COMPARATIVE ANALYSIS OF THE CYTOGENETIC STATUS OF PREGNANT WOMEN RESIDING IN THE TERRITORIES OF RADIOACTIVE, CHEMICAL AND COMBINED CONTAMINATION

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Anthropogenic impact on the biosphere has become one of the major factors dictating the conditions of our existence on the Earth. The study was aimed to perform comparative analysis of the rate of cytogenetic alterations, indicators of proliferation and destruction of the nucleus in the vaginal epithelium reflecting the reproductive health status of pregnant women aged 26–33 years living in conditions of radioactive, chemical and combined contamination of the territories of Bryansk Region. Cytogenetic status of 80 pregnant women divided into four groups, 20 individuals per group, was assessed using the micronucleus test. The rate of cytogenetic alterations, indicators of proliferation and destruction of the nucleus in the vaginal epithelium of pregnant women living in the environmentally disadvantaged territories was 1.9–4.9 times higher ($p < 0.001$) compared to that in women living in the environmentally safe (control) districts. The combined effects of radioactive and chemical contamination resulted in the significantly higher rate (increase from 12.8 to 81.4%) of cells with micronuclei, nuclear protrusions, binucleated cells, as well as cells with karyopyknosis and karyolysis compared to the effect of only one pollution factor. The findings are likely to show synergy of the effects of radiation and chemical factors on the cytogenetic status of pregnant women.

Keywords: pregnant women, cytogenetic alterations, vaginal epithelium, micronucleus test, chemical pollution, radioactive contamination, combined contamination, Chernobyl disaster, Bryansk region

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Compliance with ethical standards: the informed consent to collection of biomaterial during screening and clinical data processing was obtained from all patients.

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СРАВНИТЕЛЬНЫЙ АНАЛИЗ ЦИТОГЕНЕТИЧЕСКОГО СТАТУСА БЕРЕМЕННЫХ ЖЕНЩИН, ПРОЖИВАЮЩИХ НА ТЕРРИТОРИЯХ РАДИАЦИОННОГО, ХИМИЧЕСКОГО И СОЧЕТАННОГО ЗАГРЯЗНЕНИЯ

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Техногенное загрязнение биосферы различной природы стало одним из значимых факторов, диктующим нам условия существования на Земле. Целью настоящего исследования было провести сравнительный анализ частоты цитогенетических нарушений, показателей пролиферации и деструкции ядра во влагалищном эпителии, отражающий состояние репродуктивного здоровья беременных женщин 26–33 лет, проживающих в условиях радиационного, химического и сочетанного загрязнения территорий Брянской области. Цитогенетический статус 80 беременных женщин, разделенных на четыре группы по 20 человек, исследовали с помощью микроядерного теста. Частота цитогенетических нарушений, показателей пролиферации и деструкции ядра во влагалищном эпителии беременных женщин, проживающих на экологически неблагополучных территориях, в 1,9–4,9 раза выше ($p < 0{,}001$), чем у проживающих в экологически благополучных (контрольных) районах. Сочетанное влияние радиоактивного и химического загрязнения стало причиной значимо более высокой частоты (увеличение с 12,8 до 81,4%) клеток с микроядрами, протрузий, двуядерных клеток, клеток с двойным ядром, а также клеток с кариопикнозом и кариолизисом у беременных женщин по сравнению с влиянием только одного фактора загрязнения. Полученные результаты, по всей вероятности, указывают на синергетический характер действия радиационного и химического факторов на цитогенетический статус беременных женщин.

Ключевые слова: беременные женщины, цитогенетические нарушения, влагалищный эпителий, микроядерный тест, химическое загрязнение, радиоактивное загрязнение, сочетанное загрязнение, Чернобыльская катастрофа, Брянская область

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Соблюдение этических стандартов: информированное добровольное согласие на взятие биоматериала в процессе скрининга и обработку клинических данных было получено от каждой пациентки.

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Numerous pollutants of various nature constantly worsen the environmental situation, causing irreparable harm to public health [1–3].

Anthropogenic impact on the biosphere has become one of the major factors dictating the conditions of our existence on the Earth. Serious radiation accidents and disasters of the late 20th and early 21st centuries (Kyshtym, Windscale, Leningrad, Three Mile Island, Saint-Laurent-des-Eaux, Chernobyl, Fukushima) brought large amounts of anthropogenic radionuclides into the biosphere [3, 4–6].

As a result of the Chernobyl disaster that happened 37 years ago, large territories, where more than 5 million people lived, were contaminated [5]. The radioactive contamination density to the greater extent determined by cesium-137 (^{137}Cs) and strontium-90 (^{90}Sr) will remain radiologically significant for many decades [7].

Today, 309,000 people reside in the radioactively contaminated territories of the Bryansk region [8].

Radioecological monitoring of the Bryansk Region shows that the density of soil contamination with ^{137}Cs and ^{90}Sr in the South–Western territories (SWT) exceeds the permissible exposure limits (criteria for classifying territories as radioactively contaminated areas) [9], and the cumulative effective radiation doses of the population vary from several mSv to hundreds of mSv 37 years after the accident [10].

The environmental situation in the Bryansk region is also complicated by chemical pollution of the environment due to the work of industrial enterprises and the growing number of vehicles. According to official figures, in recent years, an increase in emissions of air pollutants, mostly of carbon monoxide (CO) and volatile organic compounds (VOCs) has been reported in the Bryansk region [11]. Such environmental situation causes deterioration of public health and makes the issue of environmental pollution a priority for the healthcare system of the region. Furthermore, it is important to note that in some territories of the Bryansk Region population is exposed to the combined effects of radioactive and chemical pollution, which leads to the massive increase in morbidity rate [12].

The described unfavorable environmental conditions in the Bryansk Region lead to the increase in the number of mutagenic factors. These can pose a real threat reflected in the increase of the population burden and the changing rate of mutation [13]. It was found that the index of cytogenetic alteration in residents of large cities is 2.0–2.4 times higher than that in rural population [14], however, pregnant women still represent the group that is most susceptible to the effects of adverse environmental factors [15–19]. According to the literature data, air pollutants (phenol, formaldehyde, heavy metals) [15–18], radioactive contamination [19, 20] not only lead to the increase in the incidence of various somatic disorders among pregnant women and worse pregnancy course, but also can cause embryo- and fetotoxicity.

The micronucleus test, that has proven itself as a reliable and simple instrument for assessment of cytogenetic disorders, has been long used for cytogenetic monitoring [21, 22]. This is explained by the fact that the method represents a universal biomarker that is reliable in terms of measuring genotoxic, mutagenic, and teratogenic environmental factors [21–23]. The capabilities of the micronucleus test can be used much more widely, since these allow one to assess not only micronuclei, but also the broad spectrum of the nucleus conditions in the exfoliative cells based on the cytogenetic alterations, indicators of proliferation and destruction of the nucleus [23, 24].

Thus, the study of the cytogenetic status of pregnant women living in the territories with various levels of radioactive and chemical contamination of the environment is very important not only for theoretical hygiene of the environment and medical ecology, but also for practical healthcare.

The study was aimed to perform comparative analysis of the rate of cytogenetic alterations, indicators of proliferation and destruction of the nucleus in the vaginal epithelium of pregnant women living in the conditions of radioactive, chemical and combined contamination in the territories of the Bryansk Region.

METHODS

Cytogenetic status of pregnant women was assessed using the micronucleus test [21–25] applied to the vaginal epithelial cells at the Bryansk City Hospital No. 1 between August and November 2023.

The study involved three groups of pregnant women living in various environmental conditions (in the territories of chemical, radioactive, and combined contamination) and the control group (women living in the ecologically safe territories). A total of 20 women aged 26–33 years with the same gestational age (27–33 weeks) and with no chronic somatic disorders or complications of pregnancy were included in each group. The non-inclusion criteria were as follows: pregnant woman's age over 35 years, various complications of pregnancy. A total of 80 women were surveyed.

The vaginal epithelium samples were obtained using the Cervex-Brush cell sampling device (Simurg; Russia) and placed in the test tube filled with specific medium, and the buccal epithelium samples were obtained using the sterile wooden spatula and applied to the slide; these were fixed in air for 2 min. The cytological vaginal epithelium preparations were produced by precipitation. All the preparations were subjected to Quik staining. The samples containing at least 2500–3000 cells were selected for analysis. Data calculation was performed per 1000 cells (the final results were expressed in ppm, ‰). A total of about 220,000 cells were assessed. Microscopy examination was performed using the Nikon Eclipse light microscope (Nikon; Japan) with 1000× magnification.

The smears were used to count cytogenetic alterations (cells with micronuclei and nuclear protrusions of various types; indicators of proliferation (binucleate cells, cells with a double nucleus, cells with more than two nuclei); indicators of destruction of the nucleus (cells with karyopyknosis, karyorrhexis and karyolysis) [23–25].

The districts and towns of the Bryansk Region were divided into four groups based on the levels of chemical and radioactive contamination. The group of environmentally safe territories included Karachevsky, Navlinsky, Kletnyansky, and Dubrovsky districts, while the group of chemically contaminated territories included the city of Bryansk and the town of Dyatkovo. The group of radioactively contaminated territories consisted of Krasnogorsky and Zlynkovsky districts, while the territories with combined radioactive and chemical contamination included the town of Novozybkov and the town of Klintsy.

The levels of the ^{90}Sr and ^{137}Cs radioactive contamination of the territories were determined based on the data [9], the average accumulated effective doses — based on the data [26], the levels of chemical contamination — based on the data of the Rostekhnadzor reports on the stationary source emissions 2-TP (air) for the decade (2010–2019) [8]. We identified the main air pollutants: carbon monoxide (CO), nitrogen oxides (NO_x), sulfur dioxide (SO_2), and VOCs (including formaldehyde, benzene, benzo[a]pyrene, styrene, pyridine, vinyl chloride, acrolein, and phenol). Chemical emissions into atmospheric air (tons/year) were recalculated for the area of the district (km^2) to be expressed in g/m^2 [8].

Table 1. Levels of chemical and radioactive contamination of the environment in the studied groups of towns and districts of the Bryansk Region (2010–2019)

№	Towns and districts of Bryansk Region	Major air pollutants					Radiactive contamination density, kBq/m²	
		Total	Of those:				^{137}Cs	^{90}Sr
			VOCs	NO_x	SO_2	CO		
		Gross gaseous pollutant emissions per area of the district, g/m²						
	Environmentally safe territories (control)							
1	Dubrovsky District	45	12	8	1	24	6.4	0.4
	Navlinsky District	57	26	7	1	23	16.7	1.2
	Kletnyansky District	68	51	4	2	11	4.7	0.4
	Karachevsky District	122	44	25	0	53	12.3	0.7
	Mean value	73	33	44	1	27.8	10	0.7
	Chemically contaminated territories							
2	Dyatkovsky District	6553	365	3809	198	2181	34	1
	City of Bryansk	30462	7671	8230	2019	12542	7.8	5.2
	Mean value	18508	4018	6020	1109	7362	20.9	3.1
	Radioactively contaminated territories							
3	Krasnogorsky District	13	2	3	1	7	268.7	8.2
	Zlynkovsky District	16	6	3	0	7	365.3	14.3
	Mean value	14.5	4	3	0.5	7	317	11.3
	Territories with combined radioactive and chemical contamination							
4	Town of Novozybkov	281	94	33	0	154	404.3	8.6
	Town of Klintsy	441	151	133	7	150	173.3	2.6
	Mean value	361	123	83	3.5	152	288.8	5.6

Statistical analysis of the data obtained was performed using the MyOffice software package (New Cloud Technologies; Russia). The sample mean (M) and standard error of the mean were used (*m*). The data distribution was tested for normality using the Shapiro–Wilk test. It was found that in the majority of cases the distribution of the indicators of cytogenetic alterations, proliferation, and destruction of the nucleus was non-normal, so the Mann–Whitney *U* test was used to verify statistical significance of differences; the differences were considered significant at $p < 0.001 – p < 0.05$.

RESULTS

The levels of air pollution with CO, SO_2, NO_x and VOCs in four groups of districts differ by hundreds and even thousands of times (Table 1). In the environmentally safe territories, the maximum gross gaseous pollutant emission value per area of the district (g/m²) is 122 g/m², while in the chemically contaminated territories, the total emission value reaches its maximum — 30,462 g/m². In the radioactively contaminated territories, the total gaseous pollutant emission value is extremely low —13–16 g/m². In the territories with combined contamination, the gaseous pollutant emission value is 281–441 g/m², which is 22–27 times higher compared to the values reported for the radioactively contaminated districts (Table 1).

The levels of CO pollution in the chemically contaminated territories vary between 2181 and 12,542 g/m², the levels of SO_2 pollution vary between 198 and 2019 g/m², the levels of NO_x are 3809–8230 g/m², and the levels of VOC pollution are 365–7671 g/m². In the radioactively contaminated territories, the values are minimal and show minor fluctuation — from 0 for SO_2 to 7 g/m² for CO, while in the territories with combined contamination, the values vary between 0 for SO_2 and 154 g/m² for CO; in the control districts these vary between 0 and 53 g/m² (Table 1).

The ^{137}Cs radioactive contamination density in the environmentally safe districts varies between 4.7 and 16.7 kBq/m², while in the chemically contaminated territories it varies between 7.8 and 34.0 kBq/m². In the radioactively contaminated territories, the average ^{137}Cs contamination density is 31.7 times higher than in the safe territories (317.0 and 10.0 kBq/m²) and 15.2 higher than in the chemically contaminated territories (317.0 and 20.9 kBq/m²), which exceeds the permissible values being the criteria for classifying territories as radioactively contaminated areas (37.0 kBq/m²) [9]. In the territories with combined contamination, the average ^{137}Cs contamination level is 288.8 kBq/m², which is slightly lower compared to the values of the radioactively contaminated territories (–8,9%), but 28.8-fold exceeds the values of the control districts and 13.8-fold exceeds the values of the chemically contaminated ones (Table 1).

The ^{90}Sr radioactive contamination density in the towns and districts of the Bryansk Region varies between 0.4 and 14.3 kBq/m² to reach its maximum in the radioactively contaminated territories (14.3 kBq/m²) and territories with combined contamination (8.6 kBq/m²), which exceeds the permissible values (5.6 kBq/м²) [9] (Table 1).

The values of the average annual effective dose (AAED90) from the Chernobyl component in the group of environmentally safe and chemically contaminated territories do not exceed 0.1 mSv per year, while in the group of territories with radioactive and combined contamination AAED90 is on average 1.1 mSv per year [26].

Table 2 provides comparative analysis of the cytogenetic alterations, indicators of proliferation and destruction of the nucleus in the vaginal epithelium of pregnant women aged 28–33 years living in various environmental conditions (chemical, radioactive, and combined contamination of the environment) and in the environmentally safe territories. The Figure presents microphotographs of the cytogenetic alterations, indicators

Table 2. Comparative analysis of the cytogenetic alterations, indicators of proliferation and destruction of the nucleus in the vaginal epithelium of pregnant women aged 28–33 years living in various environmental conditions (per 1000 cells, ‰)

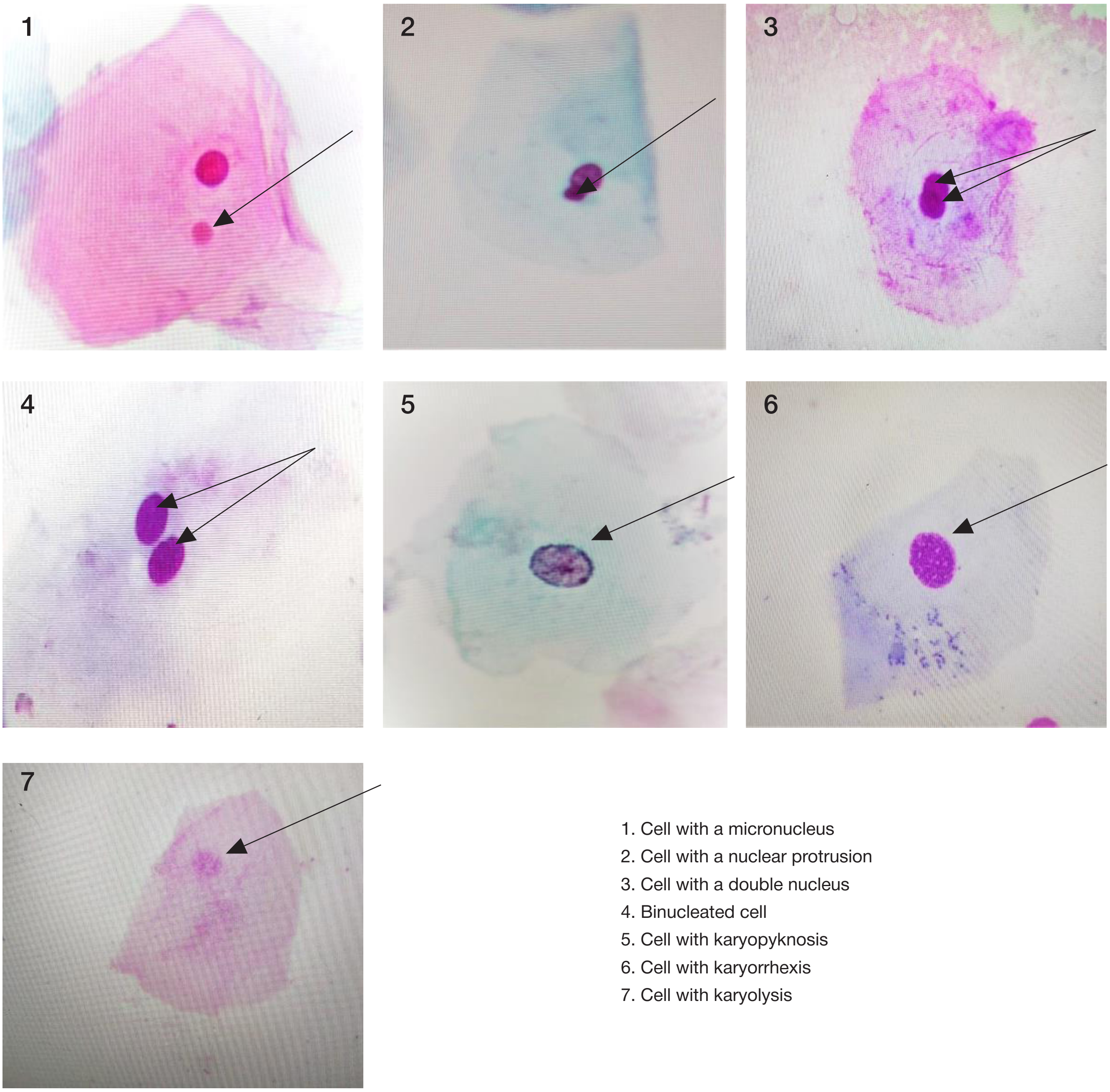
Studied territories / Studied indicator of the vaginal epithelium, ‰	Environmentally safe territories (control)	Chemically contaminated territories	Radioactively contaminated territories	Territories with combined contamination	Significance of intergroup differences based on the Mann–Whitney U test: p_1 (I–II), p_2 (I–III), p_3 (I–IV), p_4 (II–III), p_5 (II–IV), p_6 (III–IV).
	I (n = 20)	II (n = 20)	III (n = 20)	IV (n = 20)	
Cytogenetic alterations					
Cells with micronuclei	1.6 ± 0.15	5.9 ± 0.31	6.8 ± 0.35	7.9 ± 0.32	p_1***; p_2***; p_3***; p_4n/d; p_5***; p_6*
Various types of nuclear protrusions	3.8 ± 0.27	6.9 ± 0.33	8.8 ± 0.44	10.6 ± 0.43	p_1***; p_2***; p_3***; p_4**; p_5***; p_6**
Indicators of proliferation					
Binucleated cells	1.2 ± 0.22	2.7 ± 0.22	3.5 ± 0.25	4.9 ± 0.43	p_1***; p_2***; p_3***; p_4*; p_5***; p_6*
Cells with a double nucleus	0.9 ± 0.16	2.1 ± 0.22	2.4 ± 0.28	3.2 ± 0.21	p_1***; p_2***; p_3***; p_4n/d; p_5**; p_6*
Cells with two or more nuclei	0.4 ± 0.13	0.9 ± 0.20	1.1 ± 0.20	1.5 ± 0.24	p_1n/d; p_2**; p_3***; p_4n/d; p_5n/d; p_6n/d
Indicators of destruction of the nucleus					
Karyopyknosis	3.6 ± 0.35	6.4 ± 0.37	7.9 ± 0.33	9.9 ± 0.34	p_1***; p_2***; p_3***; p_4**; p_5***; p_6***
Karyorrhexis	4.8 ± 0.41	6.4 ± 0.30	7.7 ± 0.36	8.4 ± 0.29	p_1**; p_2***; p_3***; p_4*; p_5***; p_6n/d
Karyolysis	4.5 ± 0.32	7.8 ± 0.35	9.4 ± 0.27	10.6 ± 0.48	p_1***; p_2***; p_3***; p_4**; p_5***; p_6*

Note: * — the differences are considered significant at $p < 0.05$; **— the differences are considered significant at $p < 0.01$; ***— the differences are considered significant at $p < 0.001$; n/d — the differences are considered non-significant at $p > 0.05$.

of proliferation and destruction of the nucleus in the vaginal epithelium of pregnant women aged 28–33 years.

The analysis of data provided in Table 2 has shown that the rate of cytogenetic alterations based on the number of cells with micronuclei and various types of nuclear protrusions in the vaginal epithelium is significantly increased ($p < 0.001$) in the environmentally disadvantaged territories compared to environmentally safe ones. Thus, the number of cells with micronuclei and nuclear protrusions of various types in the environmentally safe territories (control) is 1.6 ± 0.15‰ and 3.8 ± 0.27‰, in the group of chemically contaminated territories it is 3.7 and 1.9 times higher (5.9 ± 0.31‰ and 6.9 ± 0.33‰), in the group of radioactively contaminated territories it is 4.3 and 2.3 times higher (6.8 ± 0.35‰ and 8,8 ± 0.44‰), and in the group of territories with combined contamination the indicators are increased 4.9- and 2.8-fold (7.9 ± 0.32‰ and 10.6 ± 0.43‰). It should be noted that the combined effects of radioactive and chemical contamination in appropriate districts resulted in the significantly higher rate of cells with micronuclei in pregnant women compared to that reported for the districts, where there was only one pollution factor (chemically contaminated territories: +33.9%, $p < 0.001$; radioactively contaminated territories: +16.2%, $p < 0.05$) (Table 2). The number of cells with various types of nuclear protrusions was also significantly higher in the territories with combined radioactive and chemical contamination (chemically contaminated territories: +53.6%, $p < 0.001$; radioactively contaminated territories: +20.5%, $p < 0.01$). Furthermore, it was found that the number of cells with nuclear protrusions of various types was significantly higher in the radioactively contaminated territories compared to the chemically contaminated territories (+27.5%, $p < 0.01$), while the increase in the rate of cells with micronuclei was non-significant (+15.2%, $p > 0.05$) (Table 2).

The pattern of proliferation indicators is virtually identical to the pattern of the rate of cells with cytogenetic alterations (Table 2). Thus, the rate of binucleated cells and cell with the double nucleus in pregnant women living in the control districts is 1.2 ± 0.22‰ and 0.9 ± 0.16‰, in the group of chemically contaminated districts it is 2.3 times higher (2.7 ± 0.22‰ and 2.1 ± 0.22‰), in the group of radioactively contaminated districts it is 2.9 and 2.7 times higher (3.5 ± 0.25‰ and 2.4 ± 0.28‰), and in the group of districts with combined contamination the values are increased 4.1- and 3.6-fold (4.9 ± 0.43‰ and 3.2 ± 0.21‰). The combined effects of radioactive and chemical contamination in appropriate territories resulted in the increased rate of binucleated cells relative to the territories, where there was only one pollution factor (chemically contaminated territories: +81.4%, $p < 0.001$; radioactively contaminated territories: +40.0%, $p < 0.05$). The rate of cells with the double nucleus was also significantly higher in the territories with combined contamination (chemically contaminated territories: +52.4%, $p < 0.01$; radioactively contaminated territories: +33.3%, $p < 0.05$). The rate of binucleated cells was significantly higher in the radioactively contaminated territories compared to the chemically contaminated ones (+29.6%, $p < 0.05$), while the increase in the rate of cells with double nuclei was non-significant (+11.4%, $p > 0.05$). Significant differences in the number of cells with two or more nuclei from controls was reported for pregnant women living in the radioactively contaminated districts (+2.8-fold, $p < 0.01$) and districts with combined contamination (+3.8-fold, $p < 0.001$). In other cases no significant patterns were revealed ($p > 0.05$), along with the persistent upward trend of the rate of cells with two or more nuclei in the districts with chemical contamination (+22.5%), radioactive and chemical (+22.2%), radioactive and combined (+36.3%), chemical and combined (+66.7%)



1. Cell with a micronucleus
2. Cell with a nuclear protrusion
3. Cell with a double nucleus
4. Binucleated cell
5. Cell with karyopyknosis
6. Cell with karyorrhexis
7. Cell with karyolysis

Fig. Microphotographs of the cytogenetic alterations, indicators of proliferation and destruction of the nucleus in the vaginal epithelium of pregnant women aged 28–33 years (Quik stain, 1000× magnification)

contamination relative to the environmentally safe districts (Table 2).

The indicators of destruction of the necleus fully reflect the overall patterns of the rate of cytogenetic alterations and the indicators of proliferation (Table 2). The rates of cells with karyopyknosis, karyorrhexis and karyolysis in the environmentally safe areas are 3.6 ± 0.35‰, 4.8 ± 0.41‰ and 4.5 ± 0.32‰, in the group of chemically contaminated territories these are 1.8, 1.3 and 1.7 times higher (6.4 ± 0.37‰, 6.4 ± 0.30‰ and 7.8 ± 0.35‰), in the group of radioactively contaminated territories — these are 2.2, 1.6 and 2.1 times higher (7.9 ± 0.35‰, 7.7 ± 0.36‰ and 9.4 ± 0.27‰), and in the group of territories with combined contamination the indicators reach their maximum and are increased 2.8-, 1.8- and 2.4-fold (9.9 ± 0.34‰, 8.4 ± 0.29‰ and 10.6 ± 0.48‰). The combined effects of radioactive and chemical contamination in appropriate districts resulted in the increased rate of cells with karyopyknosis in pregnant women relative to the districts, where there was only one pollution factor (chemically contaminated territories: +54.7%, $p < 0.001$; radioactively contaminated territories: +25.3%, $p < 0.001$). The number of cells with karyolysis was also significantly higher in the territories with combined contamination (chemically contaminated territories: +35.9%, $p < 0.001$; radioactively contaminated territories: +12.8%, $p < 0.05$). The number of cells with karyorrhexis was significantly higher in the territories with combined contamination compared to the chemically contaminated territories (+31.3%, $p < 0.001$) and higher compared to the radioactively contaminated territories, but the difference was non-significant (+9.1%, $p > 0.05$). It should be noted that the rate of cells with karyopyknosis, karyorrhexis and karyolysis is significantly higher in the radioactively contaminated districts than in the chemically contaminated ones (+23.4%, $p < 0.01$; +20.3%, $p < 0.05$; +20.5%, $p < 0.01$) (Table 2).

DISCUSSION

Comparative analysis of the cytogenetic status of pregnant women living in various environmental conditions revealed similar factor-dependent responses to the studied environmental exposures. The fact, that the studied samples belong to the territories showing many-fold differences in the chemical and radiation load, suggests that the identified differences in cytogenetic alterations, indicators of proliferation and destruction of the nucleus, reflect the effects of the chemical, radiation, and combined environmental factors.

The rate of cells with micronuclei in pregnant women living in the Bryansk Region attracts attention. Since, in accordance with the international data (HUMNxl project), the average rate of cells with micronuclei in controls is 1.1‰ [21], the indicator reported for the territories of the Bryansk Region is increased 1.45-fold relative to the control territories and 5.4–7.2-fold relative to the environmentally disadvantaged territories to reach its maximum in the territories with the combined radioactive and chemical contamination (7.9‰).

Both the differences and similarity of the data obtained by cytogenetic monitoring of exfoliative cells should be noted. Thus, according to [23], the rate of micronuclei in the urothelial, buccal and nasal cells of the control group varies between 0.24 and 0.35‰, which is significantly lower compared to both our results obtained for the control districts of the Bryansk Region (1.6‰) and international standards (1.1‰) [21]. The values of the rate of binucleated cells in the vaginal epithelium of pregnant women living in the control districts we have obtained (1.20‰) are similar to the data on the rate of binucleated cells in the urothelium of the controls (1.21‰) [23], however, the reported number of cells with karyopyknosis and karyolysis [23] is significantly higher compared to our results.

The study [25] showed that the total rate of cells with destruction of the nucleus (karyopyknosis, karyorrhexis and karyolysis) reached its maximum in the puerperas with abnormalities of the fetus living in the conditions of combined radioactive and chemical contamination.

When assessing the data obtained, it is necessary to emphasize the need to implement the complex hygienic monitoring of the environment based on the levels of chemical, radioactive and combined contamination reported over a long period, since the impact of single factors is always summed up and transformed in real-world conditions (the phenomenon of synergy) [12, 24, 25, 27].

In the reported study, the combined effects of radioactive and chemical contamination resulted in the significantly higher rates of cytogenetic alterations, indicators of proliferation and destruction of the nucleus in pregnant women living in the environmentally disadvantaged territories compared to women living in the districts where there was only one pollution factor. The findings are likely to show synergy of the effects of the radiation and chemical factors.

CONCLUSIONS

1. The rates of cytogenetic alterations, indicators of proliferation and destruction of the nucleus in pregnant women living in the environmentally disadvantaged territories are 1.9–4.9 times higher ($p < 0.001$) compared to that in women living in the environmentally safe (control) districts.

2. The number of cells with nuclear protrusions, binucleated cells, cells with karyopyknosis, karyorrhexis and karyolysis is significantly higher in the radioactively contaminated territories compared to the chemically contaminated ones (increase from 20.5 to 29.6%), which suggests the greater effect of the radiation factor resulting from the Chernobyl disaster on the cytogenetic status of pregnant women relative to the chemical factor.

3. The combined effects of radioactive and chemical contamination resulted in the significantly higher rates of cells with micronuclei (increase from 16.2 to 33.9%), nuclear protrusions (increase from 20.5 and 53.6%), binucleated cells (increase from 40.0 to 81.4%), cells with the double nucleus (increase from 33.3 to 52.4%), as well as cells with karyopyknosis (increase from 25.3 to 54.7%) and karyolysis (increase from 12.8 to 35.9%) in pregnant women living in the environmentally disadvantaged territories compared to that in women living in the districts where there was only one pollution factor. The findings are likely to show synergy of the effects of the radiation and chemical factors on the cytogenetic status of pregnant women.

4. In the towns and districts of the Bryansk Region, the average rate of cells with micronuclei in the environmentally safe territories 1.45-fold exceeds the international standards (HUMNxl project) and in environmentally unfavorable areas 5.4–7.2 times, reaching maximum values in the territories of combined radioactive and chemical contamination (7.9‰).

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