse events after pelvic irradiation.

3. Materials and methods

3.1. *Patients, treatment and control group*

The study included 40 females – 23 with cervical cancer and 17 with endometrial cancer in early stage with no lymph node metastasis present. They were recruited for the period of 2006–2008. The mean age was 57.7 ± 13.5 (range 31–75 years). They all received adjuvant external pelvic radiotherapy after radical gynecological surgery with a cobalt machine. The total dose delivered was 50 Gy with 2 Gy daily fractions. A four-field "box" technique was applied. The 2D planning target volume (PTV) inevitably included 2/3 of the vagina, 50% bladder and segments from the rectum and small intestine.

We investigated both cervical and endometrial cancer patients together because the irradiation technique and the PTV we applied were the same according to the treatment protocol of our department for postoperative radiotherapy in early stage for these cancer localizations.

The patients included had no history of previous toxic treatment or exposure.

An informed consent was obtained from every patient before the start of treatment and the study was approved by the local ethic committee.

A control group of 10 healthy females was used. Mean controls' age was 48.9 ± 12.6 (range 30–66). Our control group was found to be representative when compared with a referent control group of 57 healthy donors of the same Bulgarian laboratory and we stopped recruiting more healthy donors.[12]