

LONG-TERM FOLLOW-UP STUDY OF GENOME DAMAGE ELIMINATION IN PATIENTS WITH TESTICULAR SEMINOMA EXPOSED TO IONISING RADIATION DURING RADIOTHERAPY

Marija GAMULIN¹, Jelena KATIĆ², Mirta MILIĆ²,
Mislav GRGIĆ¹, and Aleksandra FUČIĆ²

Clinical Hospital Centre Zagreb¹, Institute for Medical Research and Occupational Health², Zagreb, Croatia

Received in January 2011
CrossChecked in February 2011
Accepted in February 2011

The rate of genome damage elimination after therapeutic exposure to ionising radiation was estimated in stage I testicular seminoma patients monitored over a seven-year follow-up. DNA damage elimination in peripheral lymphocytes of ten subjects was analysed by the chromosome aberration assay. Seven years after the end of radiotherapy, significantly increased frequency of ring and dicentric chromosomes was still detected in comparison with baseline values. These results indicate the induction of genome instability. Long-term follow-up studies of cancer patients after radiotherapy could give us valuable information on the rate of genome damage elimination after exposure to ionising radiation and about the duration and manifestation of genome instability. This may be used in health risk assessment related to the possible development of secondary neoplasia. Studies such as this could have a great value both for oncology and radiation protection management protocols, especially after accidental overexposures.

KEY WORDS: *chromosome aberration assay, dicentric chromosome, follow-up, genome instability, ionising radiation, secondary cancer*

The knowledge of genome damage elimination in humans after the exposure to ionising radiation is limited to follow-up studies of nuclear bomb or nuclear accident victims, such as Hiroshima/Nagasaki or Chernobyl (1, 2). It is also possible to follow up cancer patients decades after they have been exposed to ionising radiation through radiotherapy. Investigation of genome damage elimination after radiotherapy is of great interest for oncology (assessment of secondary cancer risk), health risk management after nuclear accidents, and for radiation protection.

Different biomarkers have been established for DNA damage assessment. Among them, chromosome aberration assay (CA) is a valuable biodosimetry

method for measuring exposure effects to xenobiotics and a good predictor of cancer risk at group level (3). Although measurement of translocation frequency can give a better insight into the permanent genome damage (4), the occurrence of clones, and possible precancerous states, this method is much less used than CA analysis, as it is costly and time-consuming.

This study included patients treated with ionising radiation for testicular seminoma. Worldwide, the highest incidence of testicular cancer is detected in young men aged 15 to 34 years (5). Young age, high survival, and long latency for secondary cancer development, which may originate from genome

damage caused by radiotherapy, makes this group a valuable source of data on the dynamics of the formation and elimination of chromosome aberrations as exposure dose and dynamics are known.

In a recent study by Hille et al. (6) blood samples were collected from patients before radiotherapy in order to detect possible genome instability related to prostate cancer. In the present study, despite the fact that the group of testicular seminoma patients was exposed to ionising radiation for diagnostic purposes before therapy (chest X-rays and CT of the abdomen and pelvis), the baseline values of CA frequencies did not differ from those reported in reference male population exposed to ionising radiation for diagnostic purposes only (7, 8). During and after radiotherapy, all testicular seminoma patients showed a significantly increased frequency of CAs such as dicentric, polycentric, and ring chromosomes. A statistically increased frequency of CA was observed one year after the received radiotherapy (7, 9).

The aim of this study was to evaluate whether seven years after radiotherapy the frequency of CAs would return to the baseline values.

SUBJECTS AND METHODS

Ten patients with clinical stage I testicular seminoma were identified from hospital records and invited to participate in this long-term follow-up study.

Their median age was 35 years (23 to 49 years) at the time of diagnosis, as described earlier by Gamulin (9). None was exposed to physical or chemical agents in their living or occupational environment. Three patients smoked. Before and during radiotherapy, the patients were not taking any drugs known to have genotoxic effects. After the tumour had been removed, they received external beam radiotherapy in the total dose of 25 Gy over 16 days (9). They were taken blood samples for the analysis of genome damage before radiotherapy (baseline values), immediately after the first irradiation, in the middle of therapy, immediately after the last irradiation, six months after radiotherapy, one year after radiotherapy, and seven years after radiotherapy as part of this follow up.

The CA assay was performed and the slides analysed according to the guidelines by the International Atomic Energy Agency (IAEA) (10). For each person, 500 metaphases were analysed under light microscope (magnification 1000x). Results were pooled and presented at group level.

The study was approved by the National Ethics Committee and was conducted following the principles of the Helsinki Declaration. All subjects were informed about the aim of the study and gave their written consent. The results of the study were reported to the subjects.

For statistical analysis we used Statistica 7.0 (StatSoft, Tulsa, USA). As Shapiro-Wilks' W-test showed that the distribution of variables was not

Table 1 Frequencies of CAs in peripheral blood lymphocytes of testicular seminoma patients. Comparison between the latest measurement and measurements obtained one year after radiotherapy and at baseline

| Sampling time | | Chromatid breaks | Chromosomal breaks | Acentric fragments | Dicentric chromosomes | Polycentric chromosomes | Ring chromosomes |
|---------------------------------|---------------|------------------|---|---|---|-------------------------|------------------|
| Seven years after radiotherapy | Mean \pm SD | 0.73 \pm 0.17 | 0.02\pm0.02^a | 0.69\pm0.13^b | 0.23\pm0.06^a | 0 | 0.15 \pm 0.06 |
| | Median | 0.6 | 0 | 0.7 | 0.2 | 0 | 0.1 |
| | Range | 0 to 1.8 | 0 to 0.2 | 0 to 1.2 | 0 to 0.5 | | 0 to 0.5 |
| One year after radiotherapy (9) | Mean \pm SD | 1.00 \pm 0.54 | 1.10 \pm 0.60 | 11.00 \pm 2.36 | 4.40 \pm 1.26 | 0.30 \pm 0.15 | 0.20 \pm 0.13 |
| | Median | 0 | 0 | 10 | 4.5 | 0 | 0 |
| | Range | 0 to 5 | 0 to 6 | 3 to 21 | 0 to 12 | 0 to 1 | 0 to 1 |
| Baseline (9) | Mean \pm SD | 0.70 \pm 0.30 | 0.70 \pm 0.21 | 0.50 \pm 0.22 | 0 | 0 | 0 |
| | Median | 0 | 1 | 0 | 0 | 0 | 0 |
| | Range | 0 to 2 | 0 to 2 | 0 to 2 | | | |

Results are expressed as group mean \pm SD, median and range per 100 metaphases. Statistical significance was tested using non-parametric Mann-Whitney U-test. Significantly increased values are printed in bold.

^a Year 7 vs baseline, $p < 0.05$

^b Year 7 vs year 1, $p < 0.001$

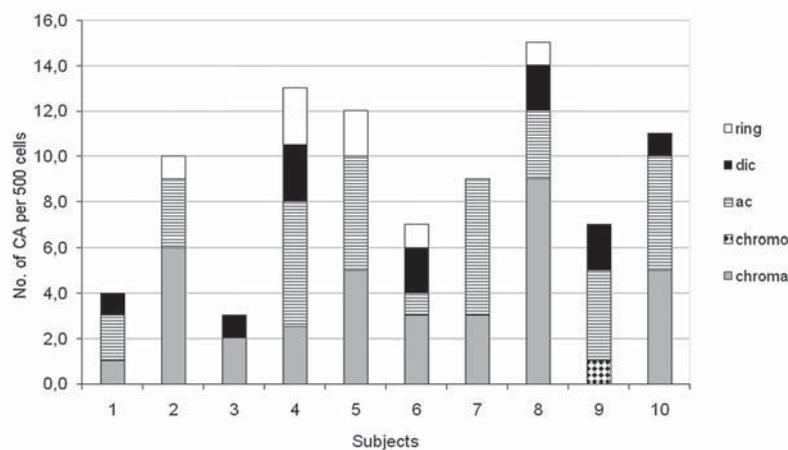


Figure 1 Frequency of CAs: ring chromosomes, dicentric chromosomes (*dic*), acentric fragments (*ac*), chromosome (*chromo*) breaks, and chromatid (*chroma*) breaks in peripheral blood lymphocytes of testicular seminoma patients ($N=10$) seven years after radiotherapy

normal, we used nonparametric Mann-Whitney U-test to test the differences in the frequencies of different types of CAs between sampling times. The threshold of statistical significance was $p<0.05$.

RESULTS

Figure 1 shows individual CA frequencies found in peripheral blood lymphocytes of testicular seminoma patients seven years after radiotherapy. The type and frequency of CAs across the follow-up window are shown in Table 1. In respect to previous sampling (9), a significant decrease in chromosome breaks and acentric fragments was detected seven years after radiotherapy. Chromatid and chromosome breaks and acentric fragments reached baseline values (before the radiotherapy).

The number of dicentric and ring chromosomes remained significantly higher than baseline. Polycentric chromosomes were not detected. Multiaberrant cells were also detected. An example of a metaphase with a dicentric chromosome and an acentric fragment is shown in Figure 2.

We observed a similar pattern of formation and elimination of acentric fragments and dicentric chromosomes during and after radiotherapy (Figure 3).

DISCUSSION

The response of mammalian cells to low doses of ionising radiation is complex and can be observed in a form of genome damage, aneuploidy, bystander

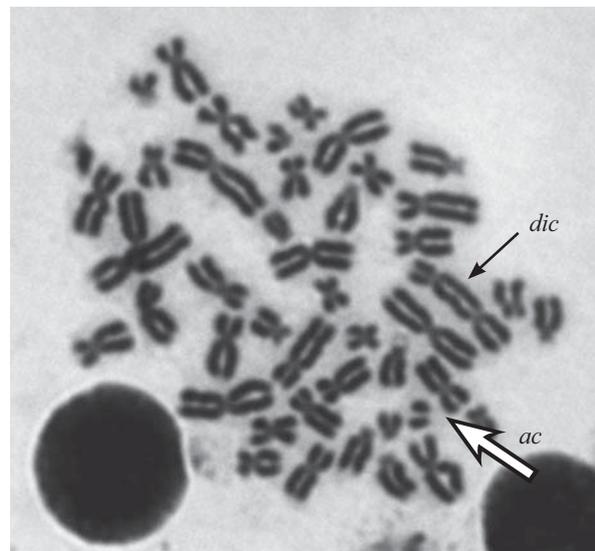


Figure 2 Metaphase with dicentric chromosome (*dic*) and acentric fragment (*ac*)

effects, methylation disturbances, and other processes that can increase health risk (11). In addition to dose rate, the efficiency of DNA repair mechanisms also depends on age and interindividual differences in radiosensitivity (12-14). In order to gain an insight into diverse biological effects of exposure to ionising radiation, different biodosimetry methods can be used, including the CA assay. Despite the advantages of the newly introduced “-omics” techniques, the CA assay still remains a standard method for the evaluation of genome damage after exposure to radiation (10). This assay is also a good predictor of cancer risk (3).

Preparedness for nuclear accidents, war, or terrorist attacks requires development of treatment protocols for victims immediately after exposure and for

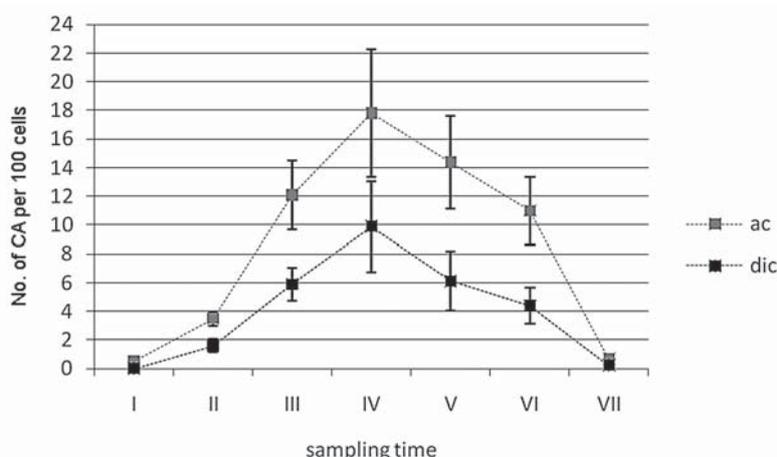


Figure 3 The frequency of chromosome aberrations (CA): acentric fragments (ac) and dicentric chromosomes (dic) in peripheral blood lymphocytes of testicular carcinoma survivors ($N=10$). Results are shown as group mean values \pm SD per 100 metaphases. Blood samples were taken before radiation therapy (baseline, I), immediately after first irradiation (II) in the middle of therapy (III), immediately after the last irradiation (IV), six months after radiotherapy (V), one year after radiotherapy (VI), and seven years after radiotherapy (VII). Data for blood samplings I-VI are taken from the PhD thesis by Gamulin (9).

survivors years after exposure. These protocols could rely on data on the dynamics of genome damage elimination after exposure to ionising radiation obtained in controlled conditions in cancer survivors who were given radiotherapy.

Our study recruited patients treated by ionising radiation after surgical removal of testicular seminoma. This type of cancer affects mainly the young male population, which means that the follow-up can take several decades. Follow-up this long is also possible due to the high survival rate of 96 % for clinical stages I-II of the disease (15). Our study did not show spontaneous genome instability before the radiotherapy, which is in line with similar studies (7, 16, 17). The total dose of 25 Gy delivered over 16 days caused a significant increase in CAs throughout radiotherapy. One year after the therapy, frequencies of dicentric and acentric chromosomes remained significantly higher from values observed before therapy (7).

Seven years after radiotherapy, the frequency of chromatid and chromosome breaks and acentric fragments returned to baseline, but the frequency of dicentric and ring chromosomes was still significantly higher. It is also interesting that acentric fragments followed the same pattern of formation and elimination as dicentric chromosomes, and in terms of biodosimetry are as informative.

According to available literature, the increased frequencies of unstable CAs usually withdraw within a year after accidental exposure to low-dose ionising radiation (18). This study is the first to show chromosomal instability long after the prolonged

exposure to a cumulative therapeutic radiation dose of 25 Gy. Genome instability and the multiaberrant cells reported in this study are hallmarks of neoplastic cells (19).

Long-term follow-up studies of subjects exposed to ionising radiation with the CA assay can provide useful hints in the understanding of the biology of genetic damage caused by ionising radiation exposure. Delayed genome instability may be relevant in medical surveillance of radiation victims and personalised cancer therapies.

Acknowledgment

This study was partly supported by the Croatian Ministry of Science, Education and Sports.

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Sažetak**VIŠEGODIŠNJA STUDIJA PRAĆENJA SMANJENJA OŠTEĆENJA GENOMA KOD BOLESNIKA SA SEMINOMOM TESTISA IZLOŽENIH IONIZIRAJUĆEM ZRAČENJU TIJEKOM RADIOTERAPIJE**

U svrhu procjene uklanjanja oštećenja genoma nakon izlaganja ionizirajućem zračenju, sedam godina nakon radioterapije kod deset ispitanika sa seminomom testisa stadija I analizirana je učestalost strukturnih aberacija kromosoma u limfocitima periferne krvi. Sedam godina nakon radioterapije zabilježena je značajno povećana učestalost dicentričnih i prstenastih kromosoma u odnosu na kontrolne vrijednosti, što upućuje na moguću pojavu odgođene nestabilnosti genoma. Višegodišnje praćenje onkoloških pacijenata nakon radioterapije omogućuje skupljanje podataka o ritmu smanjenja oštećenja genoma i izazivanju genomske nestabilnosti (njezino trajanje i značajke) nužnih u procjeni zdravstvenog rizika, kao što su sekundarne neoplazije. Rezultati ove studije veoma su vrijedni za onkologiju i u izradi preventivnih mjera i propisa iz domene zaštite od ionizirajućeg zračenja u medicini te slučajevima nuklearnog akcidenta.

KLJUČNE RIJEČI: *analiza kromosomskih aberacija, bicentrični kromosom, ionizirajuće zračenje, seminom, višegodišnje praćenje*

CORRESPONDING AUTHOR:

Marija Gamulin, MD, PhD
Clinical Hospital Centre Zagreb
Kišpatićeva 12, 10000 Zagreb, Croatia
E-mail: marija.gamulin@zg.t-com.hr