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Influence of DNA Repair Genes Polymorphism on the Karyology of Buccal Epithelium Cells in Humans Exposed to Radon

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Abstract—DNA samples from 318 children and adolescents (174 males, 144 females), exposed to radon at home and 65 children (23 males, 42 females) in the control group have been studied. Using the micronucleus test on buccal epithelial cells we identified a significant increase in the number of cells with cytogenetic and proliferative abnormalities in the exposed group. Associations between *NBS1* (rs1805794), *ATM* (rs1801516), and *ADPRT* (rs1136410) genes polymorphisms and karyological abnormalities in buccal epithelial cells were investigated. For the exposed group, an association of *NBS1* Glu185Gln polymorphism with an increased frequency of "broken egg"-type protrusions and apoptotic bodies was established. The *NBS1* 185Glu/Gln and the *ATM1* 1853Asp/Asp genotypes were associated with elevated levels of cytogenetic damage, while the *NBS1* 185Glu/Glu and *ATM* 1853Asp/Asn genotypes demonstrated a protective role. No significant associations were found in the control group.

Keywords: micronucleus test; buccal epithelial cells; ionizing radiation; radon; DNA repair genes
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INTRODUCTION

During recent years an interest to ecogenetic and biomedical problems related to the action of radon and its derivatives in everyday life have significantly increased. With the average summarized radiation dose of 3.46 mZv per year, the portion of radon isotopes was established to be 2,12 mZv per year (Onishchenko, 2008), which corresponds to 50–60% of the natural sources of radiation (Utkin, 2000). Radon is chemically inactive; however, the ionized products of its decay (polonium, bismuth, and lead radionuclides) are absorbed by dust and moisture forming α-radioactive aerosol particles (Rikhvanov, 2009). Submicron-size aerosols represent the most dangerous ones due to their ability to be deposited in the upper airways and oral cavity forming the local sources of cells' α-ionization. According to Hei et al. (1997), up to 80% of epithelial cells survive after the deposit of α-particles and the fraction of these cells is characterized by an increased mutation frequency and might provide malignant tumors formation. The role of the radon in the mortality from lung cancer in Europe was estimated to be 9% (Darby et al., 2005), in Canada—10% (Copes et al., 2007).

The main difficulties for the assessment of the genetoxic effects of radon ionization in everyday life include its low concentration in accommodations, the complexity to form a sample comprising individuals affected by the same ionization intensity, the possible influence of age, chronic diseases, and harmful habits (Oestreicher et al., 2004). This problem might be solved via sample formation comprising children and adolescents living in children's homes and boarding schools.

The use of adequate approaches for the conduction of ecogenetic studies represents another important task. Nowadays, the search for chromosomal aberrations in the peripheral blood lymphocytes (Hamza et al., 2009; Golub et al., 2010; Melnov et al., 2008), the micronucleus test in the lymphocytes culture with the cytokinetic block (Pelevina et al., 2010), fluorescent in situ hybridization (FISH) (Tawn, 2006; Oestreicher et al., 2004; Timoshevskiy et al., 2010), and Comet-assay (Hellman et al., 1999) are widely used for the assessing the mutagenic effect of environmental factors, including those of radiation. The methods mentioned above were characterized as highly precise; however, they are invasive and expensive. The micronucleus test in the buccal epithelial cells appears to be the most suitable for screening. The use of buccal epithelial cells for bioindication studies has several advantages, since the cells are exposed to atmosphere aerosols, while the karyological damage and proliferation disturbances occurring in the basal layer cells might be observed in the outer layer of cells; moreover, their obtainment is non-invasive and is maximally safe. A detailed micronucleus test allows us to detect genetoxic and proliferative effects, as well as determine the priority mecha-

Table 1. The structure of the studied groups differing in age and gender

Studied groups	Boys	Girls	Mean age, year, ($M \pm m$) $\mu \pm s$
Exposed to radon	174	144	12.30 ± 0.16
Control	23	42	15.32 ± 0.30

M—mean value, *m*—standard error of mean.

nisms of cells' death (necrosis/apoptosis) under environmental factors, including ionizing radiation (Sycheva et al., 2013).

The direct and indirect consequences of ionizing radiation are mainly determined by individual constitutive peculiarities. The functional activity of DNA reparation enzymes characterized by polymorphism in the respective genes appears to be one of the factors for differentiating radiosensitivity in humans (Goncharova et al., 2013). Hence, individual genotypes have to be assessed for the more precise estimation of gene- and cytotoxic effects from ionizing radiation.

While performing genetic and ecological monitoring in Kemerovo oblast, researchers from the Genetics Department of Kemerovo State University detected a group of children and adolescents living and studying at boarding school in Tashtagol, whose accommodations were characterized by the presence of radon concentrations above the norm; the parameter of the equivalent equilibrium volume activity of radon comprised 314.4 Bq/m^3 under the permissible value of 200 Bq/m^3 (Norms of radiation safety, 2009). The clastogenic effects of radon action expressed as the increased frequency of chromosomal aberrations compared to groups living in the regions with the normal level of radiation were established for this cohort (Druzhinin et al., 2010).

The present study aims to estimate the effect of an increased radon doses in everyday life on gene-, cytotoxic, and proliferative parameters of buccal epithelial cells assuming the presence of various polymorphic variants in the genes of the DNA reparation system.

MATERIALS AND METHODS

Two groups of children and adolescents from various regions of Kemerovo oblast with different levels of the radiation level have been studied. The group consisting of 318 individuals from boarding-school no. 3 in Tashtagol, which was exposed to radon ionization, was studied for 4 years. The control group consisted of 65 children and adolescents living in a rural area without any expressed environment pollution in terms of radiation and chemicals: the Krasnoe village of Leninsk-Kuznetskii region and the Pacha village of the Yashkinskii region. All the expeditions were conducted during the winter. The gender–age structure of the investigated samples is shown in Table 1.

The collection of the anamnestic data was performed via an oral survey and analysis of the medical history (form 025/u-87). The presence of chronic and infectious diseases, smoking, pharmaceutical administration, and roentgen-diagnostic procedures three months prior to the experiment was taken into consideration. All the individuals were assumed to be pracically healthy at the beginning of the experiment.

DNA samples extracted from the peripheral blood lymphocytes and buccal epithelial preparations served as the material for the study. The signing of the consent form by the parents or individuals looking after underaged children preceded the collection of biological material.

Cytogenetic Analysis

Cytogenetic preparations were obtained according to Thomas et al. (2009). Individuals thoroughly rinsed their mouth with purified water prior to the samples' collection. Materials were collected by buffer-containing spatula (Tris HCl, EDTA, NaCl, pH 7). Threefold washing of the cells was followed by application on preliminarily washed and heated object-plates. Preparations were fixed with Clarke's fixative, and the staining was performed with 2.5% acetic orcein and 1% light green alcohol solution. The preparations were analyzed on a Nikon E200 microscope with a magnification of $100 \times 1.5 \times 10$.

Twelve karyological parameters were registered in the preparations (Fig. 1). The micronuclei (MN) were identified according to the classic criteria (Tolbert et al., 1992). Nuclear abnormalities such as two-nuclearity, condensed chromatin, pyknosis, karyorrhexis, karyolysis, "vesicle", "broken-egg", "tongue"-type protrusions, nuclei with the central circle notch, nuclei with perinuclear and nuclear vacuoles, nuclei of atypical form, and cells with apoptotic bodies were registered according to L.P. Sycheva (2007). The frequency of cells with MN, nuclei protrusions, nuclei with atypical form, with two nuclei, and with a circular notch, as well as perinuclear and nuclear vacuoles, were expressed per mille (‰). The cells with a condensed chromatin in nucleus, karyorrhexis, karyopyknosis, karyolysis, and apoptotic bodies were assumed not to be suitable for MN registration; hence, their frequency was expressed as the number of cells detected over 1000.

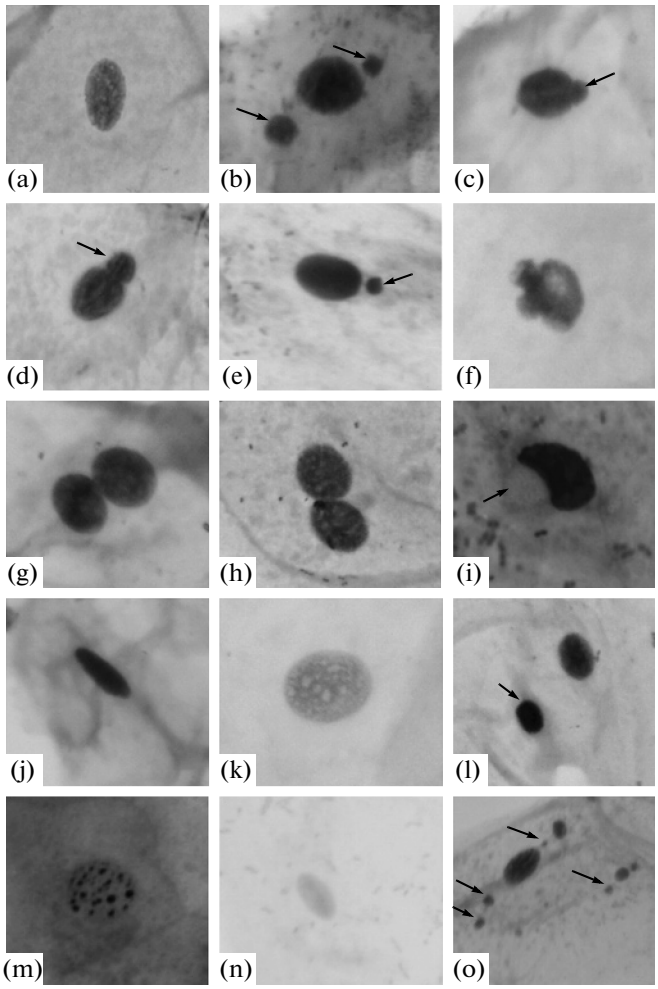

Fig. 1. Karyological parameters of the buccal epithelium. a—normal buccal epithelial cell; b—cell with two micronuclei (shown by arrow); c—cell with vesicle-type protrusion (shown by arrow); d—cell with tongue-type protrusion (shown by arrow); e—cell with broken-egg-type protrusion (shown by arrow); f—cell with an atypical nucleus form; g—cell with two isolated nuclei; h—cell with a circular notch; i—cell with a perinuclear vacuole (shown by arrow); j—cell with a condensed chromatin; k—cell with vacuolated nucleus; l—cell with karyopyknosis; m—cell with karyorrhexis; n—cell with karyolysis; and o—cell with apoptotic bodies (shown by arrow).

Molecular-Genetic Analysis

For the analysis of *NBS1* Glu185Gln, *ATM* Asp1853Asn, and *ADPRT* Val762Ala gene polymorphisms, DNA was extracted from the peripheral blood lymphocytes via the DNA-express kit. The genotyping of allelic variants of DNA reparation system genes was performed using allele-specific PCR and the SNP-express kit (Litech, Moscow) (Manual for the use of diagnostic kits..., 2012). PCR-products were detected via the electrophoretic division in 3% agarose gel followed by visualization and documentation of the obtained results using the Vilber Lourmat ECX-15.M transilluminator and Gel Imager 2 video system (OOO Helicon, Moscow).

Statistical analysis was conducted via the Statsoft Statistica 6.0. The Mann–Whitney U-criteria was used to assess the statistically significant differences in the quantitative parameters from the micronucleus test between the groups (Zaks, 1976, p. 270); the differences were assumed to be significant under $p < 0.05$. The correspondence of the observed genotype frequencies distribution to the Hardy-Weinberg equilibrium was tested with the χ^2-criteria (Weir, 1995, p. 87). The comparison of the characteristics of the genotype frequencies' distribution in the experimental and control groups was conducted using Pearson's χ^2-criteria (Lakin, 1980, p. 126). The FDR-correction for multiple comparisons for the significance level (p) (Benjamini, Yekuteili, 2001, p. 1165) was performed in order to correct the associations of different genotypes of DNA repair genes and their combinations with the karyological parameters of buccal epithelium detected via the Mann–Whitney U-test.

RESULTS

The results of the conducted analysis of the karyological parameters of buccal epithelium in the studied groups are shown in Table 2.

Cytogenetic Parameters

The micronucleus test appears to be sensitive to the action of substances of different nature, including radiation (Belyaeva et al., 2007). The micronuclei frequency in the exposed group doubled compared to the control one ($p = 0.003$). The differential analysis of the protrusion frequency demonstrated a significant twofold excess in the cells with a tongue-type protrusion ($p = 0.048$), while the excess in the vesicle-type protrusion was 2.5-fold ($p < 0.001$). The integral parameters of cytogenetic abnormalities (the summarized frequency of protrusions and cytogenetic abnormalities) in the exposed group was observed to exceed 2.5-fold the corresponding parameters relative to the compared group ($p < 0.001$ and $p < 0.001$, respectively). An excess of cells with a broken-egg-type protrusion ($p = 0.039$) and with an atypical nuclear form ($p < 0.001$) was observed in the control group (Table 2).

Parameters of Proliferation Disturbances

A statistically significant twofold increase in the mean frequency of two-nuclear cells ($p < 0.001$), a 1.5-fold increase for the cells with doubled nuclei ($p < 0.001$), and a 1.5-fold increase for the integral parameter of proliferation ($p < 0.001$) was established in the group exposed to radon (Table 2).

Table 2. Mean values ($M \pm m$) and intervals (in brackets) of the karyological parameters of the buccal epithelial cells in individuals exposed to radon and the control group

Parameters, ‰	Exposed to radon ($n = 318$)	Control ($n = 65$)
Cytogenetic parameters		
Frequency of cells with micronuclei	0.79 ± 0.07 (0–8)*	0.42 ± 0.11 (0–6)
Frequency of cells with protrusions	3.80 ± 0.18 (0–24)*	1.68 ± 0.22 (0–10)
Frequency of cells with a broken-egg-type protrusion	0.12 ± 0.02 (0–3)*	0.20 ± 0.05 (0–2)
Frequency of cells with a tongue-type protrusion	0.28 ± 0.03 (0–3)*	0.14 ± 0.05 (0–2)
Frequency of cells with a vesicle-type protrusion	3.42 ± 0.18 (0–24)*	1.34 ± 0.19 (0–10)
Summarized frequency of cytogenetic abnormalities	4.74 ± 0.2 (0–25) *	2.14 ± 0.30 (0–17)
Frequency of cells with atypical nuclei	17.58 ± 0.51 (0–61)*	22.71 ± 1.41 (3–53)
Proliferation parameters		
Frequency of cells with two nuclei	2.95 ± 0.15 (0–12)*	1.48 ± 0.25 (0–14)
Frequency of cells with the circle notch	5.48 ± 0.23 (0–28)*	3.57 ± 0.38 (0–16)
Summarized frequency	8.51 ± 0.33 (0–31) *	5.05 ± 0.47 (0–19)
Parameters of early stage of nucleus destruction (apoptosis/necrosis)		
Frequency of cells with perinuclear vacuole	20.88 ± 1.11 (0–168)	18.40 ± 2.45 (1–105)
Frequency of cells with condensed chromatin	77.36 ± 3.43 (1–353)*	139.74 ± 8.57 (41–350)
Frequency of cells with nucleus vacuolization	26.91 ± 1.77 (0–182)*	4.63 ± 1.09 (0–60)
Parameters of finished nucleus destruction (apoptosis/necrosis)		
Frequency of cells with karyorrhexis	2.59 ± 0.28 (0–46)	3.43 ± 0.77 (0–27)
Frequency of cells with karyopyknosis	10.17 ± 0.65 (0–71)*	4.11 ± 0.49 (0 –19)
Frequency of cells with karyolysis	182.98 ± 6.92 (12–735)*	251.98 ± 22.65 (11–821)
Frequency of cells with apoptotic bodies	0.47 ± 0.05 (0–7)*	0.12 ± 0.04 (0–1)

* Statistically significant differences between groups ($p < 0.05$, Mann–Whitney U-criteria).

Parameters of Nucleus Destruction

Analysis of the nucleus destruction parameters in the exposed group demonstrated a 5-fold increase in the frequency of cells with nucleus vacuolization ($p < 0.001$), a 4-fold increase in the frequency of apoptotic bodies ($p = 0.001$) and an almost 3-fold increase in the frequency of cells with karyopyknosis ($p < 0.001$) in the experimental groups compared to the control. A significant excess of the frequency of cells with a condensed chromatin ($p < 0.001$) and nucleus lysis ($p = 0.008$) was established in the control group compared to the exposed group (Table 2).

Molecular-genetic analysis of candidate genes' polymorphisms affecting radiosensitivity, including the *NBS1* (rs1805794), *ATM* (rs1801516), and *ADPRT* (rs1136410) genes was conducted. The frequency of the genotypes and alleles of the studied genes in the groups are demonstrated in Table 3. Significant differences in the *ADPRT* genotypes' distribution were established between the experimental and control groups ($\chi^2 = 7.314$, $p = 0.0258$).

A modified influence of the studied genes' polymorphisms was observed for the cytogenetic and nucleus destruction parameters. Significant associations between the studied genes' polymorphisms and the frequencies of karyological abnormalities in the buccal epithelial cells are shown in Table 4.

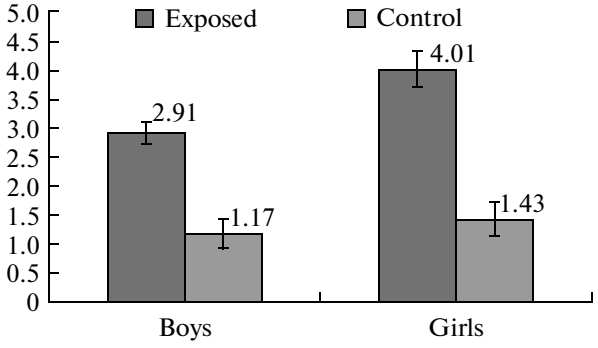

Fig. 2. The frequency of vesicle-type protrusions (‰) in the studied groups differing by gender.

Table 3. Genotype and allele frequency of the studied genes in individuals exposed to radon and control group

Groups	Genotypes (% ± m)			Alleles	
	NBS1 Glu185Gln				
	Glu/Glu	Glu/Gln	Gln/Gln	Glu	Gln
Exposed	40.98 ± 4.71	45.49 ± 4.53	13.53 ± 5.70	0.6372	0.3628
Control	54.17 ± 9.77	3542 ± 1159	10.42 ± 13.66	0.7188	0.2812
	ATM Asp1853Asn				
	Asp/Asp	Asp/Asn	Asn/Asn	Asp	Asn
Exposed	87.82 ± 1.98	11.54 ± 5.33	0.64 ± 5.64	0.9359	0.0 6 41
Control	85.25 ± 4.92	13.11 ± 11.93	164 ± 12.70	0.9180	0.0 819
	ADPRT Val762Ala				
	Val/Val	Val/Ala	Ala/Ala	Val	Ala
Exposed	38.73 ± 4.41	43.81 ± 4.22	17.46 ± 5.12	0.6063	0.3937
Control	54.55 ± 9.09	40.00 ± 10.44	545 ± 1311	0.7455	0.2545

Table 4. The frequency of karyological parameters in exposed individuals with different genotypes

Parameters \ Genotype	*ADPRT*		
	Val/Val ($n = 122$)	Val/Ala ($n = 138$)	Ala/Ala ($n = 55$)
Frequency of cells with nucleus vacuolization	34.42 ± 30.21 (0–177)	23.79 ± 2.53 (0–182)*	18.22 ± 2.94 (0–120)*
frequency of cells with karyopyknosis	12.0 ± 1.13 (0–54)	8.65 ± 0.94 (0–71)*	9.40 ± 1.37 (0–46)
	NBS1		
	Glu/Glu ($n = 109$)	Glu/Gln ($n = 121$)	Gln/Gln ($n = 36$)
Frequency of cells with a broken-egg-type protrusion	0.05 ± 0.02 (0–2)	0.19 ± 0.05 (0–3)*	0.08 ± 0.06 (0–2)
Frequency of cells with apoptotic bodies	0.31 ± 0.07 (0–4)	0.48 ± 0.09 (0–7)	0.75 ± 0.18 (0–5)*

* Statistically significant differences ($p < 0.01$) compared to major homozygotes.

A study of the association between various paired variants of the studied genes and karyological parameters has been conducted. Significant differences in the summarized frequency of protrusions in carriers of the various allelic variants of *NBS1* & *ATM* combinations were detected (Fig. 3).

DISCUSSION

A contemporary protocol of the micronucleus test includes micronucleus estimation as the main parameter of genetoxicity, since their formation is related to the acentric chromosomal fragments, budding nuclei, or to the abnormalities of the segregation of whole chromosomes at the mitotic anaphase stage, as well as the telophase incompleteness (Belyaeva et al., 2007). The nuclear protrusions of the tongue and broken-egg-type also appear to be the markers of unrepaired cytogenetic abnormalities, since their formation is caused by the formation of acentric terminal chromosomal fragments, the cleavage of internuclear bridges during the cell division, and budding nuclei (Kuzovatov, 2000). In turn, the formation of nuclear vesicles is assumed to be related to the release of amplified DNA from the cell and elimination of DNA-reparation complexes from the nucleus (Sharma, 2005).

The findings (Table 2) show a significant increase in the mean values of all the studied cytogenetic parameters, except for broken-egg-type protrusions and the nuclei of atypical form in the experimental group compared to the control sample. Analysis of the parameters of abnormal proliferation also revealed the enhancement of the mean values in the exposed group compared to the control one. The data concerning the increased frequency of two-nuclear cells caused by

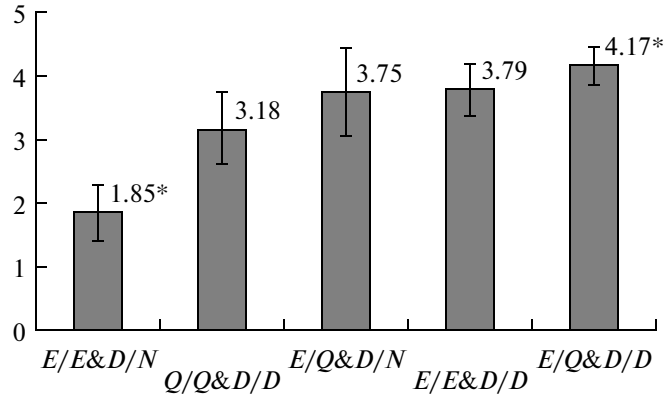

Fig. 3. Summarized frequency of protrusions (‰) in individuals with different allelic combinations between *NBS1* & *ATM*. E-Glu, D-Asp, N-Asn (according to IUPAC-IUB nomenclature of aminoacids); * Statistically significant differences between the groups ($p = 0.004$).

ionization (Koss, 1979) are known, which is confirmed by our findings.

Karyopyknosis and karyorrhexis are mostly studied among the parameters reflecting destructive modifications in the nuclei, and an increase in their frequency point to the genetoxic effect by various factors (Daniel, Olson, Stober, 1991). Moreover, the higher sensitivity of the data on nuclear abnormalities compared to micronuclei was detected for several agents (Tolbert et al., 1991; Torres-Bugarin et al., 1998). A significant increase in the frequency of the pyknotic nuclei in the experimental group compared to the control one ($p = 0.00005$) was established in the present study. The enhanced frequency of cells with nuclei vacuolization ($p < 0.001$) and the apoptotic bodies ($p = 0.001$) was shown in the experimental group compared to the control at a high level of significance. The obtained findings, including parameters such as chromatin condensation and karyolysis point to the significant increase in the mean values in the control group ($p = 0.008$ and $p < 0.001$, respectively) (Table 2). It should be noted that a decrease in the intensity of the destructive modifications (chromatin condensation, karyolysis) in the cell's subpopulation characterized by an increased level of cytogenetic abnormalities appears to be a more unfavorable process resulting in the accumulation of genetically disturbed cells (Sycheva, 2007).

The interval of variance and the mean values of detection of cytogenetic parameters, and the parameters of buccal epithelial cells' proliferation in the control sample almost coincide with the data reported by other authors reported for children and adolescents living in relatively favorable ecological conditions (Yurchenko et al., 2007; Biakhova et al., 2010; Sycheva et al., 2010), which allows us to consider the obtained characteristics as the background for the Kemerovo oblast.

It should be mentioned that the comparative analysis of the mean values of the parameters of cytogenetic, proliferative, and nuclei destruction during distinct years with the data obtained for the control group revealed statistically significant differences ($p < 0.005$) that are similar for the pooled group during the four years.

In order to estimate the influence of age-dependent peculiarities on the karyological parameters of the buccal epithelial cells' parameters under radon exposition, the studied sample was divided into age groups according to the age periods (Khrisanova, 2002): "second child's age" ($n = 151$), "adolescents" ($n = 128$), and "juvenile" ($n = 39$). The comparison of the mean values of the analyzed parameters revealed no statistically significant differences among the age groups ($p > 0.05$). Similar findings were obtained as a result of a comparison among smoking ($n = 47$) and nonsmoking ($n = 271$) individuals. Gender differences were detected in individuals exposed to radon, while comparing the mean frequencies of nuclei vesicles, the corresponding values were 4.01 ± 0.31‰ in girls and 2.91 ± 0.80‰ in boys ($p = 0.012$). No association between this parameter and gender was detected in the control group, whereas, a significant increase in the mean values of the analyzed parameter was shown in the exposed group compared to the control, with gender included as a covariate (Fig. 2), which testify to the dominating role of radon in the formation of nuclear vesicle-type protrusions.

The results obtained by karyological analysis make it possible to characterize the exposed group as the group with an increased cytogenetic risk, which, in turn, points to the necessity to study polymorphism in DNA the reparation system genes and their influence on cytogenetic instability parameters using the micronucleus test.

The association of karyological parameters with various genotypes was preceded by the study of the distribution of allelic variants of the DNA reparation system's genes in the analyzed groups (Table 3). No deviation in the genotypes' frequency of the studied genes

from the Hardy-Weinberg equilibrium was observed. The comparison of genotypes' distribution in the experimental and control groups using χ^2-criteria demonstrated an increased frequency of major homozygote *ADPRT* Val762Val and lower frequency of the minor allele homozygote in the control group.

The product of the *ADPRT* gene appears to be a key protein of base excision repair (BER) specifically bound with a damaged DNA chain providing the addition of the *XRCC1* and Lig3α complex. This enzyme also initiates the reaction of ADP-ribose polymerization (Caldecott, 2003). Ionizing radiation induces the formation of free radicals and energy deficiency in the oxidative stress cells (Zaychik, 2008). It has been established that ADPRT with Val at the 762 position was characterized by higher activity under these conditions, while enzymatic activity decreases after valine's transition to alanine (Lockett et al., 2004). Poly-ADP-ribosyl polymerases provide the NAD's transformation into poly-ADP-ribose and increased enzymatic activity exacerbated oxidative stress resulting in energy deficiency. Under the condition of an energy deficit the cells enter either apoptosis or necrosis. In the present study, we reported that major allele homozygotes were characterized by a statistically significant increase in the frequency of cells with nucleus vacuolization compared to heterozygotes ($p = 0.004$) and minor homozygotes ($p = 0.001$) (Table 4). It should be noted that *ADPRT* Val/Val-carriers demonstrated a higher mean value of the analyzed parameter ($34.42 \pm 30.21‰$) among individuals exposed to radon. Homozygous carriers of the *ADPRT* major allele were also characterized by higher frequency of cells with pyknotic nuclei compared to heterozygotes ($p = 0.004$) (Table 4).

The MRN-complex, including MRE11, RAD50, and nibrin proteins, represents the initial stage of damage recognition within reparation processes via a homologous recombination and nonhomologous agglutination of the ends, and nibrin appears to be a key regulator of this complex (Kracker et al., 2005). The *NBS1* gene (Nijmegen breakage syndrome gene 1) located at chromosome 8q21 spans over 50 kb and consists of 16 exons. As a result of the analysis of the role of *NBS1* Glu185Gln polymorphism on the cytogenetic and karyological parameters of exfoliative epithelium in the exposed group, it has been established that *NBS1* 185Glu/Glu heterozygotes demonstrated a significant increase in the mean value of cells with a broken-egg-type protrusion which belongs to nonrepaired abnormalities compared to major homozygotes ($p = 0.006$), while the minor homozygotes were characterized by a two-fold increase in the frequency of cells with apoptotic bodies compared to major homozygotes ($p = 0.006$) (Table 4). According to Zheng et al. (2011), this polymorphism was associated with an increased risk for nasopharyngeal carcinoma, which confirms the higher liability to cytogenetic abnormalities of exfoliative epithelium in minor allele carriers.

The *ATM* gene (encoding serine/threonine protein kinase) spanning 150 kb is located at 11q22-q23 and encodes proteinkinase consisting of 66 exons belonging to the family of phosphatidylinositol-3-kinases and is expressed in many tissues (Savitsky et al., 1985). ATM phosphorylate enzymes from the majority of DNA reparation pathways (Shiloh, 2003). This protein is mainly located in the nucleus in the inactive dimer form dissociated with the formation of active monomers in the case of double-ruptured DNA chains (Bakkenist, 2003). No statistically significant associations between the genotypes and the frequency of the karyological parameters of the buccal epithelium in the exposed group were detected (Table 4), which might be related to the insignificant number of minor allele carriers.

Considering the interaction of the *ATM* and *NBS1* gene products during the processes of DNA reparation (Banin et al., 1998), we analyzed the modified influence of the paired combinations of these genes on the karyological parameters of exfoliative epithelium, which allowed us to establish that differentiation of the exposed individuals by the frequencies of nuclear protrusions in the buccal epithelial cells was caused by the genetic polymorphism of the *NBS1* and *ATM* gene combinations rather than by the single effect of the *NBS1* gene (Fig. 3). Thus, *NBS1* 185 Glu/Glu homozygotes bearing the *ATM* 1853Asp/Asp genotype demonstrated a lower mean summarized frequency of nuclear protrusions compared to *NBS1* 185Glu/Gln heterozygotes and the major *ATM* homozygotes ($p = 0.004$). The combination of *NBS1* major allele homozygosity and *ATM* gene heterozygosity might be considered as a protective one, since the carriers of these genotypes under ionizing conditions reported the lowest summarized frequency of protrusions ($1.85 \pm 0.44‰$) (Fig. 3) which is close to the corresponding value in the control group (Table 2). No statistically significant differences were observed among other combinations of genotypes.

It should be noted that the analysis of the modified influence of genes' polymorphism did not reveal any statistically significant differences in any parameter of the micronucleus test in the control group. Probably, this result illustrates the absence of invariant alleles' influence on the cytogenetic status of the buccal epithelium under conditions of the absence of mutagenic factors.

Accordingly, the conducted study allowed us to establish that the group exposed to radon was characterized by an increased level of cytogenetic abnormalities compared to the control group. The increased frequency of the two-nucleus and doubled-nucleus cells under exposition to radon has to be considered as disturbances in the spindle division and cytokinesis in the buccal epithelium stem cells. The findings testify to the appropriateness of using the micronucleus test for

the assessment of ecogenetic consequences of the long-lasting radon ionization.

The enhanced frequency of cells with early and late nucleus destruction is related to the presence of major alleles of the *ADPRT* Val762Ala polymorphism. It has been shown that the *NBS1* Glu185Gln polymorphism was associated with the increased frequency of the broken-egg-type protrusions and the apoptotic bodies under ionized radiation. Individuals with *NBS1* 185Glu/Gln & *ATM* 1853Asp/Asp genotypes demonstrated a higher liability to cytogenetic abnormalities in the buccal epithelial cells, whereas the *NBS1* 185Glu/Glu & *ATM* 1853Asp/Asn genotype carriers represented the resistance cohort.

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