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HUMAN GENETICS

Study of the DNA Damage in Peripheral Blood Lymphocytes Using Micronucleus Test in Residents of the Techa Riverside Villages Who Were Chronically Exposed in Utero and Postnatally

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Abstract—The paper presents the results of the assessment of the frequency of the peripheral blood T lymphocytes with micronuclei in Techa riverside residents who were chronically exposed in the 1950s. The study was performed 40–60 years after the onset of exposure. The exposed persons consisted of two groups: individuals who were first exposed in utero and then postnatally, and individuals who had only postnatal exposure. Cumulative dose to RBM in exposed persons varied within the range 0.001–4 Gy. A comparison group was also formed. It included individuals comparable in age, sex, and living conditions, but these people were not affected by accidental exposure. Findings of the study demonstrated that the frequency of lymphocytes with micronuclei was significantly higher in exposed women as compared to exposed men. The frequency of lymphocytes with micronuclei was significantly lower in those exposed in utero relative to the postnatally exposed persons and members of the comparison group. This decrease was observed both in women and in men. The study of the contribution of the cumulative dose to RBM revealed an increase in frequency of the lymphocytes with micronuclei in women exposed at doses of 0.1–0.49 Gy.

Keywords: micronuclei, micronucleus test, chronic exposure, residents of the Techa riverside villages, in utero exposure

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INTRODUCTION

Currently, the long-term effects of chronic human exposure are a topical issue in radiation biology. It is worth noting that the data on the long-term effects of ionizing radiation on the embryo and fetus are scarce and limited to the results of examination of children born to women who underwent radiation therapy during pregnancy, as well as to monitoring the health of in utero irradiated individuals during the atomic bombing of Hiroshima and Nagasaki, Japan [1, 2]. As is known, most of the effects of irradiation are based on damage to nuclear DNA. There is convincing evidence in the literature that DNA mutations can affect the chromatin structure, which is realized in a change in the expression of neighboring genes [3].

In Chelyabinsk oblast in the period from 1949 to 1956, the Mayak Production Plant carried out the discharge of liquid radioactive waste into the Techa River, as a result of which residents of coastal villages were exposed to chronic low-intensity radiation exposure. Irradiation was combined: external gamma radiation and internal radiation mainly due to incorporation of ^{89,90}Sr and ^{137}Cs. The population of these people has been examined by the specialists at the Urals Research Center for Radiation Medicine for more than 60 years [4, 5]. Studies affecting the long-term period of radiation exposure indicate an increased mutation process in the somatic cells of the subjects. For example, the level of unstable chromosomal aberrations and TCR mutations in peripheral blood lymphocytes is increased in irradiated people [6, 7]. In addition, it was found that, in the cohort of the Techa River, the risk of developing certain types of malignant neoplasms (malignant neoplasms) and leukemia was increased [8]. Recently conducted epidemiological studies in a cohort of intrauterine irradiated residents of the Techa River revealed an increase in the excess relative risk of malignant necrosis with an increase in the postnatal radiation dose [9].

Among the affected population, one can separately distinguish a group of individuals whose irradiation began in the prenatal period and continued postnatally. In those conditions, the embryo and fetus were affected by uniform external gamma radiation. Internal irradiation was formed owing to uniform distribution ^{137}Cs received with locally produced food and river water into the body of a pregnant woman, and

Table 1. Characteristics of the subjects

Group	Age years	Sex				Ethnic group			
		men		women		Slavs		Tatars, Bashkirs	
		abs.	%	abs.	%	abs.	%	abs.	%
Exposed persons $n = 536$	66.6 ± 0.3 (45–90)	211	39.4	325	60.6	225	42	311	58
Prenatally irradiated $n = 118$	59.5 ± 0.3 (45–66)	44	37.3	74	62.7	53	45	65	55
Postnatally irradiated $n = 418$	68.6 ± 0.3 (51–90)	167	40	251	60	172	41	246	59
Comparison group $n = 300$	66.2 ± 0.4 (46–84)	93	31	207	69	159	53	141	47

uneven irradiation of the red bone marrow (RBM) due to the accumulation of ^{89,90}Sr in the bone tissue of the fetus [10].

All of the above indicates that currently the relevance of evaluating the genotoxic effects of radiation exposure in somatic cells of irradiated individuals remains. One of the recognized methods is the study of DNA damage using the micronucleus test [11].

The purpose of our study was to study the initial level of peripheral blood T lymphocytes with micronuclei in people exposed to chronic radiation (intrauterine and postnatal) exposure in the Southern Urals in the long term after the start of radiation exposure.

MATERIALS AND METHODS

Characteristics of the Surveyed Groups

The study was conducted among residents of the coastal villages of the Techa River that have been exposed to chronic radiation exposure.

Criteria for inclusion in the study group: permanent residence in one of the villages located on the coast of the Tech River from January 1, 1950 (the beginning of radioactive discharges in the Techa River) to December 31, 1960 (completion of the formation of the main part of the cumulative radiation dose); date of birth before December 30, 1960. The exclusion criteria from the study were the following: the presence of oncological, autoimmune, acute or chronic (exacerbation period) inflammatory diseases, hemoblastoses, renal or hepatic insufficiency, acute cerebrovascular disorder during the last three months; head injuries; taking antibiotics, glucocorticoids, and cytostatics in the last six months before the study; X-ray examination in the last six months.

The characteristics of the studied groups are presented in Table 1. The group of people irradiated during the period of intrauterine and postnatal development was 118 people (the group of intrauterine irradiated persons). The average age per year of examination was 59.5 ± 0.3 years (range 45–66 years). The average dose from intrauterine (0.03 ± 0.005 Gy) and postnatal (0.28 ± 0.05 Gy) RBM irradiation totaled 0.32 ± 0.05 Gy. The average dose in prenatally irradiated women was 0.30 ± 0.06 Gy; in prenatally irradiated men, 0.36 ± 0.08 Gy.

The group of postnatally exposed individuals was 418 people. This group included people born from 1914 to 1949. The average age of patients at the time of examination was 68.6 ± 0.3 years (range 51–90 years). The average absorbed dose to RBM was 0.83 ± 0.03 Gy. The average dose in postnatally irradiated women was 0.88 ± 0.05 Gy; in postnatally irradiated men, 0.76 ± 0.04 Gy.

The comparison group included 300 unirradiated persons born from 1917 to 1960, living in socioeconomic conditions similar to the group of exposed individuals. Their average age was 66.2 ± 0.4 years (range 46–84 years). They were not exposed to the hazardous effects of ionizing radiation.

Table 2 presents dosimetric information that was obtained in the biophysical laboratory of the Ural Research Center for Radiation Medicine. Cumulative doses to RBM were calculated on the basis of the TRDS 2016 dosimetric system [12].

Blood samples for the study were taken from patients of the clinical department of the Ural Research Center for Radiation Medicine in accordance with applicable international standards (Helsinki Declaration of 1964) and with the permission of the local ethics committee of the Ural Research Center for Radiation Medicine on the basis of voluntarily signed individual informed consent. Detailed information about donors was obtained from the Human Database of the Ural Research Center for Radiation Medicine.

Micronucleus Test and Preparation of Specimens

The protocol of the micronucleus test procedure included several stages: culturing peripheral blood

Table 2. Cumulative dose to RBM, Gy

Group	$M \pm$ SE	Min–max
Exposed Persons	0.73 ± 0.03	0.001–4.0
Prenatally irradiated	Prenatal radiation:	
	0.03 ± 0.005	0.00005–0.21
	Postnatal radiation:	
	0.28 ± 0.05	0.0001–2.23
	Amount:	
	0.32 ± 0.05	0.001–2.3
Postnatally irradiated	0.83 ± 0.03	0.006–4.0

T lymphocytes, blocking cytokinesis, hypotonic treatment, fixing a suspension of cells, and then preparing specimens [13].

For the study, we used samples of whole blood taken from the ulnar vein into a syringe with heparin. Preparation of working solutions and formulation of cell culture was performed under sterile conditions. Cultural vials were prepared for blood samples, each of which contained 3.3 mL of RPMI 1640 medium (Paneco, Russia), 1 mL of fetal calf blood serum (RAA Laboratories, Austria), 0.7 mL of peripheral blood, and 7 μg/mL PHA (Paneco, Russia). Vials were incubated in a CO_2 incubator at 37°C and CO_2 concentration of 5%. After 48 h from the start of incubation, 70 μL of a working solution of cytochalazine B (United States) in DMSO was added to the samples (4.5 μg/mL). After 72 h from the start of incubation, the samples were subjected to hypotonic treatment with a KCl solution (0.125 M) and fixation with a mixture of ethanol and glacial acetic acid in a ratio of 3 : 1. Then, specimens were prepared from a fixed cell suspension, which, after drying, were stained with a 2% Romanowsky–Giemsa dye for 2 h.

Peripheral blood lymphocytes with micronuclei were evaluated under 10 × 100 light microscopy using an AxioImager microscope (Germany). Cell counting was carried out using the protocol of analysis of lymphocytes with micronuclei developed by the International Collaborative Project on Micronucleus Frequency in Human Populations (HUMN) [14]. We estimated the number of lymphocytes with micronuclei per 1000 analyzed binuclear cells.

Statistical Processing Methods

Statistical processing of the obtained data was carried out using standard methods of descriptive statistics with the calculation of the median and 25th and 75th percentiles. The figures also represent the median and the 25th and 75th percentiles. Since the frequency distribution of cells with micronuclei was not normal, groups were compared using the Mann–Whitney U test. The null hypothesis of the absence of differences between the compared groups was rejected when $p \le 0.05$ and the alternative hypothesis of the presence of statistically significant differences was accepted [15]. Statistical processing of data was performed using the spreadsheet editor Microsoft Excel 2010, as well as application packages SPSS Statistics 21 and SigmaPlot 14.0.

RESULTS

The frequency of lymphocytes with micronuclei in the examined groups is presented in Table 3. In the group of exposed individuals, the frequency of cells with micronuclei was 15‰ (10–21‰), which was comparable to the group of unirradiated individuals—16‰ (11–21‰). It is noteworthy that, in prenatally irradiated people, the frequency of lymphocytes with micronuclei (11‰) was significantly lower than in postnatally irradiated (16‰) and unirradiated people (16‰), $p = 0.0001$.

Since the age range was narrower in the group of prenatally irradiated individuals, and the average age was less than that in the groups of postnatally irradiated and unirradiated individuals, the revealed differences could have been determined by age. In order to confirm or reject this hypothesis, we formed a coeval group of prenatally irradiated individuals and a comparison group, and then we compared the median values of cell frequency with micronuclei. These results are shown in Table 4. The frequency of lymphocytes with micronuclei in the comparison group remained significantly higher ($p = 0.001$) than in the group of prenatally irradiated individuals. The results of the analysis allowed us to conclude that age was not a causative factor in the differences in the frequency of cells with micronuclei in the group of prenatally irradiated people and the comparison group.

Figure 1 shows the frequency of lymphocytes with micronuclei depending on the gender of donors. As can be seen, in irradiated men, the frequency of cells with micronuclei is significantly reduced relative to irradiated women, $p = 0.0001$. In the group of unnirradiated men, the frequency of cells with micronuclei

Table 3. The frequency of cells with micronuclei, ‰

Group	Median (25–75%)	
Exposed Persons	15 (10–21)	
Prenatally irradiated	11 (8–17)	$p_{1,2} = 0.0001$
Postnatally irradiated	16 (11–23)	
Comparison group	16 (11–21)	

p_1—statistically significant differences from the group of postnatally exposed individuals; p_2—statistically significant differences from the comparison group.

Table 4. The frequency of cells with micronuclei in the group of prenatally irradiated individuals and the comparison group of a similar age, ‰

Group	Age, years	Median (25–75%)
Prenatally irradiated $n = 118$	59.5 ± 0.3 45–66	11 (8–17) $p = 0.001$
Comparison group $n = 122$	59.6 ± 0.3 45–66	15 (10–21)

p—statistically significant differences from the comparison group.

was also reduced relative to women, but no statistical significance was revealed, $p = 0.131$.

Tables 5 and 6 present the results of a study of the frequency of lymphocytes with micronuclei separately in women and men. As can be seen from Table 5, in the study of all irradiated women, there were no differences from the comparison group. When considering prenatally irradiated women, the frequency of lymphocytes with micronuclei was significantly lower than that of postnatally irradiated and unirradiated women (12‰ versus 18 and 16‰, respectively, $p = 0.0001$ and $p = 0.004$). In postnatally irradiated women, the frequency of cells with micronuclei is significantly higher than in unirradiated women (18 vs. 16‰, $p = 0.04$).

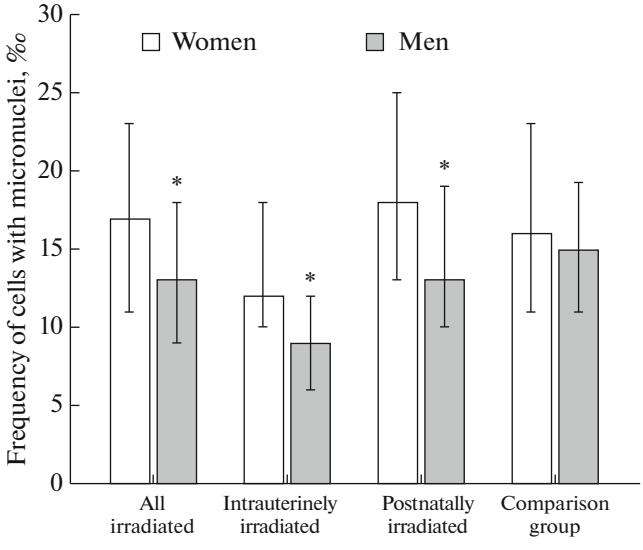

Fig. 1. The frequency of lymphocytes with micronuclei depending on gender (median and 25–75%). *—statistically significant differences between women and men.

In the group of irradiated men, the frequency of cells with micronuclei was lower than in unirradiated men (Table 6). In prenatally irradiated men, lymphocytes with micronuclei were less common than in the group of postnatally irradiated men; the same patterns were obtained when compared with the comparison group (9 vs. 13 and 15‰, respectively, $p = 0.0001$).

The results of a study of the frequency of lymphocytes with micronuclei depending on the cumulative radiation dose to RBM are presented in Figs. 2 and 3. As can be seen from Fig. 2, the frequency of lymphocytes with micronuclei was significantly increased in those women whose doses to RBM were in the range from 0.1 to 0.49 Gy (relative to the comparison group and the lowest dose range, $p = 0.03$ and $p = 0.02$). Figure 3 shows that in men there were no significant differences in the frequency of lymphocytes with micronuclei depending on the dose of ionizing radiation to RBM.

DISCUSSION

Investigations devoted to the study of distant cytogenetic effects in people exposed to chronic radiation exposure as a result of living in coastal villages of the Techa River have been conducted at the Ural Research Center for Radiation Medicine for more than 50 years. The irradiated population showed a higher level of unstable chromosomal aberrations (UCA) compared with the comparison group. And the highest level of UCA was found in individuals with a history of chronic radiation syndrome [6]. In addition to classical cytogenetics methods, a micronuclear test was used to examine the irradiated population to assess the

Table 5. The frequency of cells with micronuclei in women, ‰

Group	Median (25–75%)	
Exposed Persons	17 (11–23)	
Prenatally irradiated	12 (10–18)	$p_1 = 0.0001$ $p_2 = 0.004$
Postnatally irradiated	18 (13–25)	$p_2 = 0.04$
Comparison group	16 (11–23)	

p_1—statistically significant differences from the group of postnatally exposed individuals; p_2—statistically significant differences from the comparison group.

Table 6. The frequency of cells with micronuclei in men, ‰

Group	Median (25–75%)	
Exposed Persons	13 (9–18)	$p_2 = 0.039$
Prenatally irradiated	9 (6–12)	$p_1 = 0.0001$ $p_2 = 0.0001$
Postnatally irradiated	13 (10–19)	
Comparison group	15 (11–19)	

p_1—statistically significant differences from the group of postnatally exposed individuals; p_2—statistically significant differences from the comparison group.

adaptive capabilities of cell systems [16]. In these studies, it was shown that, in residents of radioactively contaminated regions, the initial level of micronuclei in T lymphocytes did not differ from the comparison group, and the radiosensitivity and adaptive response ability of irradiated T cells was significantly reduced.

In our study, when considering the group of all irradiated individuals, no differences were found in the frequency of lymphocytes with micronuclei from the comparison group. Since the sample included individuals whose irradiation began in utero and continued postnatally and those whose irradiation affected only the postnatal period, we examined the frequency of cells with lesions in each of these groups. In the group of prenatally irradiated individuals, the frequency of lymphocytes with micronuclei was 11‰, which is significantly lower than in the group of postnatally exposed individuals and in the comparison group (in both groups, the median frequency of lymphocytes with micronuclei was 16‰). These differences were not related to the age of patients, since when selecting individuals from the comparison group

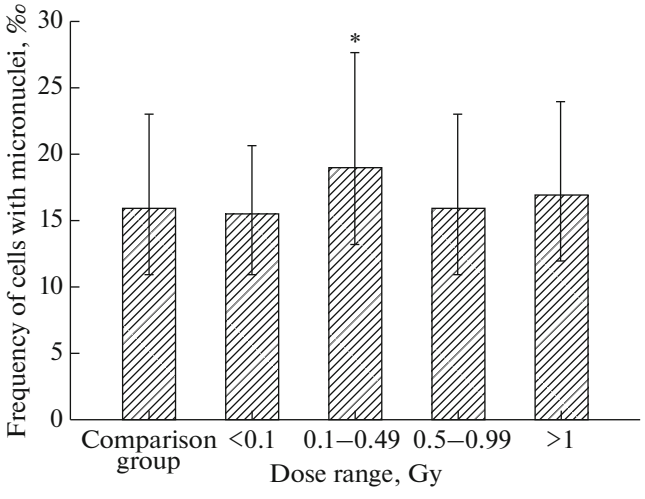

Fig. 2. The frequency of lymphocytes with micronuclei in women in different dose ranges (median and 25–75%). *—statistically significant differences from the comparison group and the group "<0.1."

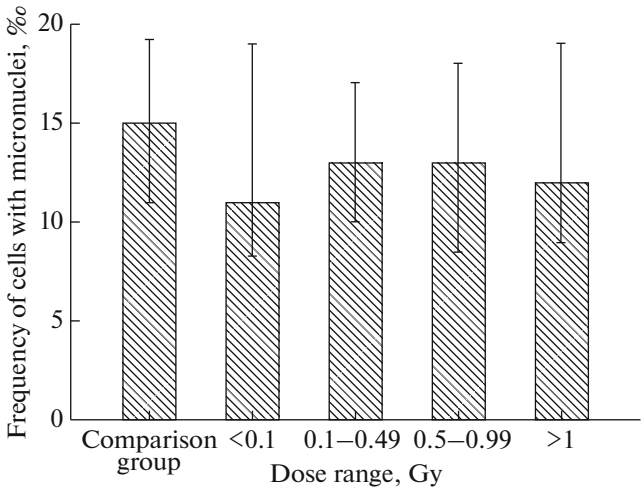

Fig. 3. The frequency of lymphocytes with micronuclei in men in different dose ranges (median and 25–75%).

of the same age with prenatally irradiated individuals, they remained ($p = 0.001$).

It is known that sex can affect the frequency of micronuclei formation [17]. When comparing the frequency of cells with micronuclei in women, there were more of them than in men (Fig. 1). These results are consistent with published data. When studying the mechanisms of micronucleus formation, FISH staining of the chromosomal material is used, owing to which it becomes clear what DNA material the micronuclei contain. So, in a study [18], it was shown that 72% of micronuclei in women contain the X chromosome. Some studies indicate that women most often have an inactivated X chromosome in micronuclei [19].

Since the frequency of lymphocytes with micronuclei depends on gender, we compared the results for irradiated and unirradiated individuals according to gender. The tendency for a decrease in lymphocytes with micronuclei in prenatally irradiated individuals of different sexes has persisted. In prenatally irradiated women and men, the frequency of cells with micronuclei was significantly lower than in the corresponding gender comparison groups.

Thus, we see a clear pattern of a decrease in the frequency of lymphocytes with micronuclei in prenatally irradiated individuals. In the scientific literature, information on distant cytogenetic effects in prenatally irradiated people is scarce. In the study of the Japanese population which was exposed to prenatal radiation 40 years ago, no increased frequencies of chromosomal aberrations were detected, contrary to the authors' expectations. A slight increase in the frequency of chromosomal aberrations (less than 1%) was observed in irradiated patients at doses below 0.1 Sv. To interpret the results, the authors of [20] proposed the following hypothesis. The fetus has two subpopulations of progenitor cells of lymphoid origin, which differ in radiosensitivity. One of the subpopulations is extremely sensitive to the induction of chromosomal translocations and cell death; at doses above 50 mSv, these cells most often die quickly. The other subpopulation of cells is more radioresistant to the induction of chromosomal translocations and death, possibly because of the good repair ability of the cells. The authors suggest that the hypothesis will help resolve the debate that a significant risk of leukemia in children is associated with prenatal X-ray irradiation at low doses, while animal studies, which mainly include exposure to high doses, usually do not confirm this [20].

In the study of the relative risk of cancer in prenatally exposed residents of the coastal villages of the Techa River, there was no conclusive evidence that chronic low-dose exposure of the embryo and fetus increased the risk of developing solid cancer in childhood or adulthood. Both in relation to morbidity and mortality, there was a tendency toward a decrease in relative risk with an increase in the dose to the fetal soft tissues. The authors of [21] suggest that the effects can be explained by the induction of lethal genetic changes at higher doses of prenatal exposure in embryo and fetal cells, which are capable of high proliferative activity, and the subsequent elimination of compromised offspring during the prenatal and early postpartum period.

It is noteworthy that, in postnatally irradiated women, the frequency of cells with micronuclei was higher than in the group of unirradiated women. Perhaps, these results are associated with an increase in the frequency of violations of X-chromosome segregation and its incorporation into the micronucleus, as indicated above. Also, we cannot exclude the contribution of long-lived subpopulations of T lymphocytes, which can enter their first post-radiation mitosis many years after irradiation and retain cytogenetic markers of radiation exposure for many years [4].

When studying the frequency of cells with micronuclei in subgroups of people with different doses of radiation, it was found that, in irradiated women at doses of 0.1–0.49 Gy, this indicator was increased relative to unirradiated individuals and subgroups of people with the lowest doses of RBM irradiation (less than 0.1 Gy). Doses of 0.1–0.5 Gy are in the range of average doses at which various long-term effects of chronic exposure by epidemiological methods are recorded. It is also noteworthy that the effect was found only in women. Possibly, the revealed increase in the frequency of cells with micronuclei is associated with sex differences in human radiosensitivity [22].

The studies established the relationship of an increased frequency of micronuclei in binuclear T cells with chemical toxicants and some physical agents; in addition, there is a dependence on age, gender, diet, the use of certain pharmaceuticals and alcohol, and smoking [17]. In the case of the present study, which was carried out a long time after the start of radiation exposure in residents of the Techa River, the formation of micronuclei in peripheral blood lymphocytes can be determined both by the influence of ionizing radiation and by some of the above factors. Nevertheless, according to published data, ionizing radiation is a powerful genotoxicant and, depending on the radiation dose, can make a significant contribution to the development of long-term effects of radiation, such as radiation-induced genomic instability [23].

Thus, the initial level of peripheral blood T lymphocytes with micronuclei in prenatally irradiated residents of the coastal villages of the Techa River was significantly lower than in the group of postnatally exposed individuals and in the comparison group. The frequency of lymphocytes with micronuclei in irradiated women was significantly higher than in irradiated men. Women irradiated postnatally showed a higher frequency of lymphocytes with micronuclei compared to the group of unirradiated women. When studying the effect of the cumulative radiation dose to RBM, an increase in the frequency of lymphocytes with micronuclei was observed in women exposed to doses of 0.1–0.49 Gy.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in a study involving people are in accordance with the ethical standards of the institutional and/or national research ethics committee and the 1964 Helsinki Declaration and its subsequent changes or comparable ethical standards. All participants in the study signed an informed consent.

REFERENCES

1. Preston, D.L., Cullings, H., Suyama, A., Funamoto, S., et al., Solid cancer incidence in atomic bomb survivors exposed in utero or as young children, *J. Natl. Cancer Inst.*, 2008, vol. 100, no. 6, pp. 428—436. https://doi.org/10.1093/jnci/djn045
2. Boice, J.D., Jr. and Miller, R.W., Childhood and adult cancer after intrauterine exposure to ionizing radiation, *Teratology*, 1999, vol. 59, no. 4, pp. 227—233.
3. Gorbunova, V. and Seluanov, A., DNA double strand break repair, aging and the chromatin connection, *Mutat. Res., Genet. Toxicol. Environ. Mutagen.*, 2016, vol. 788, pp. 2—6. https://doi.org/10.1016/j.mrfmmm.2016.02.004
4. *Mediko-biologicheskie i ekologicheskie posledstviya radioaktivnogo zagryazneniya reki Techa* (Medical, Biological, and Environmental Consequences of Radioactive Pollution of the Techa River), Akleev, A.V. and Kiselev, M.F., Eds., Moscow: Medbioekstrem, 2001.
5. Pastukhova, E.I., Shalaginov, S.A., and Akleev, A.V., The frequency of multiple pregnancy among the population of radioactively contaminated regions in the Chelyabinsk oblast, *Vopr. Radiats. Bezop.*, 2011, no. 4(64), pp. 45—53.
6. Vozilova, A.V., Shagina, N.B., Degteva, M.O., and Akleyev, A.V., Chronic radioisotope effects on residents of the Techa River (Russia) region: cytogenetic analysis more than 50 years after onset of exposure, *Mutat. Res., Genet. Toxicol. Environ. Mutagen.*, 2013, vol. 756, nos. 1—2, pp. 115—118. https://doi.org/10.1016/j.mrgentox.2013.05.016
7. Akleev, A.V., Veremeeva, G.A., and Kyoizumi, S., Long-term effects of chronic radiation exposure on the level of somatic mutations in peripheral blood cells, *Radiats. Biol., Radioekol.*, 1998, vol. 38, no. 4, pp. 573—585.
8. Akleev, A., V., Krestinina, L.Yu., Preston, D., Devis, F., et al., Radiation risk of malignant neoplasms in residents of the riverside villages of the Tech River, *Med. Radiol. Radiats. Bezop.*, 2008, vol. 53, no. 4, pp. 13—37.
9. Krestinina, L.Yu., Kharyuzov, Yu.E., Epiphanova, S.B., Tolstykh, E.I., et al., Cancer incidence after in utero exposure to ionizing radiation in Techa river residents, *Radiat. Res.*, 2017, vol. 188, no. 3, pp. 314—324. https://doi.org/10.1667/RR14695.1
10. *Posledstviya radioaktivnogo zagryazneniya reki Techi* (Consequences of Radioactive Pollution of the Techa River), Akleev, A.V., Ed., Chelyabinsk: Kniga, 2016.
11. Fenech, M., The cytokinesis-block micronucleus technique and its application to genotoxicity studies in human populations, *Environ. Health Perspect.*, 1993, no. 3, pp. 101—107. https://doi.org/10.1289/ehp.93101s3101
12. Degteva, M.O., Shagina, N.B., Vorob'eva, M.I., et al., Contemporary view on radioactive pollution of the Techa River in 1949—1956, *Radiats. Biol., Radioekol.*, 2016, vol. 56, no. 5, pp. 523—534. https://doi.org/10.7868/S0869803116050039
13. Akhmadullina, Yu.R., Radiosensitivity of peripheral blood T-lymphocytes in first-generation offspring whose fathers were exposed to chronic radiation, *Cand. Sci. (Biol.) Dissertation*, Moscow, 2014.
14. Fenech, M., Chang, W.P., Kirsch-Volders, M., Holland, N., et al., HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures, *Mutat. Res.*, 2003, vol. 534, nos. 1—2, pp. 65—75. https://doi.org/10.1016/S1383-5718(02)00249-8
15. Glantz, S.A., *Primer of Biostatistics*, New York: McGraw—Hill, 1997, 4th ed.
16. Akleev, A.V., Aleshchenko, A.V., Gotlib, V.Ya., et al., Adaptive response of blood lymphocytes of the inhabitants of the South Ural chronically exposed to radiation, *Radiats. Biol., Radioekol.*, 2004, vol. 44, no. 4, pp. 426—431.
17. Fenech, M. and Bonassi, S., The effect of age, gender, diet and lifestyle on DNA damage measured using micronucleus frequency in human peripheral blood lymphocytes, *Mutagenesis*, 2011, vol. 26, no. 1, pp. 43—49. https://doi.org/10.1093/mutage/geq050
18. Tucker, J.D., Nath, J., and Hando, J.C., Activation status of the X chromosome in human micronucleated lymphocytes, *Hum. Genet.*, 1996, no. 4, pp. 471—475.
19. Norppa, H. and Falck, G.C., What do human micronuclei contain?, *Mutagenesis*, 2003, vol. 18, no. 3, pp. 221—233.
20. Ohtaki, K., Kodama, Y., Nakano, M., Itoh, M., et al., Human fetuses do not register chromosome damage inflicted by radiation exposure in lymphoid precursor cells except for a small but significant effect at low doses, *Radiat. Res.*, 2004, vol. 161, no. 4, pp. 373—379.
21. Akleyev, A., Deltour, I., Krestinina, L., Sokolnikov, M., et al., Incidence and mortality of solid cancers in people exposed in utero to ionizing radiation: pooled analyses of two cohorts from the Southern Urals, Russia, *PLoS One*, 2016, vol. 11, no. 8. https://doi.org/10.1371/journal.pone.0160372.eCollection
22. Alsbeih, G., Al-Meer, R.S., Al-Harbi, N., Bin Judia, S., et al., Gender bias in individual radiosensitivity and the association with genetic polymorphic variations, *Radiother. Oncol.*, 2016, vol. 119, no. 2, pp. 236—243. https://doi.org/10.1016/j.radonc.2016.02.034
23. Morgan, W.F., Radiation-induced genomic instability, *Health Phys.*, 2011, vol. 100, no. 3, pp. 280—281. https://doi.org/10.1097/HP.0b013e3182082f12