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Assessing the level of chromosome aberrations in peripheral blood lymphocytes in long-term resident children under conditions of high exposure to radon and its decay products

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Abstract

In this study, the frequency and spectrum of chromosomal aberrations were analysed in samples of peripheral blood from 372 (mean age = 12.24 ± 2.60 years old) long-term resident children in a boarding school (Tashtagol city, Kemerovo Region, Russian Federation) under conditions of high exposure to radon and its decay products. As a control group, we used blood samples from people living in Zarubino village (Kemerovo Region, Russian Federation). We discovered that the average frequencies of single and double fragments, chromosomal exchanges, total number of aberrations, chromatid type, chromosome type and all types of aberrations were significantly increased in the exposed group. This is evidence of considerable genotoxicity to children living under conditions of high exposure to radon compared to children living under ecological conditions without increased radon radiation.

Introduction

Estimating the biomedical long-term effect of small doses of ionising radiation is a complicated issue that affects not only radiobiology but also social and economic spheres. Greater than 60% of the ionising radiation a person receives each year can be caused by natural sources of radiation, and >50% of this radiation can be caused radon and its decay products (1). Therefore, maintaining radon safety is one of the most critical challenges in modern ecology and genetic toxicology.

Radon (^{222}Rn) is a naturally radioactive noble gas. It is generated from uranium, a chemical element that is widespread in the earth's crust. Radon is electrically neutral and is not itself a potential health threat, but its decay daughter products, ^{218}Po, ^{214}Po, ^{214}Pb and ^{214}Bi, are electrically charged and can affix themselves to tiny dust or smoke particles in indoor air. These particles can be inhaled into the lung where they may penetrate the epithelial cells that cover the bronchi and alveoli. These short-lived, unstable decay daughter products, particularly ^{218}Po and ^{214}Po, emit alpha particles that can interact with biological tissues in the lungs and induce DNA damage (2,3). There are many epidemiological studies on the connection between radon influence and some cases of cancer, particularly lung cancer (3–11).

However, it is important to note that current models estimating the risk of radiation-related hazards are based on the analysis of data collected from irradiated miners. Currently, it is not clear whether this risk model can be used for studying the inhabitants of domestic areas under high-radiation conditions (11).

Estimating the effects of radon exposure on the population in the territories of regions with developed mining industries is of particular interest. The Kemerovo Region is such a territory (12). Therefore, a portion of the population living in the coal-mining area may be exposed to a long-term radiation risk from radon and its decay products. In addition to several methods of radiation monitoring, the level and nature of such effects should be determined using biological indication methods. The World Health Organization recommends estimation of the frequency and spectrum of structural chromosome aberrations in cultured lymphocytes for the biological indication of the effects of radiation on human populations (13). The importance of these indicators of genotoxic effects is evidenced by

the finding that the frequency of chromosome mutations detected in somatic cells is a marker of susceptibility to cancer (14–18).

Materials and Methods

Group description

Blood samples were obtained from 372 (mean age = 12.24 ± 2.60 years old) long-term resident children in a boarding school (Tashtagol city, Kemerovo Region, Russian Federation). This area is characterised as a wood-mountain area with a low level of air pollution from chemical agents, such as polycyclic aromatic hydrocarbons and heavy metal salines, which may induce some cytogenetic abnormalities. We used the bioindicative methods of air and water analyses, including the Ames test to assess the air mutagenic activity and test to induce dominant lethal mutations in *Drosophila* with water and air samples, to exclude chemical mutagenesis factors.

This region is characterised by the intensive mining of minerals, such as iron ore and coal; therefore, some portions of the territory are contaminated by ash and slag (19). In addition, the measurement of radon volume activity performed in the rooms of the boarding school showed an excess critical concentration (>200 Bq/m^3) (Table 1).

The optimal opportunity to estimate the significance of a radiation factor as a potential modifier of the spontaneous level of chromosomal aberrations is achieved by the age requirement during the formation of a study group. From this perspective, the group of children and adolescents may be the most useful, and in this case, the influence of factors, such as bad habits and occupational exposure to industrial hazards, is minimised. In addition, the compact residence of all members of the sample permits the maximum similarity of the studied group for nutrition and living conditions.

In the control group, 186 children (mean age = 14.43 ± 2.58 years old) were included. They live in settlements without increased radon levels (Table 1). The gender and age characteristics are presented in Table 2. Nutrition for the control group has been similar as for the exposed group.

The children receiving medical treatment, as well as having received an X-ray examination 3 months prior to collection of the material, were not included in the study. For each person, informed consent signed by the parents or persons with custody of the minors was obtained. The research has been performed in accordance with the requirements of the Ethics Committee of the Kemerovo State University.

Radiological investigation

The level of external exposure dose of γ-radiation in the residential and public rooms in the residence and education areas of the children was measured during the collection of biological material. The measurements were performed by the staff of the state organization 'Skarabey' (Kemerovo, Russian Federation) that has a license to perform such measurements. The certified gamma radiation dosimeters DBH-04A, DKG-02U (Arbiter) (SPC «Doza», Ltd, Zelenograd, Russia) and search gamma-radiometer SRP-88 were used. All measurements conformed to the normative-methodological material (NRB-99/2009).

Measurements of the volume of radon activity in the air of the residential rooms and classrooms were performed using the radon radiometer PPA-01M-01 (Alfarad) («Zashita», Ltd, Moscow, Russia) in the Air 1 mode, which includes air sampling by a built-in blower for 3 min and analysis of the sample with the subsequent show of the integral value for 20 min. USK «Progress» (Yekaterinburg, Russia) with a set of carbon absorbers was used for the integrated assessment of radon concentration. The integral method is based on the exposure of the absorbers with activated charcoal for 1 day and then measuring *in vitro* of the activity of the radon adsorbed in the activated carbon. The individual effective dose was calculated by the following formula:

$$E = \sum d_i \times m_i \times C_i, \text{ mSv / year}$$

(d_i—dose coefficient for the i radionuclide in its preoral intake with foods; C_j—the average specific activity of the j radionuclide in the i component of the diet, Bq/kg; m_i—the average annual consumption of the i product, kg/year).

Cytogenetic investigation

Cytogenetic investigation was performed using the assessment of chromosome aberrations. The metaphase chromosomes were prepared using the standard method of lymphocytes cultivation (20). The whole blood obtained from the ulnar vein was cultivated. Volumes of 0.5 ml blood, 0.1 ml phytohaemagglutinin (PanEco, Moscow, Russia), 6 ml RPMI-1640 (PanEco) and 1.5 ml embryonic veal serum were added to a culture flask. The duration of the cultivation was 48 h. Then, colchicine at a final concentration of 0.5 μg/ml was added to the culture, and the flasks were placed in an incubator for 2 h. At the end of the cultivation cycle, the preparations were centrifuged for 10 min at 1000 rpm,

Table 1. The radon volume activity in buildings of the studied settlements

Settlement	Date of measurement	Number of measuring points	Average radon volume activity, Bq/m^3, mean ± standard error	Limit variation, Bq/m^3–Bq/m^3
Exposed group				
Tashtagol	20.12.2007	11	235 ± 44	68–583
Tashtagol	06.02.2008	6	415 ± 53	232–617
Tashtagol	13.05.2008	5	200 ± 42	101–334
Tashtagol	04.02.2009	7	730 ± 77	192–1285
Tashtagol	11.02.2009	22	441 ± 88	110–1373
Tashtagol	02.03.2010	10	905 ± 134	680–1143
Tashtagol	02.03.2011	18	347 ± 101	74–749
Control group				
Krasnoye village	25.01.2008	12	106 ± 18	39–203
Pacha village	16.05.2008	6	64 ± 22	20–135
Zarubino village	14.03.2011	10	119 ± 33	39–203
Zarubino village	25.01.2011	10	64 ± 13	39–203
Zarubino village	06.04.2011	17	119 ± 27	53–172

the supernatant was removed, the pellet was resuspended. The pellets were placed in a hypotonic solution of 0.55% KCI for 10–15 min at 37°C. The fixation of the material was performed in cooled fresh Carnoy's fixative (methanol and acetic acid in the ratio 3:1). The cell suspension was pipetted onto clean, cooled slides moistened with water. The preparations were encoded and stained with 2% Giemsa solution.

Counting of the aberrations was performed using light microscopy at ×1000 magnification (oil immersion) without karyotyping. The selection of metaphases included in analysis and criteria for cytogenetic abnormalities conformed to the generally accepted recommendations (20). We identified these cytogenetic abnormalities as, frequency of aberrant cells (%), frequency of aberrations/100 cells (%), average frequencies of single and double fragments (%), frequency of chromatid and chromosome interchanges (%), frequency of dicentric chromosomes with fragments (%), frequency of ring chromosomes (%) and translocations (%), number of aberrations chromatid and chromosome type (%), and rogue cells (%). On average, 225 metaphases (100–500) were analysed for each individual. The results were entered into an electronic database.

Assessment of the background mutagenic activity was performed using standard methods: the Ames test (21) and the assay for dominant lethal mutations (22).

Table 2. Gender and age of children/teenagers included in the exposed and control groups

Person	Number	Age (mean ± SD)	Age (min–max)
Exposed group			
Total	372	12.24 ± 2.60	8–18
Male	195	12.14 ± 2.63	8–18
Female	177	12.36 ± 2.58	8–18
Control group			
Total	186	14.43 ± 2.58	8–19
Male	83	13.90 ± 2.58	9–19
Female	103	14.85 ± 2.51	8–19

Statistical analysis

Statistical analysis was performed using the program StatSoft STATISTICA 6.0. We used the Kolmogorov–Smirnov test to verify the compliance of the data with the normal distribution. The data analysis was performed using the non-parametric statistics block. Group comparisons were performed using the *U*-rank Mann–Whitney test. Spearman's correlation coefficient was used to calculate the correlation.

Results

The average volume radon activity in the residential areas of the children of exposed group was 468 ± 77 Bq/m^3 during all years of the investigation. It exceeds the similar parameter for the control group (94 ± 23 Bq/m^3). Gamma background (from natural sources of radiation) in the exposed group was 11–18 microroentgen/h (control group: 12–14 microroentgen/hour). The individual effective dose inhalation exposure due to isotopes of radon and its short-lived decay products was ~27 mSv/year. Full results of the measurements of volume radon activity are presented in Table 1.

Air probes for the Ames test showed not mutagenic effects on cultures of *Salmonella typhimurium* TA98 and TA100. Full results are presented in Table 3. Test for the induction of dominant lethal mutations in *Drosophila melanogaster* showed an increased level of early embryonic lethality in the air probes from Tashtagol, which explains to occurrence of some agents that influence the physiological features of fly embryonic growth. Full results are presented in Table 4. The number of eggs laid after exposure to water from Tashtagol and Krasnoye village did not differ (23).

Results of the chromosome aberration studies in the cohort of long-term resident children under conditions of high radon exposure and the control group are presented in Table 5.

The primary cytogenetic indicator, the frequency of aberrant cells, was significantly increased in the exposed group (4.26 ± 2.47 vs. 2.74 ± 1.64%—in control group). In addition, there were also

Table 3. Results of the assessment of mutagenic activity in the air probes (the Ames test)

Conditional sample volume, m^3/Petri dish	The rate of excess average number of *Salmonella typhimurium* colonies per Petri dish in the experience to the negative control			
	TA98		TA100	
	Metabolic activation (+)	Metabolic activation (–)	Metabolic activation (+)	Metabolic activation (–)
Krasnoye village				
0.04	**1.23**	**1.06**	**0.58**	**1.08**
0.11	**1.21**	**1.06**	**0.82**	**0.95**
0.32	**0.98**	**0.90**	**0.84**	**0.96**
0.96	**1.17**	**0.77**	**0.84**	**0.83**
Tashtagol				
0.04	**1.29**	**0.62**	**0.91**	**0.82**
0.11	**1.46**	**1.06**	**0.86**	**1.11**
0.32	**1.25**	**1.02**	**0.75**	**0.86**
0.96	**0.90**	**1.15**	**0.92**	**0.98**
Positive control				
Ethidium bromide	**5.85**[a]			
DDDTDP ($C_{14}H_{10}N_4O_4$)		**1.28**[a]		
Sodium azide				**>17.39**[a]
Negative control (the average number of colonies per dish)				
5% DMSO	24.0	26.0	128.0	12.0

DMSO, dimethyl sulphoxide.
[a]Significant mutagenic effect.

Table 4. Results of the assessment of the level of early embryonic lethality in *Drosophila melanogaster* in the air probes

Settlement	Number of laid eggs	Early embryonic lethality		Late embryonic lethality	
		Number of non-hatching eggs			
		N	%	*N*	%
Tashtagol	1953	154	7.89	12	0.61
Krasnoye village	1729	46	2.66	16	0.93
Control (distilled water)	1034	13	2.74	5	0.37

Table 5. Frequency and spectrum of chromosome aberrations identified in the studied groups

Cytogenetic index, %	Exposed group, mean ± SD			Control group, mean ± SD		
	Total (n = 372)	Smoking (n = 58)	Non-smoking (n = 314)	Total (n = 186)	Smoking (n = 26)	Non-smoking (n = 160)
Frequency of aberrant cells	4.26 ± 2.47*	5.48 ± 2.35*	4.04 ± 2.43*	2.74 ± 1.64	2.69 ± 1.42	2.75 ± 1.67
Frequency of aberrations/100 cells	4.38 ± 2.57*	5.76 ± 2.52*	4.12 ± 2.50*	2.80 ± 1.71	2.71 ± 1.44	2.81 ± 1.75
Aberrations chromatid type	3.04 ± 2.20*	4.20 ± 2.19*	2.83 ± 2.14*	2.08 ± 1.28	1.96 ± 1.04	2.10 ± 1.32
Single fragments	3.02 ± 2.20*	4.16 ± 2.21*	2.82 ± 2.14*	2.05 ± 1.26	1.92 ± 0.98	2.07 ± 1.31
Chromatid interchanges	0.02 ± 0.12	0.04 ± 0.17	0.02 ± 0.10	0.03 ± 0.12	0.04 ± 0.14	0.03 ± 0.12
Aberrant chromosome type	1.33 ± 1.09*	1.54 ± 1.03*	1.30 ± 1.09*	0.72 ± 0.79	0.75 ± 0.67	0.71 ± 0.81
Double fragments	1.12 ± 0.94*	1.28 ± 0.84*	1.09 ± 0.96*	0.66 ± 0.78	0.67 ± 0.68	0.66 ± 0.79
Chromosome interchanges	0.22 ± 0.43*	0.26 ± 0.47*	0.21 ± 0.42*	0.06 ± 0.18	0.08 ± 0.19	0.05 ± 0.18
Dicentric chromosomes with fragment	0.04 ± 0.15	0.04 ± 0.14	0.04 ± 0.16	0.02 ± 0.11	0.02 ± 0.10	0.02 ± 0.12
Dicentric chromosomes without fragment	0.05 ± 0.17	0.04 ± 0.17	0.04 ± 0.16	0.01 ± 0.06	0	0.01 ± 0.09
Ring chromosomes	0.08 ± 0.31	0.09 ± 031	0.08 ± 0.30	0.01 ± 0.08	0.02 ± 0.10	0.01 ± 0.08
Translocations	0.05 ± 0.16	0.06 ± 0.19	0.04 ± 0.15	0.01 ± 0.10	0.04 ± 0.14	0.01 ± 0.09
Rogue cells	0.02 ± 0.10	0.02 ± 0.09	0.02 ± 0.10	0.01 ± 0.07	0.02 ± 0.10	0.01 ± 0.07

Significant difference between groups: *P < 0.001.

significant increases of the frequencies of single and double fragments (3.02 ± 2.20 vs. 2.05 ± 1.26% and 1.12 ± 0.94 vs. 0.66 ± 0.78%, respectively), chromosome interchanges (0.22 ± 0.43 vs. 0.06 ± 0.18%), number of aberrations chromatid and chromosome type (3.04 ± 2.20 vs. 2.08 ± 1.28% and 1.33 ± 1.09 vs. 0.72 ± 0.79%) and a nearly 2-fold increase of the frequency of aberrations/100 cells (4.38 ± 2.57 vs. 2.80 ± 1.71%) in the exposed groups. The percentage of chromatid interchanges in the control group exceeded this value in the exposed group, but this was not significant (P = 0.651). The remaining indicators showed an increase in the exposed group, but these differences were also not significant (Table 5).

Sex does not influence the frequency of any type of chromosome abnormalities.

We identified a significant increase of the frequencies of double fragments (1.28 ± 1.06 vs. 0.99 ± 0.82%, P = 0.020) and aberrations chromosome type (1.49 ± 1.19 vs. 1.21 ± 0.99%, P = 0.049) in the second childhood group (9–12 years old boys, 9–11 years old girls) compared to the first childhood (4–8 years old) in the control and exposed groups. There is trend towards the increasing frequency of double fragments in the older age group from Tashtagol. There was a tendency to positive correlation for the duration of residence under high radon conditions with the total frequency of aberrations (Figure 1A), with frequency of double fragments (Figure 1B) and with the aberrant chromosome type (Figure 1C). We found significant differences between smoking (58 people) and non-smoking (314 people) children for several cytogenetic parameters in the exposed group. We eliminated smoking children from the study cohort to exclude the influence of smoking and performed the comparison of non-smoking children. We showed that smoking does not significantly influence the differences between groups but lead to an increase in the level of total aberrations (Table 5). This is evidence of the leading role of radiation by radon and its decay products in the increased level of cytogenetic abnormalities in the exposed group.

Discussion

Based on the presented data, the radon level in the indoor air of a boarding school during the winter period exceeded the permissible level (up to 200 Bq/m^3) for operating buildings. We also found a marked decrease of radon volume activity during spring, but even under conditions of improved ventilation, the radon concentration remained relatively high (Table 2). The average radon volume activity in the living rooms of the exposed group ranged from 235 to 905 Bq/m^3 in the winter and from 200 to 347 Bq/m^3 in the spring. The average value of the radon volume activity over 5 years was 463 ± 98 Bq/m^3, which is much higher than the control settlements.

Therefore, the living and education conditions in the boarding school of Tashtagol do not correspond to the radiation safety standards, and this group is chronically exposed to excessive doses of radon, which can induce genotoxic effects.

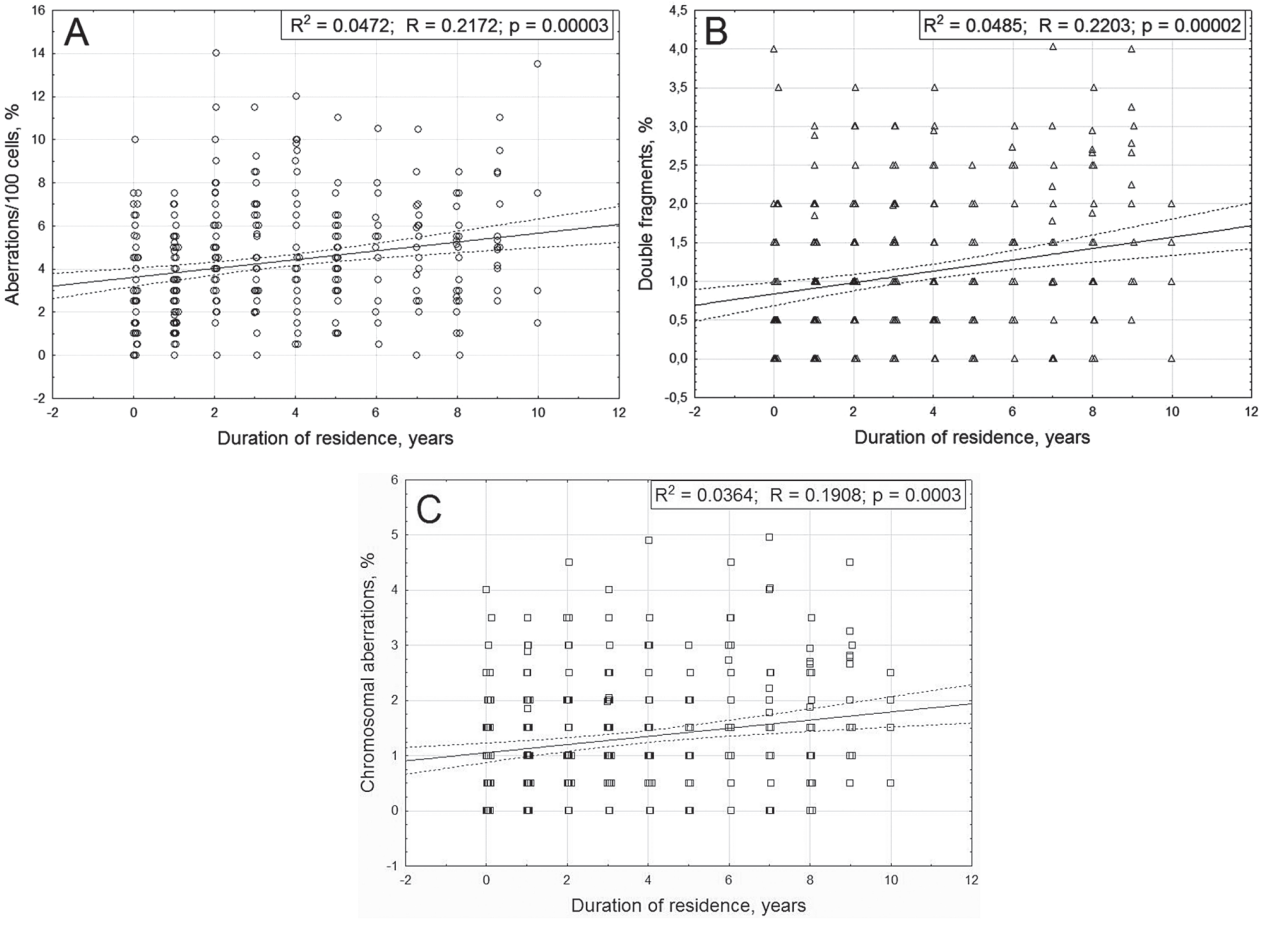

Figure 1. Correlations of the total frequency of aberrations (**A**), double fragments (**B**) and aberrations chromosome type (**C**) with duration of residents in conditions of high radon concentration and its decay products.

A number of studies showed the genotoxic risk, including clastogenic, of radon exposure. Uranium miners or other miners exposed to high doses of radon emission are characterised by a higher level of cytogenetic damage in their peripheral blood lymphocytes compared to control groups (24–26). In addition, the high values of aberrant metaphases and the high frequency of chromatid breaks in miners are significantly associated with cancer risk (27).

On the other hand, some articles (28–33) show the opposite relationship between radon exposure and the level of DNA damage and the cancer risk. This fact is evidence of the lack of knowledge about this problem and the necessarily for more extensive research.

In contrast to occupational exposure, the effects of radon exposure on the frequency of chromosome aberrations in residential conditions are contradictory. Possible reasons for this are a relatively low concentration of radon in dwellings and a limited number of individuals surveyed (34–36). A study conducted in the UK showed a significant association between the frequency of mutations at the hprt-locus and indoor air radon concentration. However, in subsequent studies using the expanded sample and determining the cytogenetic translocations for BCL-2, there was no correlation between the frequency of mutations and radon level (37). Swedish researchers used a DNA-comet assay to assess the primary DNA damage in single cells. They analysed the blood samples from 125 people living in 45 houses with different indoor air radon concentrations (35–1025 Bq/m^3), as well as with different concentrations of radon in the drinking water (10–2410 Bq/l). The increased radon concentration in the air (>200 Bq/m^3) was associated with increased DNA damage in lymphocytes ($P < 0.05$). There was also no correlation between radon concentration in the drinking water and the level of cytogenetic damage (38). Other studies used fluorescence *in situ* hybridization technology to assess the induction of stable chromosome interchanges in a sample of residents in homes with different radon concentrations (27,38,39). Virtually none of the cited publications found significant increases of translocation frequency in the persons living in conditions of very high air radon concentrations. However, the frequency of unstable chromosome interchanges, such as dicentric and ring chromosomes, was significantly increased in people exposed to high radon concentrations (200 Bq/m^3) compared to a control group (2.45 ± 0.50 and 1.03 ± 0.15%; $P < 0.05$) (34).

The effectiveness of cytogenetic tests to assess clastogenic effects induced by increased radon concentrations in indoor air has been shown in several studies. Interestingly, the proportion of aberrant metaphases in the sample studied here was nearly twice as many as in the study from Slovenia (40) (4.26 vs. 2.03%).

Notably, the duration of the stay of the surveyed children in the boarding school can reach 18–20 h a day, particularly during the cold period that lasts for 5–6 months in the continental climate of Western Siberia. The wintertime radon concentration is the highest because of the poor ventilation and energy-saving technologies used.

The increased frequency of dicentric and ring chromosomes under conditions of radon exposure from 0.043 to 0.35% (41) and from 0.15 to 0.44% (42), respectively. In our study, we found that the frequency of dicentric and ring chromosomes increasing in the exposed group, but was not statistically significant. The overall

frequency of chromosome interchanges, including dicentric chromosomes, ring chromosomes and translocations, differentiable by routine staining in the exposed group was significantly higher than in controls (0.21 ± 0.42 and 0.05 ± 0.18%, respectively, $P < 0.05$).

Chromosome interchanges are often used as markers of ionising radiation exposure. In this study, we found no significant effect of other factors capable of modifying the chromosome aberration frequency, such as the action of chemical mutagens, morbidity, sex and age. Assessing the combined influence of radon exposure and smoking showed that the average frequency of acentric fragments and chromatid type aberrations under conditions of the combined influence of these factors were significantly increased compared with the group exposed to radon only (Table 5). Our findings are consistent with the available scientific publications. Recent studies showed that the percentage of acentric fragments and chromatid type aberrations under conditions of combined radon (189 Bq/m^3) and smoking exposure were significantly ($P < 0.0001$) increased compared to the control (non-smoking individuals) (1.63 ± 0.87 and 1.41 ± 1.04% vs. 0.926 ± 0.67 and 0.827 ± 0.66%, respectively) (43).

Conclusion

We found that long-term resident children under conditions of high exposure to radon and its decay products are characterised by an increased frequency of several cytogenetic abnormalities, including chromosome interchanges that occurred almost four times often as the control group. It should be emphasised that among these abnormalities, we found dicentric and ring chromosomes, often regarded as specific markers of radiation exposure. We also found a modifying effect of smoking and residence duration under conditions of low-level ionising radiation exposure on the incidence of these damages. Therefore, the method of chromosome aberration analysis showed high efficiency for the evaluation of chronic low-level ionising radiation exposure on children and adolescents under domestic conditions.

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