THE EFFECT OF CHRONIC EXPOSURE ON THE PARAMETERS OF CYTOGENETIC MARKERS OF SENESCENCE IN THE RESIDENTS OF THE TECHA RIVERSIDE SETTLEMENTS

Akhmadullina YuR[1,2] ✉, Vozilova AV[1], Krivoshchapova YV[1]

[1] Urals Research Center for Radiation Medicine of the Federal Medical Biological Agency, Chelyabinsk, Russia

[2] Chelyabinsk State University, Chelyabinsk, Russia

The understanding of the exposure effects on the human health could be improved by analyzing the influence of the chronic low dose rate exposure on the senescence of the immune system cells. It will also help to develop the measures aimed at the mitigation of the adverse effects. The objective of the study is to investigate the influence of the chronic low dose rate exposure on the senescence of the immune system cells using the cytogenetic markers. In the course of the research the authors evaluated the cellular senescence markers — genome instability and telomere depletion — in T-lymphocytes of the individuals exposed in the Southern Urals (exposure doses were 0.001 Gy — 4.7 Gy, the age of examined people was 40–89 years). The data analysis has demonstrated that the effect of chronic exposure on the T-cell senescence was indirect. Unstable chromosome aberrations occurred statistically significantly more frequently in exposed people aged 40–59 years (p = 0.012). Frequency of lymphocytes with micronuclei in exposed individuals differed in men and women (p = 0.001). Statistically significant decrease in the telomere length was revealed (for the chromosome arms 1q, 3p, 3q, 20p, 20q, 13q, 15p, 22q (p < 0.05); 19p, 21q (p < 0.01)).

Keywords: markers of cellular senescence, ionizing radiation, unstable chromosome aberrations, micronuclei, the Techa River, telomeres

Funding: the study was supported by the RSF grant (project № 22-25-20145 “Exploring the Mechanisms Underlying the Effects of Tolerance to Food Antigens on the Glucose Utilization”.

Acknowledgments: the authors would like to express their gratitude to Savkova N.F., senior laboratory assistant, for technical and laboratory support.

Author contributions: Akhmadullina YR — developing the criteria of the analysis, slide staining and assessment for the manuscript section on micronuclei, statistical analysis, literature review, manuscript writing; Vozilova AV — study conception and design, research priority setting, literature review, staining and analysis of cytogenetic slides for the manuscript section on unstable chromosome aberration, statistical analysis, manuscript writing; Krivoshchapova YaV — developing the criteria of the analysis, slide staining and assessment for the manuscript section on the telomere regions of the chromosomes, statistical analysis, literature review, manuscript writing.

Compliance with the ethical standards: the study was approved by the Ethics Committee of the Urals Research Center for Radiation Medicine (protocol No. 1 dated 22 January 2024); individuals who underwent cytogenetic examinations gave the informed consent to blood sampling and further assessment.

✉ **Correspondence should be addressed:** Yulia Rafisovna Akhmadullina
Vorovskogo, 68A, Chelyabinsk, 454141, Russia; akhmadullina.yul@yandex.ru

Received: 02.04.2024 **Accepted:** 31.05.2024 **Published online:** 19.06.2024

DOI: 10.47183/mes.2024.018

ВЛИЯНИЕ ХРОНИЧЕСКОГО ОБЛУЧЕНИЯ НА ПОКАЗАТЕЛИ ЦИТОГЕНЕТИЧЕСКИХ МАРКЕРОВ СТАРЕНИЯ У ЖИТЕЛЕЙ ПРИБРЕЖНЫХ СЕЛ РЕКИ ТЕЧА

Ю. Р. Ахмадуллина[1,2] ✉, А. В. Возилова[1], Я. В. Кривощапова[1]

[1] Уральский научно-практический центр радиационной медицины Федерального медико-биологического агентства, Челябинск, Россия

[2] Челябинский государственный университет, Челябинск, Россия

Исследование влияния хронического низкоинтенсивного облучения на старение клеток иммунной системы имеет важное значение для понимания последствий воздействия облучения на здоровье человека и разработки мер минимизации негативных эффектов. Целью работы было исследовать влияние хронического низкоинтенсивного облучения человека на старение клеток иммунной системы с использованием цитогенетических маркеров. В работе оценили маркеры старения — нестабильность генома и истощение теломер в Т-лимфоцитах у облученных людей на Южном Урале (дозы облучения от 0,001 до 4,7 Гр, возраст — от 40 лет до 89 лет). Анализ данных показал, что хроническое воздействие повлияло на старение Т-клеток опосредованно. Частота нестабильных хромосомных аберраций у облученных лиц была статистически выше в 40–59 лет (p = 0,012). Частота лимфоцитов с микроядрами у облученных лиц наиболее различалась у мужчин и женщин (p = 0,001). Выявили статистически значимое снижение показателей длины теломер у облученных лиц (для хромосомных плеч 1q, 3p, 3q, 20p, 20q, 13q, 15p, 22q (p < 0,05); 19p, 21q (p < 0,01)).

Ключевые слова: маркеры клеточного старения, ионизирующая радиация, нестабильные хромосомные аберрации, микроядра, река Теча, теломерные районы хромосом

Финансирование: государственное задание ФМБА РФ на выполнение прикладной научно-исследовательской работы по теме: «Изучение влияния хронического низкоинтенсивного облучения на преждевременное старение клеток иммунной системы человека».

Благодарности: авторы выражают благодарность старшему лаборанту Н. Ф. Савковой за техническую и лабораторную поддержку.

Вклад авторов: Ю. Р. Ахмадуллина — разработка критериев анализа, покраска и анализ стекол для раздела про микроядра, статистика, анализ литературы, написание статьи; А. В. Возилова — идея исследования, постановка научных задач, анализ литературы, покраска и анализ цитогенетических препаратов для раздела про НХА, статистика, написание статьи; Я. В. Кривощапова — разработка критериев анализа, покраска и анализ стекол для раздела про теломерные участки хромосом, статистика, анализ литературы, написание статьи.

Соблюдение этических стандартов: исследование одобрено этическим комитетом УНПЦ РМ (протокол № 1 от 22 января 2024 г.). У лиц, участвующих в цитогенетических исследованиях, было получено информированное согласие на забор образцов крови и дальнейшие исследования.

✉ **Для корреспонденции:** Юлия Рафисовна Ахмадуллина
ул. Воровского, д. 68А, г. Челябинск, 454141, Россия; akhmadullina.yul@yandex.ru

Статья получена: 02.04.2024 **Статья принята к печати:** 31.05.2024 **Опубликована онлайн:** 19.06.2024

DOI: 10.47183/mes.2024.018

For more than 60 years people chronically exposed as a result of the releases of liquid radioactive waste (LRW) of the “Mayak” PA into the Techa River in the Southern Urals have been undergoing medical examinations at the URCRM. The specific feature of this exposure situation is the combination of internal exposure due to the intake and accumulation of $^{89,90}Sr$ radionuclides in the body, and external γ-exposure due to the river water. The critical organ was the red bone marrow (RBM), where the hematopoietic precursor-cells were exposed at a wide dose range. Since the LRW started to be released into the Techa River in 1948, the maximum dose rates were recorded in 1951–1952, and exposure doses were formed by 1960. However, chronic internal low dose exposure proceeds up to date. As the youngest of the exposed people are ≥60 years old, therefore, it is possible to study the effect of ionizing radiation on the ageing of a human being [1].

Human body is a complex system with multiple levels. It has its own ontogenesis program. Its implementation is regulated by a group of genes and multiple pathways of interaction and interdependence of their products. An important stage in ontogenesis is the ageing of the body. Nowadays the proportion of the elderly people (aged 60+) in the world population is increasing drastically. That is why, scientists and physicians are interested in studying the mechanisms of the natural ageing of a human being, factors that cause premature ageing, and mechanisms that can postpone or delay in time its development [2].

No doubt, in addition to the natural processes the environmental conditions also influence the ageing of a body. Active application of ionizing radiation (IR) sources for medical and diagnostic purposes leads to a question whether radiation can affect the premature ageing [3, 4].

Investigation of any process is based on the identification of the markers that have qualitative or quantitative attributes. Generally accepted markers of ageing include the following: genomic instability, telomere depletion, epigenetic genome damage, changes in the proteostatus of the cells, changes in the regulation of nutrients, mitochondrial dysfunctions, depletion of the stem cell pool, impaired intracellular interaction [3, 5].

In our study we assess the chromatin status of the nuclear DNA in PHA-stimulated peripheral blood T-lymphocytes of the exposed individuals. The parameters that we have selected are indicative of the two above-mentioned markers of ageing — genomic instability (frequency of unstable chromosome aberrations and cells with micronuclei) and telomere depletion (assessment of the chromosome telomere length).

Unstable chromosome aberrations (UCA) include such rearrangements as dicentrics, ring chromosomes, acentric rings. Numerous studies confirmed the UCA dependence on the age of a person, as the number of errors in repair increases with age and so does the number of cells with UCA [6–9].

The analysis of the cytokinesis-blocked T-lymphocytes with micronuclei is an additional tool for a secondary assessment of the chromosome abnormalities. It is used in case of the exposure to various genotoxic environmental factors. Micronucleus (MN) is the structure that contains chromatin formed out of either unrepaired DNA breaks or as a result of the incorrect segregation of the sister chromatid of a single or several chromosomes. Some studies demonstrate that spontaneous MNs occur more frequently with age [10].

The length of the telomere regions of the chromosomes or “biological clock” of a cell is another marker of the cellular senescence. Daughter cells lose their capacity to divide and die due to gradual depletion of the chromosome telomeres in cell line division (Hayflick limit). The phenomenon of the telomere shortening in the cells of the body with the age of a person formed the basis of the method of biological age determination [11–12].

Thus, to evaluate the effect of chronic exposure on the DNA ageing of the PHA-stimulated human T-lymphocytes we have chosen cytogenetic methods that allow assessing the status of the chromosome DNA of the immune system cells, the precursors of which started to be exposed already in the RBM more than 50 years ago, and this exposure is still going on.

The objective of the study is to investigate the effect of chronic low dose rate human exposure on the immune cell ageing using cytogenetic markers.

METHODS

The study design: to form comparable subgroups of people of different age among exposed and unexposed individuals; to analyze the parameters of the genomic instability in each age subgroup and to compare the values between themselves. To evaluate the time-dependent changes in chromosome aberrations in age subgroups of exposed and unexposed people (assessment of the nuclear DNA instability).

Characteristics of the studied individuals

People affected by chronic combined exposure in the Southern Urals (hereinafter referred to as ‘donors”) were chosen as the object of the research. Nuclear chromatin of the PHA-stimulated peripheral blood T-lymphocytes were the subject of the research.

The study comprised the individuals born before 1959 inclusively, with the total RBM external and internal doses from 0.001 to 4.7 Gy calculated with the TRDS-2016 [13]. These were people of both sexes, mainly of three ethnicities — Russians, Tartars, and Bashkirs.

The study also involved people living in similar social and economic conditions in the Southern Urals who were not affected by accidental exposure (“comparison group”). The comparison group for the study of the telomere length consisted of the TRC members whose dose to RBM did not exceed 0.01Gy.

The following cytogenetic criteria were used to select the donors: people who had autoimmune diseases or cancers in past medical history, or chronic inflammatory diseases in the exacerbation phase, or people taking cytostatic drugs or antibiotics were excluded from the study.

Information on the health status of the exposed people has been provided by the department “Database “MAN”. Individual exposure doses were calculated at the Biophysics laboratory. The data on the oncopathology in the past medical history of the examined people were provided by the Epidemiology laboratory (Urals Research Center for Radiation Medicine).

In accordance with the existing International Norms and Regulations (Declaration of Helsinki, 1964) and with the approval of the Ethics Committee of the URCRM all the donors provided informed consent to blood sampling and further study.

The study of the UCA frequency

Two groups of donors were formed to assess the frequency of UCA. The comparison group consisted of 83 people. The study group included 570 individuals with RBM doses 0.001–4 Gy. The age of the studied individuals varied in the range 40–89 years. The studied group of exposed people was subdivided into two subgroups. The 1st subgroup comprised the individuals with doses 0.001–0.2 Gy inclusive. The 2nd subgroup included

Table 1. Characteristics of the examined individuals and results of the evaluation of UCA frequency in the studied groups (median, 25% and 75%)

Age sub-groups, years	Comparison group	Exposed individuals		
		0.001–4 Gy	0.001–0.2 Gy	0.3–4 Gy
40–59	0 *n* = 17	0 0–0.27 *p* = 0.012 *n* = 100	0 0–0.4 *p* = 0.002 *n* = 39	0 *p* = 0.04 *n* = 61
60–69	0 p_1 = 0.064 n = 44	0 0–0.26 *n* = 285	0 0–0.133 *n* = 58	0 0–0.2 *n* = 227
70–89	0 0–0.62 p_2 = 0.023 *n* = 22	0 0–0.22 *n* = 185	0 0–0.18 *n* = 43	0 0–0.255 *n* = 142
Total	0 n = 83	0 0–0.20 *p* = 0.04 *n* = 570		

Note: *p* — statistically significant differences with the comparison group in one and the same age-group; p_1 — statistically significant differences between age-groups 40–59 years and 60–69 years; p_2 — statistically significant differences between age-groups 40–59 years and 70–89 years.

persons with doses 0.3–4 Gy. The exposed and unexposed individuals were ranked according to their age at the time of examination. Three age-groups were distinguished: 40–59 years, 60–69 years, and the oldest group 70–89 years (Table 1). In our previous studies no dependence of the UCA frequency on sex of the studied individuals was registered. That is why the groups were mixed in terms of sex, most of the studied individuals were women (up to 70%).

The study of the frequency of cells with MN

Characteristics of the studied individuals are given in Table 2.

In total the comparison group included 113 women and 44 men. The age of the studied individuals ranged from 52 to 82 years. Women predominated in all the age-groups. The group of exposed individuals consisted of 573 persons (354 women and 219 men). The age of the studied individuals was 50–89 years. Cumulative doses to the RBM ranged from 0.001 to 4 Gy.

We have also conducted a pilot study with a view to find out whether the X-chromosome chromatin into the micronucleus. It involved 6 exposed women aged 73–82 with RBM doses 0.73–1.93 Gy (study group), and two unexposed women aged 63 and 65 years (comparison group).

Assessment of the telomere length

Two groups of donors were formed to measure the telomere length. The comparison group consisted of 27 people with RBM doses 0–0.01 Gy. Out of these people 23 persons had doses 0.0001–0.01 Gy, and four individuals did not have accidental exposure at all. The group of exposed individuals included 26 people with RBM doses 0.6–4.7 Gy. The age of the examined individuals was 61–84 years. Characteristics of the examined donors are given in Table 3.

To analyze the effect of sex on the telomere length the groups were matched in terms of the number of examined people and their age. The group “Women” consisted of 11 donors aged 61–73 with RBM dose 0–1.4 Gy. The group “Men” included 11 donors aged 61–72 with RBM dose 0–1.4 Gy.

Assuming that the non-radiation factor “sex” could affect the relative telomere length, to estimate the exposure effect on the relative length of the telomere regions two groups of female donors were formed. The comparison group consisted of 20 people with RBM doses 0–0.01 Gy, out of comparison group members 18 women had the dose 0.0001–0.01 Gy, and 2 women did not have accidental exposure at all. The donors’ age was 62–80 years. The group of exposed women included 22 individuals with RBM doses 0.6–4.7 Gy. Their age at examination was 70–84 years.

Preparing the chromosome metaphase slides

Preparing and staining of the slides

Cytogenetic slides from the PHA-stimulated peripheral blood T-lymphocytes of the donors were prepared according to the protocol that includes four consecutive steps: cell culturing to

Table 2. Characteristics of the persons examined using the MN assay

Age groups, years	Female		Male	
	Comparison group	Exposed individuals	Comparison group	Exposed individuals
	Age, years number of person	Age, years number of person, RBM dose, Gy	Age, years number of person	Age, years number of persons, RBM dose, Gy
50–59	57 *n* = 23	57 *n* = 45 0.002–2.9	56 *n* = 8	56 *n* = 22 0.007–1.0
60–69	65 *n* = 45	65 *n* = 177 0.004–3.7	65 *n* = 20	65 *n* = 125 0.004–2.2
70–89	74 *n* = 45	75 *n* = 132 0.001–4.0	75 *n* = 16	73 *n* = 72 0.02–2.1

Table 3. Characteristics of the donors with measured telomere length

Age, years	Female		Male	
	Comparison group	Exposed individuals	Comparison group	Exposed individuals
	Age, years number of persons, RBM dose, Gy	Age, years number of persons, RBM dose, Gy	Age, years number of persons, RBM dose, Gy	Age, years number of persons, RBM dose, Gy
61–84	62–80 *n* = 20 0–0.01	70–84 *n* = 22 0.6–4.7	61–72 *n* = 7 0–0.01	71–76 *n* = 4 0.6–1.35

the metaphase (for 52 hours, colcemid in the final concentration 0.1 mg/ml was added 3 hours before the end of the culturing), hypotonic treatment of the metaphase cells (one hour prior to fixation); metaphase cells fixation (3 parts of ethanol, 1 part glacial acetic acid), and then obtaining the chromosome preparations. Metaphase chromosomes were stained with 2% Gimza solution for 10 min. Then the slides were washed to remove the stain and dried at room temperature [14].

The preparations were analyzed under light microscopy without caryotyping using the AxioImager A2, Z2 microscope. The analysis included cells with 46 chromosomes, with no overlapping or with 1–2 overlappings maximum. Dicentrics, ring chromosomes, acentric rings were the focus of the attention of the researchers. From 100 to 500 cells were analyzed per each studied individual.

Obtaining the preparations of binucleated lymphocytes to detect micronuclei

The protocol of the micronuclei test consists of several steps: culturing the PHA-stimulated peripheral blood lymphocytes, cytokinesis block, hypotonic treatment, fixation of suspension cells, preparation of cytogenetic slides [15]. Preparations were stained with 1% Gimza solution for 20 min. The preparations were analyzed under light microscopy using the AxioImager A2 microscope. 1000 binucleated cells were analyzed per donor.

The chromosomes of the micronuclei were studied with locus-specific fluorescence *in situ* hybridization. Fluorescent probes for centromeric region of X-chromosome (cenX) (Metasystems, Germany) were used. Fluorescent staining was performed in accordance with the manufacturer's protocol which requires DNA denaturation of the preparation and the probe, hybridization for 24 hours at 37 °C, post-hybridization wash-out of the unbound probes. For counter-staining of the chromatin 15 μl of the DAPI antifade (Metasystems, Germany) were applied onto each slide. Then slides were covered with the coverslip and stored at –20 °C.

The analysis of FISH-stained preparations was performed with fluorescent microscope AxioImager Z2 (Zeiss; Germany) with fluorescent filters and Isis Metasystems software module to process fluorescent images. The analysis of centromere signals of X-chromosome was focused on the presence and amount of the signals in a micronucleus. Micronuclei with one or more centromere signals were considered centromere-positive (cen X+). Micronuclei without centromere signals were considered centromere-negative (cen X–).

Detection of the telomere regions of the metaphase chromosomes

Slides with metaphase cells were prepared in accordance to the protocol described for the assessment of the UCA. To perform fluorescent staining with Q-FISH slides were cleared from cytoplasm, the DNA of the probe and preparation was denaturated. Hybridization was preformed according to the manufacturer's protocol with genuine solutions. To estimate the length of the chromosome telomere DAKO probes (Denmark) were used. Centromere signal of the chromosome #2 (Metasystems, Germany) was used as a reference one.

Fluorescently stained preparations were analyzed on AxioImager Z2 fluorescent microscope (Zeiss, Germany) with DAPI and SpO (spectrum orange) filters. Thirty cells were studied per person. The telomeres were measured with the telomere module of the Isis software. The measurement results were expressed in percentage ratio of the telomere signal length (T) to that of the centromere signal (C) – (%T/C). The method of the telomere length measurement is described in detail in [16].

The telomere lengths for the metacentric (#1, #3, #19, #20) and acrocentric (#13, #14, #15, #21, #22) chromosomes are given in the Results section.

The main focus of the research was on the relative telomere length in metacentric and acrocentric chromosomes as the most diverse ones in terms of the coefficient of the chromosome arms ratio and in terms of the chromosome length on the whole. The chromosomes of various size were also compared within the groups of metacentric and acrocentric chromosomes. Among metacentric chromosomes, chromosome #1 is the largest. It contains approximately 8% of the whole DNA-material of the cell. The smallest metacentric chromosome is #20. It has about 2.5% of the cell DNA. The group of acrocentrics includes chromosomes #13, #14, #15. Each of them contains about 3.5–4% of the cell DNA, while the smallest acrocentrics — chromosomes #21, #22 — have 1.5–2% [17].

Methods of the statistical processing of data

The normality of data distribution was checked with Kolmogorov-Smirnov test. Common methods of variation statistics with the calculation of the median, 25th and 75th percentile were used for the statistical processing of the obtained results. The values in the groups were compared with Mann–Whitney U test.

The relationship between the frequency of UCA, age and dose were determined using the linear regression equations.

Spearman correlation coefficient was used to determine the relationship between the frequency of lymphocytes with micronuclei and age. To assess the effect of a set of factors on the frequency of lymphocytes with micronuclei in the studied individuals a univariant general linear model was applied. The frequency of micronuclei with centromere signals of the X-chromosome was calculated as a percentage of all the micronuclei. The analysis was performed with the help of the chi-square criterion. The differences were considered statistically significant at $p < 0.05$. Statistical processing of the obtained results was performed with the software package Sigmaplot (SYSTAT Software; USA), STATISTICA, version 10.0 (USA) and PAST, version 4.03

RESULTS

The study of the frequency of UCA

The comparison of the parameter values revealed that exposed people have statistically significantly more exchange

Table 4. Frequency of lymphocytes with micronuclei in groups of exposed women of every studied age (median, 25–75%, min-max)

Age groups	Comparison group	Exposed individuals		
		0.001–4 Gy	0.001–0.2 Gy	0.3–4 Gy
50–59	15 9–21 (5–65)	15 11–20 (6–36)	12 10–18 (6–24)	16 14–24 (7–36)
60–69	17 12–26 (4–48)	16 12–22 (2–55)	15 11–19 (2–42)	16 12–25 (3–55)
70–79	16 10–22 (0–40)	18 13–23 (3–47)	19 15 – 25 4–42	17 12–23 (3–47)
80–89	15 11–21 (10–24)	20 17–25 (4–44)	20 18–27 (16–39)	20 15–23 (4–44)

aberrations than unexposed individuals, $p = 0.04$ (Table 1). Weak linear correlation of the studied parameters with the bone marrow dose was observed in the combined group of exposed individuals ($R = 0.125$, $p = 0.005$).

In the comparison group the studied parameter increases slightly with age (mean — 0, 0.18, and 0.30 per 100 cells, respectively). The frequency of CA in unexposed individuals in the age subgroups “60–69 years” and “70–79 years”, is increased relative to the subgroup “40–59 years” ($p_1 = 0.06$, $p_2 = 0.02$). Despite the increase in the frequency of exchanges in the subgroup “70–79 years”, the differences with the subgroup “60-69 years” are statistically insignificant.

As for the exposed individuals, then there was no increase in the studied parameter with age. On the contrary, UCA in all the age subgroups occurred with equal frequency ($p_1 = 0.69$, $p_2 = 0.37$). It was proved once again by the absence of linear correlation ($R = 0.0002$, $p = 0.76$).

When we compared the frequency of exchange aberrations in the control subgroups and subgroups of exposed people, we revealed a statistically significant increase of the parameters only in the subgroup of exposed individuals aged 40–59 years ($p = 0.038$). The parameter values in exposed individuals from the age subgroup “60-69 years” exceeded those in the respective control group but the differences were statistically insignificant. As for the older age, then the frequency of cells with CA in these groups was similar.

The construction of the linear regression allowed noticing weak dependence of the UCA frequency on age for the comparison group (equation 1). For the group of exposed individuals there was no linear dependence of the UCA frequency on age (equation 2). Weak dependence of the UCA frequency on RBM dose (equation 3) was revealed, and it agrees with the published data [1,18].

$$UCA = 0.013\,A – 0.670\ (R = 0.242\ p = 0.04) \quad (1)$$
$$UCA = 1.56 \times 10^{–6}\,A + 0.241\ (R = 0.0002\ p = 0.76) \quad (2)$$
$$UCA = 0.109\,D + 0.152\ (R = 0.126\ P = 0.005) \quad (3)$$

where UCA — frequency % of exchange aberrations A — age at last birthday, D — dose to RBM, Gy.

Study of the frequency of cells with micronuclei

According to the data presented in Table 4 it is clear that the frequency of lymphocytes with micronuclei is 15‰, 17%, 16‰ and 15‰ in exposed women aged 50–59, 60–69, 70–79, and 80–89, respectively. No statistically significant differences were noted.

No statistically significant differences in the frequency of lymphocytes with micronuclei were observed between the groups of exposed women and comparison groups, and between the groups of exposed women themselves.

It is clear from Table 5 that the frequency of lymphocytes with micronuclei in exposed men aged 50–59 is 11‰, in those aged 60–69 it makes up 12‰. As for men aged 70–79 the frequency of lymphocytes with micronuclei is 15‰. This value does not differ from that in the group of unexposed men. The study of the frequency of lymphocytes with micronuclei in the dose groups of exposed men revealed no statistically significant differences with the comparison group and between the groups of exposed men themselves.

The study of the dependence of the frequency of lymphocytes with micronuclei on sex revealed that in the groups of exposed individuals the studied parameter was statistically significantly higher in women relative to men (18‰ (13–25‰) vs 13‰ (10–19‰), $p = 0.001$).

In the course of the analysis of the differences in the frequency of lymphocytes with micronuclei between men and women in various age subgroups it was noted that parameter values differed statistically significantly in the age subgroups 60–69 and 70–79 ($p = 0.0001$ and $p = 0.033$, respectively). In the age period 50–59 years the differences between men and women followed the trend ($p = 0.119$).

Multivariate analysis was used to study the influence of the combination of radiation and non-radiation factors. The results

Table 5. Frequency of lymphocytes with micronuclei in groups of exposed men of every studied age (median, 25–75%, min-max)

Age groups	Comparison group	Exposed individuals		
		0.001–4 Gy	0.001–0.2 Gy	0.3–4 Gy
50–59	15 8–20 (3–21)	11 9–18 (5–41)	11 8–20 (5–29)	12 10–22 (5–41)
60–69	13 11–19 (5–37)	12 8–19 (2–38)	15 11–22 (3–34)	12 8–18 (2–38)
70–79	15 10–18 (3–21)	15 11–19 (4–41)	16 11–19 (8–28)	15 11–19 (4–41)

Table 6. The effect of radiation exposure, age and sex on the frequency of lymphocytes with micronuclei

Factor	Parameters of the model
adjusted model	$F = 9.5$ $p = 0.0001$
age	$F = 3.75$ $p = 0.053$
RBM dose	$F = 0.3$ $p = 0.599$
sex	$F = 22.24$ $p = 0.0001$

of the analysis are given in Table 6. As it can be seen from the table, the sex of the studied individuals had the greatest effect on the frequency of lymphocytes with micronuclei. No dependence of the frequency of lymphocytes with micronuclei on the RBM dose was observed, $p = 0.599$. The dependence of the frequency of lymphocytes with micronuclei on age was similar to the trend, $p = 0.053$.

Table 7 presents the frequency of micronuclei containing centromeres of the X-chromosome.

It is seen from table 7 that in the group of exposed individuals 46.6% of micronuclei contained centromere-positive chromatin of the X-chromosome. This value was statistically significantly higher than that in the comparison group — 31%, $\chi^2 = 4.78$, $p = 0.04$. Inter-individual variability of the values in exposed women was observed. The frequency of micronuclei with X-chromosome centromere signal varies from 22.8% to 59%.

Individual variability was also observed in the frequency of micronuclei containing different number of centromeres. For instance, in the group of exposed people there was one donor whose centromere-positive micronuclei contained predominately one centromere; three donors who mainly had 2 signals in centromere-positive micronuclei. In the donors from the comparison group centromere-positive micronuclei contained predominately one centromere. These findings show that there has been anaphase X-chromosome nondisjunction or lagging, which is likely to result in chromosome elimination to the micronucleus.

The study of the length of the chromosome telomere regions

The length of the telomere regions of the metacentric and acrocentric chromosomes in men and women is given in Table 8.

It can be seen from table 8 that the relative length of the telomere regions was statistically significantly higher in men than that in women for metacentric chromosomes (#1, #3, #19, #20) and acrocentric chromosomes (#13, #14, #15, #21, #22), differences with $p < 0.05$ were observed for the chromosome arms 1q, 3p, 20q, 13q, 15q, 21q.

Table 9 presents the median of the telomere lengths for metacentric (#1, #3, #19, #20) and acrocentric (#13, #14, #15, #21, #22) chromosomes in the comparison group and chronically exposed women.

It is clear from Table 9 that on the whole the telomeres of the metacentric chromosome were shorter in chronically exposed women than those in the cells of the people from the comparison group. Statistically significant differences were observed for the 1q, 3p, 3q, 19p, 20p, 20q arms of the metacentric chromosomes, and for the 13q, 15p, 21q, 22q arms of the acrocentric chromosomes.

DISCUSSION

More than 100 thousand residents of the riverside settlements were affected by combined chronic exposure due to the releases of LRW into the Techa River.

Since nowadays a natural decline in the size of the cohort is registered, and at the same time rather big amount of cytogenetic data has been collected it is important to evaluate how chronic exposure influenced the cellular ageing processes. We have used several different cytogenetic methods in the study. They all reflect the status of the chromosome DNA in peripheral blood T-cells. To reach the set objective it was of utmost importance to characterize the status of chromatin at various levels of its structure organization and its behavior in the cellular cycle.

The analysis of data provided in table 1 demonstrates that UCA — are the events that occur occasionally in stimulated T-cells of the residents of the Southern Urals. For example, 60% of the examined individuals in the comparison group in three age periods (the age-range is 40–89) did not have cells with UCA except for the chromosome-exchanges aberrations in members of the oldest age-group. It should also be noted that according to the protocols of the cytogenetic study the exchange aberrations in members of the comparison group were represented mainly by dicentrics without paired fragments which proved that cells had undergone the first mitosis *in vivo*. Only older donors had ring chromosomes and acentric fragments. Similar situation for the dicentrics without paired fragments was observed in exposed individuals. It is known that unstable chromosomes that developed due to the exposure of

Table 7. Frequency of micronuclei with X-chromosome centromere signals, %

No. of the Donor	Cells	Number of MN	Cen X+, %	1 signal, %	2 signals, %	3 signals, %	4 signals, %	Cen X-, %
1	2683	134	59	25.3	68.3	5.1	1.3	41
2	2234	70	40	28.6	71.4	–	–	60
3	1941	27	22.2	66.7	33.3	–	–	77.8
4	633	22	54.5	50	41.7	–	8.3	45.5
5	3191	55	49	37	55.6	7.4	–	51
6	1000	35	22.9	12.5	87.5	–	–	77.1
Total “Exposed persons”		343	46.6	30.6	64.3	3.75	1.25	53.4
7	1667	46	32.6	73.4	13.3	–	13.3	67.4
8	3000	127	30.7	66.7	30.7	–	2.6	69.3
Total “Comparison group”		173	31.2	68.5	26	–	5.5	68.8

Table 8. Dependence of the telomere region length (%T/C) in metacentric and acrocentric chromosomes on sex (median, 25% and 75%)

No. of the chromosome	Chromosome arm	Male, $n = 11$			Female, $n = 11$		
		Median	Percentile		Median	Percentile	
			25%	75%		25%	75%
1	p	21.7	11.1	44.3	19.1	10.1	37.6
	q	23.1*	9.8	42	16	8.8	33.6
3	p	21.4*	10.3	46.2	16.8	9.7	30.8
	q	16.4	8.2	40.8	14.8	7.6	29.4
19	p	13.7	7.7	34.1	12.7	6.6	24.7
	q	12.8	6.7	33.2	11.3	6.4	26
20	p	18.3	8.8	33	16.3	9.5	29.2
	q	15.4*	8.5	32.9	11.9	5.9	20.1
13	p	19	9.5	43.4	18.7	10.9	33.3
	q	19.3*	8.5	40.8	14	7.7	29.1
14	p	16.1	8.9	37.6	16.1	9.3	32.6
	q	17.6	8.5	35.3	14.5	7.1	30
15	p	15.3	8.3	32.3	18.1	9.1	35.1
	q	15.5*	6.9	31	11.9	6.4	23.4
21	p	15.1	7.6	39	16.7	8.5	36.1
	q	12.5*	6.5	32.2	11	5.1	27.1
22	p	16.7	8.4	41	15	7.8	32
	q	13.3	6	32.4	12.3	5.9	26

Note: * — statistically significant sex-dependent difference of the values, $p < 0.05$.

the hematopoietic stem cells (HSC) in the bone marrow are eliminated eventually together with the cells that contain them. However, the UCA frequency in exposed individuals exceeds that in the comparison group in the long-term after the onset of radiation exposure. Evidently, the majority of aberrant cells registered in the long-term period after the onset of radiation exposure were exposed in the bone marrow (their precursors), entered the blood flow, underwent differentiation in the thymus, and remained in quiescent state until the PHA-stimulation *in vitro*.

Micronuclei can be formed as a result of the damage associated with both unrepaired chromosome DNA breaks, and during chromosome segregation impairment which is predetermined by changes in the cytosine methylation in centromeric regions of the chromosome, impairment in the

Table 9. The median of the telomere regions (%T/C) in metacentric and acrocentric chromosomes in women from the comparison group and from the group of chronically exposed people (median, 25% and 75%)

No. of the chromosome	Chromosome arm	Comparison group, $n = 20$			Exposed persons, $n = 22$		
		Median	Percentile		Median	Percentile	
			25%	75%		25%	75%
1	p	17.3	7.2	36.6	16	7	35.7
	q	14.6**	6.3	31.2	13.3	5.9	28.8
3	p	16.0*	6.7	31.3	14	6	30.3
	q	12.7**	5.5	26.8	12.2	5.2	24.5
19	p	11.7**	4.7	24	10.5	4.4	22
	q	10.8	5	24.5	9.4	4.5	21.2
20	p	14.4*	7.6	27.6	13.6	7.6	25.8
	q	10.0*	4.7	22.7	9.9	4.5	19.9
13	p	16.7	7.9	32.5	16.6	8	31.1
	q	13.4*	5.5	30.2	12.5	5.2	27.6
14	p	17.1	8.7	32.1	15.5	8.3	30
	q	13.3	5.4	29.4	12	4.8	26.6
15	p	16.6*	7.6	31	15.7	7.5	28.9
	q	11.3	4.8	24	10.4	4.2	21
21	p	16	7.1	32.8	15.2	7	30.1
	q	10.1**	4.5	22.9	9.2	4	30.1
22	p	15	6.9	4.7	13.5	6.1	27.1
	q	10.6*	4.7	24.9	10.1	4.5	21.3

construction of the kinetochore protein and microtubes, and with many other events [19]. In the study [20], having performed the analysis of the age-dependent frequency of cells with micronuclei in unexposed residents of the Southern Urals, we have revealed monotonous increase in the parameter values in men and women aged ≤ 69 years. After the age of 70 this value remains stable or slightly decreases. Similar results concerning the frequency of cells with micronuclei in the age group 60–69 years have been obtained by other researchers. They showed that at the age of 50–69 the frequency of micronuclei reaches its maximum, and then levels off [21–23].

In the current research the age of the examined exposed individuals was 50–89 years. It is the age when micronuclei frequency reaches the plateau. That is why, the frequency of lymphocytes with micronuclei in exposed people is not associated with the increase in age in this age range either.

The study of the sex effect on the frequency of lymphocytes with micronuclei demonstrated that this value is statistically significantly higher in women than in men, $p = 0.001$. When we compared the frequency of lymphocytes with micronuclei between men and women in different age groups it turned out that there were statistically significant differences in the groups of exposed individuals aged 60–69 and 70–79. Thus, it can be said that the differences in the frequency of micronuclei between men and women are more pronounced in the group of exposed individuals, than in the group of unexposed ones. The studies show that the differences in the ratio “frequency of micronuclei in men/women” become more pronounced in people aged 40 and older [21]. However, the mechanisms that lead to such age-dependent increase is not completely clear. In the published papers there are some data stating that sex chromosomes are eliminated to the micronuclei with age. In our study we have noted that micronuclei with X-chromosome are rather frequent and make up 22–59% of the total number of micronuclei. Moreover, micronuclei contain X-chromosomes both with single and several centromeres. It is indicative of the chromosome nondisjunction in the mitotic anaphase. Probably, in our case radiation can have certain impact even without linear dependence of micronuclei frequency on the RBM dose.

In the study [24] the chromosome composition of the micronuclei in exposed women was analyzed using mFISH technique. The study has also demonstrated that X-chromosome is often observed in micronuclei composition. On the average in the group of exposed women the micronuclei are composed of bigger number of chromosomes relative to the comparison group. The amount of chromosome signals per micronucleus in exposed women doubles that in the control group ($p = 0.001$). At the same time mean frequency of micronuclei per 1000 estimated binucleated lymphocytes does not differ in women of these two groups. These results point indirectly at increased frequency of chromosome damages and their elimination from the nuclear genome to micronuclei. The mechanisms of micronucleus formation are similar in the group of exposed women and in the comparison group. Nevertheless, exposed women have some specific features of mono- and multicolored micronuclei formation. Thus, we can assume that there are mechanisms of the segregation impairment of the sex chromosomes and chromosomes with aberrations.

In accordance with the obtained data the relative telomere length in each donor varies within a wide range. Telomeres differ in length both within one cell, and within one chromosome pair. These data agree with the findings of the previous studies [16, 25] and published research data [26–28]. Our findings have demonstrated that the telomere length does not depend on a chromosome or arm where it is located [16]. In the course of the study we revealed the dependence of the chromosome telomere length on sex: the telomeres were statistically significantly longer in the studied groups of chromosomes in men than those in women.

In the present study in the course of the analysis a statistically significant decrease in the thelomere signals in metacentric and acrocentric chromosomes of exposed women was noted relative to the comparison group. Statistically significant differences were observed for the chromosome arms 1q, 3p, 3q, 20p, 20q, 13q, 15p, 22q ($p < 0.05$); 19p, 21q ($p < 0.01$). These data are in line with the published research data that show that low dose exposure shortens the telomeres. These changes persist even in 20–70 years after the exposure [29, 30]. It is of interest to note that the effect of ionizing radiation exposure on the telomere length is dubious. Some studies demonstrate that the telomeres lengthen in exposed people, which is associated with the elevated expression of the telomerase (the authors associate it with the increased risk of malignant neoplasms development). If the telomeres are short, then it is associated with the replicative ageing of the cells [31, 32]. Moreover, the results we have obtained could be the evidence of the fact that ionizing radiation induced immune cell death in the period of maximum exposure. It resulted in compensatory proliferation of cell differons in hematopoietic organs, and thus, in telomere shortening.

The analysis of the obtained data allows us to draw a conclusion that chronic radiation exposure that occurred in the Southern Urals influences T-cell aging indirectly, as one of the numerous factors. Perhaps, the selection criteria of donors for the cytogenetic study, which could contribute to the selection of the most radio-resistant donors to the group of exposed individuals, influenced the results. For instance, according to the selection criteria, only people without cancer, autoimmune disease, and diabetes in past medical history could be included into the study. Taking into account the fact that the above-mentioned diseases start manifestating more frequently in elderly and old age, then it is rather probable that radiosensitive individuals among the exposed ones could have bigger chance to develop the radiation exposure effects, and therefore, when the groups of donors were being formed, these people did not meet the study inclusion criteria [20–33].

CONCLUSIONS

Based on the conducted cytogenetic study it can be said that chronic radiation exposure that have occurred in the Southern Urals is just one of the factors that influence indirectly the cellular senescence of the peripheral blood T-lymphocytes. The frequency of unstable chromosome aberrations in exposed individuals was statistically significantly higher in the age group 40–59 years ($p = 0.012$). There was a weak linear correlation of the studied parameters and the dose to the bone marrow in the pooled group of the exposed individuals ($R = 0.125$, $p = 0.005$). The frequency of lymphocytes with micronuclei in exposed individuals was similar to that in the comparison group. The frequency of lymphocytes with micronuclei differed in exposed men and women ($p = 0.001$). In 22-59% of the exposed women micronuclei contained X-chromosome. No dependence of the frequency of lymphocytes with micronuclei on the dose to the bone marrow was observed. Statistically significant decrease in the telomere length was found in the exposed individuals (for the chromosome arms 1q, 3p, 3q, 20p, 20q, 13q, 15p, 22q ($p < 0.05$); for chromosome arms 19p, 21q ($p < 0.01$)). In the studied groups the value of the relative length of the telomere regions was statistically significantly higher in men than that in

women (for chromosome arms 1q, 3p, 20q, 13q, 15q, 21q). We cannot exclude the fact that the selection criteria of the elderly donors for the cytogenetic study that is performed many years after the onset of exposure could promote the inclusion into the study group the most radio-resistant exposed people, and it can have counterintuitive results.

References

1. Akleev AV, editor. Posledstviya radioaktivnogo zagryazneniya reki Techa. Chelyabinsk: Kniga, 2016; p. 400. Russian.
2. Aunan J, Watson MM, Hagland HR, et al. Molecular and biological hallmarks of ageing. British Journal of Surgery. 2016; 103 (2): 29–46.
3. Richardson R. Ionizing radiation and aging: rejuvenating an old idea. Aging. 2009; 1 (11): 887–902.
4. Little MP, Brenner AV, Grant E J, et al. Age effects on radiation response: summary of a recent symposium and future perspectives. International Journal of Radiation Biology. 2022; 2: 1–11.
5. López-Otín C, Blasco MA, Partridge L. The hallmarks of aging. Cell. 2013; 153 (6); 1194–217.
6. Bauchinger M. Quantification of low-level radiation exposure by conventional chromosome aberration analysis. Mutation Research. 1995; 339: 177–89.
7. Lyubimova NE, Vorobtsova IE. The Effect of Age and Low-Dose Irradiation on the Chromosomal Aberration Frequency in Human Lymphocytes. Radiation Biology. Radioecology. 2007; 47 (1): 80–5. Russian.
8. Sevankaev AV, Khvostunov IK, Snigiryova GP, et al. Comparative Analysis of Cytogenetic Examination of Control Groups of Subjects Carried out in Different Russian Laboratories. Radiation Biology. Radioecology. 2013; 53 (1): 5–24. Russian.
9. Sigurdson A, Hauptmann M, Bhatti P, et al. International study of factors affecting human chromosome translocations. Mutation Research. 2008; 652 (2): 112–21.
10. Fenech M, Bonassi S. The effect of age, gender, diet and lifestyle on DNA damage measured using micronucleus frequency in human peripheral blood lymphocytes. Mutagenesis. 2011; 26 (1): 43–9.
11. Olovnikov AM. Starenie est' rezul'tat ukorocheniya «differoteny» v telomere iz-za kontsevoy nedoreplikatsii i nedoreparatsii DNK. Izvestiya AN SSSR, Ser. biol. 1992; 4: 641–3. Russian.
12. Aubert G, Lansdorp PM. Telomeres and aging. Physiological Reviews. 2008; 88 (2): 557–79.
13. Degteva MO, Napier BA, Tolstykh EI, et al. Enhancements in the Techa River Dosimetry System: TRDS-2016 D code for reconstruction of deterministic estimates of dose from environmental exposures. Health Physics. 2019; 117 (4): 378–87.
14. IAEA. Cytogenetic dosimetry: applications in preparedness for and response to radiation emergencies. Vienna, Austria: IAEA, 2011; p. 229.
15. Fenech M. Cytokinesis-block micronucleus cytome assay. Nat Protoc. 2007; 2 (5): 1084–104.
16. Krivoshchapova YaV, Vozilova AV. The study of the telomere length of the chromosomes in T-lymphocytes of the exposed individuals. Radiation Safety Problems. 2022; 3 (107): 71–9. Russian.
17. King RC, Stansfield WD, Mulligan PK. A Dictionary of genetics. 7th ed. Oxford University Press, 2006; p. 608.
18. Pilinskaya MA. Tsitogeneticheskie effekty v somaticheskikh kletkakh lits, postradavshikh vsledstvie Chernobyl'skoy katastrofy, kak biomarker deystviya ioniziruyushchikh izlucheniy v malykh dozakh. Mezhdunarodnyy zhurnal radiatsionnoy meditsiny. 1999; 2: 60–6. Russian.
19. Fenech M, Kirsch-Volders M, Natarajan A, et al. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis. 2011; 26 (1): 125–32.
20. Akhmadullina YuR. Study of the age dependence of the spontaneous frequency of lymphocytes with micronuclei in residents of the South Urals. Environment and Human: Ecological Studies. 2021; 11 (2): 230–45. Russian.
21. Fenech M, Neville S, Rinaldi J. Sex is an important variable affecting spontaneous micronucleus frequency in cytokinesis-blocked lymphocytes. Mutation Research. 1994; 313: 203–7.
22. Bolognesi C, Abbondandolo A, Barale R, et al. Age-related increase of baseline frequencies of sister chromatid exchanges, chromosome aberrations, and micronuclei in human lymphocytes. Cancer Epidemiology. 1997; 6 (4): 249–56.
23. Jones KH, York TP, Juusola J, et al. Genetic and environmental influences on spontaneous micronuclei frequencies in children and adults: a twin study. Mutagenesis. 2011; 26 (6): 745–52.
24. Akhmadullina YuR. Sostav mikroyader v T-limfotsitakh u zhenshchin, podvergshikhsya khronicheskomu radiatsionnomu vozdeystviyu. Radiation Biology. Radioecology. 2022; 62 (6): 591–601. Russian.
25. Vozilova AV, Krivoshchapova YaV. Investigation of the Frequency of Inversions and Complex Translocations in T-Lymphocytes in Irradiated Residents of the Southern Urals. Radiation Biology. Radioecology. 2022; 62 (4): 408–15. Russian.
26. Ning Y, Xu JF, Li Y, et al. Telomere length and the expression of natural telomeric genes in human fibroblasts. Human Molecular Genetics. 2003; 12 (11): 1329–36.
27. Ferrucci L, Gonzalez-Freire M, Fabbri E, et al. Measuring biological aging in humans: A quest. Aging Cell. 2020; 19 (2): e13080.
28. Shaffer LG, Tommerup N, editors. An International System for Human Cytogenetic Nomenclature (2005) («ISCN 2005»). Recommendations of the International Standing Committee on Human Cytogenetic Nomenclature. Basel: S. Karger, 2005; p. 132.
29. Ilyenko I, Lyaskivska O, Bazyka D. Analysis of relative telomere length and apoptosis in humans exposed to ionising radiation. Exp Oncol. 2011; 33 (4): 235–8.
30. Lustig A, Shterev I, Geyer S, et al. Long term effects of radiation exposure on telomere lengths of leukocytes and its associated biomarkers among atomic-bomb survivors. Oncotarget. 2016; 7 (26): 38988.
31. Reste J, Zvigule G, Zvagule T, et al. Telomere length in Chernobyl accident recovery workers in the late period after the disaster. Journal of Radiation Research. 2014; 55 (6): 1–12. DOI: 10.1093/jrr/rru060.
32. Berardinelli F, Nieriab D, et al. Telomere loss, not average telomere length, confers radiosensitivity to TK6-irradiated cells. Mutation Research. 2012; 740: 13–20.
33. Vozilova AV. Assessment of the effect of chronic exposure on premature aging of human T-lymphocytes based on unstable chromosome aberrations. Extreme medicine. 2023; 2: 85–90. Russian.

Литература

1. Аклеев А. В., редактор. Последствия радиоактивного загрязнения реки Теча. Челябинск: Книга, 2016; 400 с.
2. Aunan J, Watson MM, Hagland HR, et al. Molecular and biological hallmarks of ageing. British Journal of Surgery. 2016; 103 (2): 29–46.
3. Richardson R. Ionizing radiation and aging: rejuvenating an old idea. Aging. 2009; 1 (11): 887–902.
4. Little MP, Brenner AV, Grant E J, et al. Age effects on radiation response: summary of a recent symposium and future perspectives. International Journal of Radiation Biology. 2022; 2: 1–11.
5. López-Otín C, Blasco MA, Partridge L. The hallmarks of aging. Cell. 2013; 153 (6); 1194–217.

6. Bauchinger M. Quantification of low-level radiation exposure by conventional chromosome aberration analysis. Mutation Research. 1995; 339: 177–89.
7. Любимова Н. Е., Воробцова И. Е. Влияние возраста и низкодозового облучения на частоту хромосомных аберраций в лимфоцитах человека. Радиационная биология. Радиоэкология. 2007; 47 (1): 80–5.
8. Севанькаев А. В., Хвостунов И. К., Снигирёва Г. П. и др. Сравнительный анализ результатов цитогенетических обследований контрольных групп лиц в различных отечественных лабораториях. Радиационная биология. Радиоэкология. 2013; 53 (1): 5–24.
9. Sigurdson A, Hauptmann M, Bhatti P, et al. International study of factors affecting human chromosome translocations. Mutation Research. 2008; 652 (2): 112–21.
10. Fenech M, Bonassi S. The effect of age, gender, diet and lifestyle on DNA damage measured using micronucleus frequency in human peripheral blood lymphocytes. Mutagenesis. 2011; 26 (1): 43–9.
11. Оловников А. М. Старение есть результат укорочения «дифферотены» в теломере из-за концевой недорепликации и недорепарации ДНК. Известия АН СССР, Сер. биол. 1992; 4: 641–3.
12. Aubert G, Lansdorp PM. Telomeres and aging. Physiological Reviews. 2008; 88 (2): 557–79.
13. Degteva MO, Napier BA, Tolstykh EI, et al. Enhancements in the Techa River Dosimetry System: TRDS-2016 D code for reconstruction of deterministic estimates of dose from environmental exposures. Health Physics. 2019; 117 (4): 378–87.
14. IAEA. Cytogenetic dosimetry: applications in preparedness for and response to radiation emergencies. Vienna, Austria: IAEA, 2011; p. 229.
15. Fenech M. Cytokinesis-block micronucleus cytome assay. Nat Protoc. 2007; 2 (5): 1084–104.
16. Кривощапова Я. В., Возилова А. В. Исследование длины теломерных районов хромосом в Т-лимфоцитах облученных лиц. Вопросы радиационной безопасности. 2022; 3 (107): 71–9.
17. King RC, Stansfield WD, Mulligan PK. A Dictionary of genetics. 7th ed. Oxford University Press, 2006; p. 608.
18. Пилинская М. А. Цитогенетические эффекты в соматических клетках лиц, пострадавших вследствие Чернобыльской катастрофы, как биомаркер действия ионизирующих излучений в малых дозах. Международный журнал радиационной медицины. 1999; 2: 60–6.
19. Fenech M, Kirsch-Volders M, Natarajan A, et al. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis. 2011; 26 (1): 125–32.
20. Ахмадуллина Ю. Р. Изучение возрастной зависимости спонтанной частоты лимфоцитов с микроядрами у жителей Южного Урала. Социально-экологические технологии. 2021; 11 (2): 230–45.
21. Fenech M, Neville S, Rinaldi J. Sex is an important variable affecting spontaneous micronucleus frequency in cytokinesis-blocked lymphocytes. Mutation Research. 1994; 313: 203–7.
22. Bolognesi C, Abbondandolo A, Barale R, et al. Age-related increase of baseline frequencies of sister chromatid exchanges, chromosome aberrations, and micronuclei in human lymphocytes. Cancer Epidemiology. 1997; 6 (4): 249–56.
23. Jones KH, York TP, Juusola J, et al. Genetic and environmental influences on spontaneous micronuclei frequencies in children and adults: a twin study. Mutagenesis. 2011; 26 (6): 745–52.
24. Ахмадуллина Ю. Р. Состав микроядер в Т-лимфоцитах у женщин, подвергшихся хроническому радиационному воздействию. Радиационная биология. Радиоэкология. 2022; 62 (6): 591–601.
25. Возилова А. В., Кривощапова Я. В. Исследование частоты инверсий и комплексных транслокаций в Т-лимфоцитах у облученных жителей Южного Урала. Радиационная биология. Радиоэкология. 2022; 62 (4): 408–15.
26. Ning Y, Xu JF, Li Y, et al. Telomere length and the expression of natural telomeric genes in human fibroblasts. Human Molecular Genetics. 2003; 12 (11): 1329–36.
27. Ferrucci L, Gonzalez-Freire M, Fabbri E, et al. Measuring biological aging in humans: A quest. Aging Cell. 2020; 19 (2): e13080.
28. Shaffer LG, Tommerup N, editors. An International System for Human Cytogenetic Nomenclature (2005) («ISCN 2005»). Recommendations of the International Standing Committee on Human Cytogenetic Nomenclature. Basel: S. Karger, 2005; p. 132.
29. Ilyenko I, Lyaskivska O, Bazyka D. Analysis of relative telomere length and apoptosis in humans exposed to ionising radiation. Exp Oncol. 2011; 33 (4): 235–8.
30. Lustig A, Shterev I, Geyer S, et al. Long term effects of radiation exposure on telomere lengths of leukocytes and its associated biomarkers among atomic-bomb survivors. Oncotarget. 2016; 7 (26): 38988.
31. Reste J, Zvigule G, Zvagule T, et al. Telomere length in Chernobyl accident recovery workers in the late period after the disaster. Journal of Radiation Research. 2014; 55 (6): 1–12. DOI: 10.1093/jrr/rru060.
32. Berardinelli F, Nieriab D, et al. Telomere loss, not average telomere length, confers radiosensitivity to TK6-irradiated cells. Mutation Research. 2012; 740: 13–20.
33. Возилова А. В. Оценка влияния хронического облучения на преждевременное старение Т-лимфоцитов человека на основе нестабильных хромосомных аберраций. Медицина экстремальных ситуаций. 2023; 2: 85–90.