ORIGINAL ARTICLE

Biodosimetry of Persons Chronically Exposed to Low and Therapeutic Doses of Ionizing Radiation

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ABSTRACT

Dynamic changes of the chromosomal aberrations and the DNA damage were analyzed in individuals exposed to low and therapeutic doses of radiation. The investigation included 37 persons living in areas where the radioactive sources were discovered 10–12 years ago. It was established by biodosimetry methods that the examined persons had absorbed dose of 0.2–0.7 Gy or had increased number of chromosomal aberrations, though insufficient to determine a dose. Clinical examination, chromosomal analysis, and assay of DNA damage by the comet (single-cell gel electrophoresis) assay were carried out. There was no correlation between the doses received 10 years ago and the cytogenetic changes with clinical outcome. The effect of the local fractionated gamma-irradiation with doses of 40–70 Gy was studied in cancer patients with localized head and neck tumors. The study of chromosomal abnormalities, the DNA damages by the comet assay, and the micronuclei detection of the buccal cells revealed a statistically significant correlation between the initial cytogenetic indices in cancer patients and their dynamic changes during and after the radiation exposure. In addition, the correlation was detected between the initial cytogenetic parameters and the functional stage of red blood system. Our results allow us to conclude that there is a need for further research to estimate the individual radiation risk to optimize and individualize the subsequent medical management of radiotherapy.

Key words: Chromosome aberrations, DNA damage, irradiation, micronuclei, radiotherapy

Introduction

The study of the impact of ionizing radiation (IR) on biological objects began almost with the discovery of radiation. Immediately, besides its beneficial use, its destructive effects on living organisms, including human, were discovered and documented. Application of IR in various fields of human activity is progressively increasing in parallel with growing risk of biological effects. Exposure of humans to radiations may occur not only from atomic bomb, nuclear accidents, radiation contamination of environment, or human errors but also through routine diagnostic and therapeutic procedures. Many reports focus on low-dose effects as they do not cause clinical changes and appear only after a long time and more often, by way of cancer or associated diseases.[1-4] Being a strong mutagen, IR may cause changes in genome of the living organisms. Cytogenetic indices are excellent biomarkers for detection of the

effects of IR.[5,6] Estimation of the radiation dose is one of the most important steps for the treatment of victims of radiation accidents. Among different types of radiation-induced lesions, DNA double-strand breaks are considered the most relevant of the deleterious effects of IR. Comet assay, also known as single-cell gel electrophoresis assay, is a rapid and sensitive method which is employed in the detection of DNA double-strand breaks in individual cells. This assay can also be applied to determine the DNA repair efficiency, which involves the incision and subsequent rejoining of damaged strands. Therefore, this assay may provide important information regarding the molecular mechanisms that are responsible to counteract radiation impact.[7]

There has been significant increase in the use of IR for medical procedures both in diagnostics and treatment in recent times. Although the general radiobiological principles underlying external beam and radionuclide therapy are similar, there exist significant differences in the biophysical and radiobiological effects caused by these two types of radiations. Due to this,

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it is important to accurately assess the absorbed dose for the physician to have a real picture of changes in patients caused by irradiation using either external beam or radionuclide. Such information will help better management of the patient as well as its follow-up. The main contingent for investigation of radiation impact is professionals, being influenced due to their occupation, inhabitants of regions with increased radiation background,[8-12] and patients undergoing radiotherapy or multiple diagnostic procedures.[13-17] Here, we compared the response of human cells in the form of chromosomal and DNA damage dynamics to comparable radiation doses from whole-body or local irradiation.

Materials and Methods

Radiation contamination was detected in Georgia due to safety lapses in the regulation of the territorial military bases in the late 1990s, following the withdrawal of Soviet troops from Georgia. In 2002–2004 in two regions of Georgia, Daba Vaziani and Dedoplistskaro in the frame of ISTC Project G-564, it was established by cytogenetic investigations that 19 individuals had received radiation doses exceeding 0.2 Gy. Increased dicentric frequency exceeding the background was also observed in another group of 30 persons. However, exact dose was not established as it was <0.2 Gy. Hence, these individuals were included in the risk group but were not under any follow-up monitoring. In our project, we have examined 37 inhabitants from the risk group of two above-mentioned regions and 12 head and neck cancer patients undergoing radiotherapy after their informed consent. Blood and buccal cells were collected and subjected to detection of chromosomal aberrations, DNA strand breaks in lymphocytes, and yield of micronuclei in buccal cells. In each case, about 150–300 metaphases, 150–200 lymphocytes for DNA-comet assay, and 1000 buccal cells for micronuclei were analyzed. Biodosimetry was carried out with conventional dicentric chromosomal assay in the first mitosis of peripheral blood lymphocyte in 48–52-h cultures. According to standard method[6] and by our own calibration curve,[18] we estimate the absorbed dose of IR at 0.2 Gy or above. In addition, DNA strand break damage was investigated by comet assay. This method enables the detection of DNA strand breaks in cells.[19] For the detection of DNA damage through comet assay, blood leukocyte cells were suspended in low melting point agarose and embedded within a thin agarose gel on a microscope slide. Microgels were submersed in cell lysis buffer and were washed by deionized water. Later, slides were electrophoresed and stained with luminescent dyes (ethidium bromide or 4',6-diamidino-2-phenylindole) for light microscopic study.

For the cancer patients, we investigated the exfoliative cells of oral cavity, henceforth referred to as buccal cells, for micronuclei in buccal cells (MnB) and other nuclear anomalies. The yield of MnB was determined by Stich method.[20] Cells were scraped off from the inner surface of the cheek with wooden spatula. These cells were evenly distributed on the slides, dried, and fixed (3:1; ethyl alcohol: acetic acid). The preparations underwent weak acidic hydrolysis 1 N HCl at room temperature. After this, preparations were washed with distilled water and stained with

Schiff's reagent, followed by fast green. Light microscopy was used for the analysis.

To determine the possible physiological criteria of assessment of cancer risk and predictors of individual radiosensitivity, the functional state of the red blood system (RBS) was studied in cancer patients. The functional state of RBS was evaluated by specially developed method based on analysis of the dynamics of population spectrum of erythrocytes of peripheral blood (EPB) – EPB distribution according to their living resources.[21,22] As minimally sufficient set of parameters, describing living resources of EPB, the following two parameters were applied: spherulation degree (Q) and volume (V). The first parameter is a good approximation and could be viewed as a degree of the erythrocytes spherulation (Q), determined as the relation of cell volume (V) to the volume of sphere with the same surface area (S). The second parameter chosen was cell volume – V.

$$Q = \frac{V}{V_{SF}} = 6 \times \pi^{1/2} \times \frac{V}{S^{3/2}}$$

Spherulation degree characterizes cell deformability and along with the volume determines the probability of overcoming by them of a barrier of reticuloendothelial system.

In 12 cancer patients undergoing radiotherapy, biodosimetry was performed to determine the levels of chromosome aberrations and micronuclei and extent of DNA damage before and after first and last irradiations. Radiotherapy was performed on linear accelerator **(**6 MV photon energy) with three-dimensional conformal planning system 2 Gy/fraction (20–33 fractions) with a total 40–70 Gy.

Statistical analysis was performed by nonparametric statistic methods, Mann–Whitney U-test and Wilcoxon-matched pairs test.

Results

We have not been able to reexamine all individuals, living in areas where the radioactive sources were discovered 10–12 years ago and who were included in the risk group in 2004. This examined group included individuals who had received an estimated dose 0.2–0.7 Gy or had increased number of chromosomal aberrations, though insufficient to determine a dose. Out of 19 people subjected to dose estimation in 2004, eight (estimated doses 0.3, −0.5 Gy) died from various cancers. Some people changed their place of residence. We could reexamine nine persons who were subjected to dose estimation and also 28 inhabitants from the risk group. In total, 37 individuals from Daba Vaziani and Dedoplistskaro were studied.

Biodosimetry was carried out in all above-mentioned cases. In a 31-year-old man, whose previous established dose was 0.3 Gy, we found 3 dicentrics (0.01 per cell) in a 300 analyzed metaphases and stable chromosome aberrations (marker chromosomes). According to our calibration curve, the possible dose of exposure should be <0.2 Gy but more than our control

data (0.0015 ± 0.0006). Two dicentrics were found (0.008 per cell) in 250 metaphases of a 38-year-old man, a native resident of Daba Vaziani. We were interested in one patient with previously established dose of 0.7 Gy. This patient suffered from the first degree of disability. Clinical examination of this patient has revealed slight enlargement of lymph nodes, gastritis, colitis, and lymphocytosis. Two dicentric chromosomes without acentric fragments were detected in 200 metaphases. No stable aberrations were observed. In individuals who have been examined this year, no significant cytogenetic, clinical, or hematological disorders have been observed.

We have also investigated DNA damage by comet assay. We did not observe any significant difference in the DNA damage produced in the exposed residents compared to the unexposed individuals (8%–12%). Since the comet assay detects the DNA break breaks, it is possible that the breaks formed due to initial irradiation might have been eliminated from the system. All investigated individuals were examined by physician-oncologist, and peripheral blood tests were conducted. In some of them, anemia and lymphocytosis were detected. Other disorders in hemogram were not identified.

Cancer as a cause of death among the eight individuals deserves particular attention. Comparing the chronic irradiation of 10–12 years ago (0.2–0.7 Gy) and the current clinical outcome, differential response was seen in patients who received similar amounts of radiation.

The effect of local irradiation was studied in cancer patients with tumors of the same anatomical localization. There were two groups of radiotherapy patients with head and neck cancer. Six patients were in the first group (five males and one female, 50–65 years) with cancers of planocellulare of larynx[4] and nasopharynx,[2] first-stage local disease. In the second group, there were six patients with the same sex and age distribution, with the same localization of cancer, but with II–IV stages and local spread of disease. Two of them had metastases in local lymphatic nodes. In the first group, investigation before irradiation did not show any difference compared with our control data on all parameters. Even though the patients displayed cancer clinically, chromosomal abnormalities and DNA damage remained well within the baseline data determined for healthy unexposed individuals. However, following the first course of irradiation (as part of radiotherapy), all end-points studied showed significant increase with differential sensitivity among patients (MnB level 4.33 ± 0.99; amount of comets –26%–30%, chromosomal aberrations [acentric single and paired fragments] 0.02–0.05 per cell, number of dicentrics - 0.02–0.03per cell). After the last irradiation, most of the patients did not show increase in the tested parameters. Only in patient number 4 and 6, higher dicentrics (0.05 and 0.07per cell) were detected. According to our data, this corresponds to the absorbed dose of 0.6 and 0.8 Gy, respectively, during the whole-body irradiation. Together with unstable aberrations in patient number 6, marker chromosomes in 4 metaphases were detected and amount of micronuclei increased from 0.5 up to 2. No major changes in DNA damage were detected.

After 4 months of radiotherapy, clinical data were obtained on the patients' conditions. As can be expected from the chromosomal data obtained in patient number 4 and 6, postradiation complications were seen from the clinical data. For patient number 4 (cancer of larynx – 44 Gy in 22 fractions), swelling and pain in the throat were observed. For patient number 6 (cancer of larynx – 66 Gy in 33 fractions), tumors decreased marginally. After a gap of 1 month, laryngectomy was performed. The clinical status of the other patients was good. The data are presented in Table 1.

In the second group, changes in all estimated parameters were observed even before the irradiation [Table 2]. This indicates the presence of more complicated and more developed stage of disease. Increase of all parameters during radiotherapy was observed in all cases. The stable aberrations (marker chromosomes) were observed in several cases. The dynamics of radiation-induced cytogenetic changes is reflected in Figure 1, and the testing of the statistical significance in Table 3.

It is apparent that there was a significant difference in the cytogenetic parameters studies in patients from Groups 1 and 2. However, only Group 1 showed increased chromosome aberrations and micronuclei upon radiotherapy. A difference in response was visible among the two groups during the process of radiotherapy. In the second group of patients, a sharp statistically significant increase of all cytogenetic characteristics was observed [Figure 1 and Table 3]. Further, the attention should be paid to the fact that in the first group of patients the level of background DNA-comet was significantly lower compared to Group 2 [Figure 1, before, Table 3], while in the initial levels of the micronuclei and dicentric chromosomes, inter-group difference was not observed. Taking into account the above stated, correlations in peripheral blood erythrocytes (PBE) morphometric characteristics (volume, shape) with background values of cytogenetic properties were studied. It was identified that standard deviation of volume of PBE young fractions (PBE
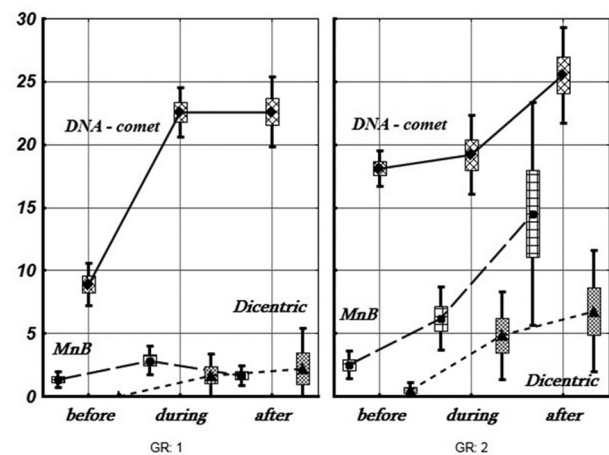

Figure 1: The dynamics of radiation-induced cytogenetic changes in Groups I and II X-axis: Stage of irradiation, Y-axis: Mean values: DNA-comet in %, dicentrics per cell, MnB – per 1000 cells (standard error, 0.95 confidence interval)

Table 1: Dynamics of estimated parameters of first group cancer patients undergoing radiotherapy

Chromosomal aberrations/cell			MnB/1000 cells			DNA-comet %		
Before RT	During RT	After RT	Before	During	After	Before	During	After
Not done	0.016 ab/cell	0.016 ab/cell	1	4.5	2	8.5	21	18
0.015 ab/cell	0.045 ab/cell 0.02 dic/cell	0.02 ab/cell 0.01 dic/cell	2	2.5	2	8.5	21.5	22
0.012 ab/cell	0.018 ab/cell	0.015 ab/cell	1.5	3	2	11.8	23.7	24
0.014 ab/cell	0.03 dic/cell 0.05 ab/cell	0.05 dic/cell 0.06 ab/cell	2	2	0.5	9.2	22	24
Not done	0.01 dic/cell	0.018 ab/cell	1	3.5	1	7	21.3	22
0.012 ab/cell	0.1 ab/cell 0.04 dic/cell	0.07 dic/cell + 2 marker chromosomes	0.5	1.5	2.5	8.3	25.8	25.6

RT: Radiotherapy, MnB: Micronuclei in buccal cells, ab: Aberrations, dic: Dicentric chromosomes

Table 2: Dynamics of estimated parameters of second group cancer patients undergoing radiotherapy

Chromosomal aberrations/cell			MnB/1000 cells			DNA-comet (%)		
Before RT	During RT	After RT	Before	During	After	Before	During	After
0.03 ab/cell	0.02 dic/cell 0.04 ab/cell	0.036 dic/cell	4	6	17	19	20	23
0.01 dic/cell 0.08 ab/cell	0.05 dic/cell 0.06 ab/cell	0.06 dic/cell	3	4	7	16	16	20
0.03 ab/cell	0.01 dic/cell 0.06 ab/cell	0.09 dic/cell 0.06 ab/cell	3	5	10	18	16	30
0.01 dic/cell 0.02 ab/cell	0.1 dic/cell 0.06 ab/cell + marker chromosomes	0.04 dic/cell 0.05 ab/cel	2	8	14	17.6	22	27
0.01 dic/cell	0.04 dic/cell	0.03 dic/cell + 2 marker chromosomes	1	4	9	18	18	25
0.02 ab/cell	0.07 dic/cell 0.06 ab/cell + marker chromosomes	0.15 dic/cell	2	10	30	20	23	28

RT: Radiotherapy, MnB: Micronuclei in buccal cells, ab: Aberrations, dic: Dicentric chromosomes

Table 3: Statistical significance of radiation-induced changes DNA-comet, micronuclei, and dicentric values in the first and second group of patients

	Wilcoxon matched pairs test					
Value	First group pairs			Second group pairs		
	Before - during	Before - after	During - after	Before - during	Before - after	During - after
DNA-comet						
Z	2.041*	2.201*	0.733	1.0954	2.2013*	2.201*
P	0.041*	0.027*	0.463	0.273	0.027*	0.027*
Dicentric						
Z	1.825	1.603	0.730	2.201*	2.201*	0.943
P	0.067	0.108	0.465	0.027*	0.027*	0.345
MnB						
Z	2.022*	0.730	1.677	2.201*	2.201*	2.201*
P	0.043*	0.465	0.093	0.027*	0.027*	0.027*

Marked tests are significant at $P < 0.050$

with low sperulation degree) has a tendency of correlation with DNA-comets background values ($r = 0.7$, $P = 0.064$). Correlations with other cytogenetic characteristics are not observed.

Discussion

Detection of pattern of cytogenetic changes among individuals exposed to low doses of radiation in contaminated environment and patients undergoing radiotherapy enabled us to compare effect of IR on different human beings after whole-body and local irradiations. Whole-body irradiated persons were residents of the regions where the radioactive sources were detected 10–12 years ago. Absorbed dose in range of 0.2–0.7 Gy was determined by biodosimetry in 19 persons and selected persons with high level of chromosomal aberrations. In this work, we

have analyzed the most recent results of these individuals and find that whole-body irradiation with identical doses is causing very heterogeneous response in different individuals. This might depend on different factors, such as immunological stage, age, and sex. There are several data that organism response on IR can depend also on genetic polymorphism.[16,17,23]

To estimate the effect of local irradiation, we have monitored the dynamics of chromosomal and DNA damage in patients with head and neck cancer undergoing fractionated radiotherapy with total dose of 40–70 Gy. We observed more evident individual differences among estimated specific radiation biomarkers: dicentrics and other chromosomal damage, micronuclei in exfoliate buccal cells, and amount of DNA-comets. Despite one and the same tumor localization and identical received dose of radiation, changes in the studied parameters were not homogeneous.

Considering the heterogeneity in the response of patients as well as individuals to IR, caution should be exercised and appropriate treatment regimen should be planned for effective therapeutic outcome. Individual radiosensitivity might play a significant role in the management of radiotherapy patients even for similar cancer types. We believe that the biomarkers we have chosen are more appropriate for the determination of geno- and cyto-toxic effects of IR. IR induces chromosomal aberrations in a variety of human tissues. The methods used in the present study were successfully used in the past to determine the individual radiosensitivity.[24] Moreover, our approach of using buccal exfoliated cells for micronucleus analysis may provide a complementary method for measuring DNA damage and cytotoxic effects.[25,26]

There is a strong association between DNA damage and radiosensitivity as observed by other authors.[27-29] The correlation between initial cytogenetic parameters and functional stage of RBS substantiates as observed in our study highlights the importance of further research to estimate the individual radiation risk. As can be seen from the current study, even after 10–12 years of chronic general exposure to small doses of radiation and during the process of radiotherapy, radiation response may depend on individual sensitivity. It is believed that the application of multiple/appropriate biomarkers will help in optimizing and individualizing subsequent medical management of radiotherapy in the future.

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Conflicts of interest

There are no conflicts of interest.

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