Study of Genetic Effects in Somatic Cells of Children Living on the Contaminated Territories in Belarus

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Due to the Chernobyl APS accident in April 1986, a series of serious radiological consequences were brought in Belarus: the pollution of her territory with radionuclides with gamma-, beta- and alpha-radiation, and "hot" particles found in human organisms [1]; the high irradiation doses obtained by children in first period after the catastrophe; the chronic influence of complex of radionuclides with various biological effects on the organisms for many years. Constant external and internal exposure and their combined action on organisms and their cells, including the blood formation system, required the development of new approaches to evaluate mutation process in lymphocytes of peripheral blood of children [2].

The application of the conventional cytogenetic method analysing chromosomal aberrations in metaphase was necessary in the first stage of examination of children population in 1986-1990 because it enabled to apply the biological dosimetry to the estimation of radiation doses of individuals and groups. In the course of time after the catastrophe, the information obtained by this method has decreased. Besides, by this method of metaphase analysis, it is difficult to study simultaneously both the chromosomal aberrations induced in a series of cellular generations of lymphocytes and the hereditary cellular-lethal effect, comparing the level of chromosomal aberrations with that of gene mutations based on comparatively small volumes of children blood. We proposed and proved a new scheme applying micronucleus technique to cytogenetic examination. This method permits to take into account all the above mentioned deficiencies of the conventional method [2].

We organised genetic monitoring of Belarus children in the following two ways:

1. The monitoring (individual and group) was carried out with the children in the same settlement (for example, Bragin), which made possible to observe the dynamics of mutation process in peripheral blood cells.
2. The genetic examination (individual and/or group) was carried out with the children in several settlements with different radioecological situation in the same period of time (as it was in Komarin and Malejki settlements of the Bragin district), which made possible to fulfil comparative analysis of mutation frequency.

1. Dynamics of *in vivo* mutation in lymphocytes of peripheral blood of the children in Bragin town (1988-1994)

At the first stage of cytogenetic examination, blood smears were taken from the children in the radiocontaminated zones in order to study non-proliferating lymphocytes in peripheral blood. Presently, this method of blood analysis is one of the most popular laboratory tests used at the screening stage without the process of cell cultivation. The structural changes of lymphocyte populations obtained by this method is considered a high-sensitive index of irradiation at low doses [3,4].

It is known that cell division is necessary for micronuclei to be formed. Consequently, we can consider that the frequency of micronuclei registered in circulating blood lymphocytes is governed by the damages of blood-forming cells *in vivo*, possibly, as a result of genetic injuries on the level of stem cells and/or preceding cells of hemopoiesis which develop to recognizable cells of peripheral blood.

We have been investigating structural changes in lymphocyte populations such as the level of mono- and polynuclear cells with micronucleus, number of micronuclei in them, as well as of disturbances in blood cell morphology in all three groups of lymphocytes; small, wide-cytoplasmic lymphocytes and lymphocytes with plasmatization of cytoplasm, which represent the polymorphism of these cells in children [6,7].

In this connection, since 1988, the cytogenetic monitoring of children in Bragin town has been organized based on two age groups: the first group (1-7 years old) and the second (7-15 years old). It is necessary to note that there were different characteristics of the same age groups examined in different years. The young group, examined in 1988, consisted of equal number of children born before and after the Chernobyl catastrophe, whereas the old group (7-15) wholly was born before the catastrophe. The children of 1-7, examined in 1990, constituted a mixed group, and the children of 7-15 were born before the catastrophe. The groups, examined in 1994, were uniform: the children in the young group were born after the catastrophe, and those in old group - before the catastrophe.

Thus, comparison of the results of cytogenetic examination of children "by horizontal" within one year and "by vertical" in dynamics (1988-1994) allows to answer the question concerning the differential sensitivity of hereditary material of children lymphocytes in dependence of age, period of birth before or after the accident, period of living on radiocontaminated territory, and others.

1.1. Study of the spontaneous level of *in vivo* mutation in lymphocyte population of peripheral blood of the children in the control group

The quantitative and qualitative assessment of cytogenetic effects of ionizing radiation is impossible without the knowledge of spontaneous level of *in vivo* mutation observed in lymphocyte populations of peripheral blood of the Belarus children. The spontaneous level of mutation not only determines the reliability of increase of induced structural mutations in lymphocytes of the children in Bragin town and other areas, but also create the basis to carry out the population studies with the given method.

The first information about the spontaneous frequency of micronuclei in erythrocytes in peripheral blood of healthy people as compared with their level in ill patients was obtained by hematologists. The principal attention in those studies was paid to the qualitative but not quantitative characteristics [5]. Just in 1976, Countryman and Heddel introduced lymphocytes as the next cellular indicator of micronuclei in man [8].

After the Chernobyl catastrophe, papers appeared which indicated the increased micronuclei level in lymphocytes of peripheral blood of the affected people [9]. However, uncertainty of information about the spontaneous level of *in vivo* mutation in lymphocytes of human peripheral blood restrains the advance of population-genetic investigations.

Therefore, it is quite natural to do special studies and analysis to know the spontaneous level of micronuclei in lymphocytes of children peripheral blood. So, we carried out cytogenetic studies of spontaneous level of structural and quantitative chromosomal injuries in lymphocytes of the children of two principal age groups, living permanently in Minsk. In 1988-1990-1994, we examined 139 children of age from 1 to 15 years. The summary data is shown in Table 1.

The analysis of cytogenetic structure of lymphocytes of the children in Minsk has shown that polynuclear lymphocytes represent only a small fraction of the whole lymphocyte population. In this connection, mononuclear lymphocytes have been chosen as the principal object of study. A complicated

Table 1. Dynamics of *in vivo* mutation mononuclear lymphocytes of peripheral blood of the children in Minsk city.

Year	Age group of children* /number of examined	Number of analysed lymphocytes	Number of cells with micronuclei		Number of micronuclei in cells		Distribution of cells by the number of micronuclei				
			Absolute number (AN)	x ± Sx (%)	AN.	x ± Sx (%)	1	2	3	4	5
1988	1 (n=20)	8705	61	0.7±0.1	61	0.7±0.1	61	-	-	-	
	2 (n=18)	3226	28	0.9±0.2	28	0.9±0.2	28	-	-	-	-
1990	1 (n=15)	3974	21	0.5±0.1	21	0.5±0.1	21	-	-	-	-
	2 (n=20)	1863	25	1.3±0.3	25	1.3±0.3	25	-	-	-	-
1994	1 (n=15)	1339	21	1.6±0.3	21	1.6±0.3	21	-	-	-	-
	2 (n=18)	769	15	1.9±0.5	15	1.9±0.5	15	-	-	-	-

*:1 - the young age group of 1-7 years, 2 - the old age group of 7-15 years.

Table 2. Dynamics of *in vivo* mutation in binuclear lymphocytes of peripheral blood of the children in Minsk city.

Age group*	Number of analysed lymphocytes	Number of binuclear cells		Number of cells with micronuclei		Number of micronuclei in cells		Distribution of cells by the number of micronuclei				
		AN	x±Sx	AN	x±Sx	AN	x±Sx	1	2	3	4	5
1988												
1(n=20)	8705	105	1.2±0.1	-	-	-	-	-	-	-	-	-
2(n=18)	3226	64	1.9±0.2	-	-	-	-	-	-	-	-	-
1990												
1(n=15)	3974	24	0.6±0.1	-	-	-	-	-	-	-	-	-
2(n=20)	1863	43	2.3±0.3	-	-	-	-	-	-	-	-	-
1994												
1(n=15)	1339	38	2.8±0.4	-	-	-	-	-	-	-	-	-
2(n=18)	759	38	4.9±0.8	-	-	-	-	-	-	-	-	-

*:1 - the young age group of 1-7 years, 2 - the old age group of 7-15 years.

picture has been revealed for mononuclear lymphocytes with micronuclei. First of all, we are to note that only cells with one micronucleus were found during the years examined. However, the variations in the frequency of micronucleus cells were observed. The significant difference in the level of micronucleus cells between the young and the old groups was found only in 1990, whereas in 1988 and 1994 the levels of such cells were approximately equal among two groups. On the other hand, growth of the frequency of micronucleus cells was revealed with time in both groups.

There were no substantial differences between the children born before and after the Chernobyl accident. We also could not find dependence of micronucleus induction on sex.

Regretfully, among all the analysed of lymphocyte populations *in vivo*, a considerable number of binuclear cells were found, which are extremely rare in healthy children (Table 2). In 1988, a increasing tendency of binuclear lymphocyte frequency was observed in the old children groups compared with the young group. Then this difference turned out statistically significant in the following years. The highest indices were revealed in the children born before the Chernobyl accident. As is seen in Table 2, cells with micronuclei were not found among binuclear lymphocytes. Thus, lymphocytes populations in peripheral blood of the examined children in Minsk is characterized by increasing tendencies of the number of mononuclear cells with micronucleus and the number of binuclear cells *in vivo*.

As we noted earlier, there are a limited number of investigations dedicated to the quantitative and qualitative *in vivo* assessment of micronuclei in lymphocyte populations of human peripheral blood. The data on the spontaneous level of micronuclei in lymphocytes were obtained in other researches in which a sufficient number of adults were examined in China. Thus, when 183 persons of both sexes in the age of 45.6±13.9 were examined, 0.16±0.35 micronuclei were found per 1000 analysed lymphocytes [10]. Consequently, approximately equal frequency of micronuclei was registered both in the children of Minsk and in the adults of China. However, unlike in the Chinese population, our investigations discovered high occurrence of binuclear lymphocytes. Apparently, the peculiarities of mutation process in lymphocyte populations of the children in Minsk are linked to the unfavourable radioecological situation.

In 1996, one more article was published in which *in vivo* micronuclei analysis of peripheral lymphocytes was used in combination with an application of the FISH method to an adult group of various ages [11]. Nevertheless, due to the fact that the number of examined people is insufficient, it is not clear whether the levels of micronuclei and polynuclear cells depend on age, sex and other factors. However, the data obtained in the present study can be used to estimate the levels of spontaneous and induced mutation in lymphocytes of peripheral blood of the Belarus children.

1.2. Dynamics of *in vivo* mutation in lymphocytes of peripheral blood in the children of Bragin town

The cytogenetic monitoring of the children of Bragin town in 1988-1994 discovered certain regularities in the dynamics of mutation process in lymphoid cells *in vivo* (Tables 3-5).

The most substantial results of them are as follows:

1. Significant increase of the level of cytogenetic injuries, as compared with corresponding control groups, in lymphocyte populations of peripheral blood of the children of all age groups regardless of their birth date (before or after the Chernobyl accident); the number of polynuclear lymphocytes, the levels of micronucleus cells, and the number of micronuclei in them.
2. Statistically significant differences from the control, not only in quantitative aspects - the frequency of mutation in lymphocytes, but also in qualitative one - distribution spectrum by the number of registered injuries in one cell. According to the results of micronuclei analysis, the common phenomenon in the children groups in the radiocontaminated areas is the presence of subpopulation of cells with several micronuclei - 2, 3, 4 and more, while they are absent in the control preparations. This can serve as a proof of contact of the given children with dense ionizing radiation.
3. Significant difference in the frequency of *in vivo* mutation in lymphocytes of peripheral blood between in the children born before the Chernobyl catastrophe and in the children born on radiocontaminated areas of Bragin town after the catastrophe. The number of cytogenetic injuries grew with the course of time (i.e. depended on the accumulated dose), and in 1994 it increased by about one order as high as in 1986.
4. The level of mutation frequency registered in the children born after the Chernobyl accident also increased with the course of time, or with the increase of accumulated dose. But it is in a lesser scale than in the children born before the accident.

Table 3. Dynamics of *in vivo* mutation in mononuclear lymphocytes of peripheral blood of the children in Bragin town.

Year	Age group of children* /number of examined	Number of analysed lymphocyte	Number of cells with micronuclei		Number of micronuclei in cells		Distribution of cells by the number of micronuclei				
			AN	x±Sx	AN	x±Sx	1	2	3	4	5
1988	1 (n=23)	4444	113	2.5±0.2	144	3.2±0.2	91	16	4	1	1
	control (n=20)	8705	61	0.7±0.1	61	0.7±0.1	61	-	-	-	-
	2 (n=22)	7802	248	3.1±0.1	345	4.4±0.2	175	55	12	6	-
	control (n=18)	3226	28	0.9±0.2	28	0.9±0.2	28	-	-	-	
1990	1 (n=18)	6371	272	4.2±0.2	397	6.2±0.3	180	64	23	5	-
	control (n=15)	3974	21	0.5±0.1	21	0.5±0.1	21	-	-	-	-
	2 (n=17)	16622	677	4.0±0.1	942	5.6±0.1	484	131	54	6	2
	control (n=20)	1863	25	1.3±0.3	25	1.3±0.3	25	-	-	-	-
1994	1(n=15)	1707	65	3.8±0.4	95	5.5±0.5	41	18	6	-	-
	control (n=15)	1339	21	1.6±0.3	21	1.6±0.3	21	-	-	-	-
	2 (n=25)	1331	323	24.2±1.1	561	42.1±1.3	164	98	48	8	5
	control (n=18)	769	15	1.9±0.5	15	1.9±0.5	15	-	-	-	-

*:1 - the young age group of 1-7 years, 2 - the old age group of 7-15 years.

Table 4. Dynamics of *in vivo* mutation in binuclear lymphocytes of peripheral blood of the children in Bragin town.

Age group*	Number of analysed lymphocyte	Number of binuclear cells		Number of cells with micronuclei		Number of micronuclei in cells		Distribution of cells by the number of micronuclei				
		AN	x±Sx	AN	x±Sx	AN	x±Sx	1	2	3	4	5
1988												
1(n=23)	4444	477	10.7±0.6	24	0.5±0.1	33	0.7±0.1	16	7	1	-	-
C(n=20)	8705	108	1.2±0.1	-	-	-	-	-	-	-	-	-
2(n=22)	7802	756	9.6±1.0	78	0.9±0.1	109	1.3±0.1	50	25	3	-	-
C(n=22)	3225	64	1.9±0.1	-	-	-	-	-	-	-	-	-
1990												
1(n=18)	6371	629	9.8±0.1	98	1.5±0.1	122	1.9±0.1	78	17	2	1	-
C(n=15)	3974	24	0.6±0.1	-	-	-	-	-	-	-	-	-
2(n=17)	16622	1515	9.1±0.2	282	1.6±0.0	396	2.3±0.0	199	61	15	5	2
C(n=20)	1863	43	2.3±0.3	-	-	-	-	-	-	-	-	-
1994												
1(n=15)	1707	64	3.7±0.4	5	0.2±0.0	5	0.2±0.0	6	-	-	-	-
C(n=15)	1339	38	2.8±0.0	-	-	-	-	-	-	-	-	-
2(n=25)	1331	247	18.5±0.8	36	2.7±0.4	44	3.3±0.4	29	6	1	-	-
C(n=18)	769	38	4.9±0.7	-	-	-	-	-	-	-	-	-

*:1 - the young age group of 1-7 years, 2 - the old age group of 7-15 years.

Table 5. Dynamics of *in vivo* mutation in trinuclear lymphocytes of peripheral blood of the children in Bragin town.

Age group*	Number of analysed lymphocyte	Number of trinuclear cells		Number of cells with micronuclei		Number of micronuclei in cells		Distribution of cells by the number of micronuclei				
		AN	x±Sx	AN	x±Sx	AN	x±Sx	1	2	3	4	5
1988												
1(n=23)	4444	83	1.8±0.1	83	1.8±0.1	83	1.8±0.1	83	-	-	-	-
C(n=20)	8705	-	-	-	-	-	-	-	-		-	-
2(n=22)	8702	130	1.6±0.1	11	0.1±0	16	0.2±0	6	5	-	-	-
C(n=18)	3226	49	1.5±0.4	-	-	-	-	-	-	-	-	-
1990												
1(n=18)	6371	198	3.1±0.2	29	0.4±0	39	0.6±0	21	6	2	-	-
C(n=15)	3974	-	-	-	-	-	-	-	-	-	-	-
2(n=17)	16622	455	2.7±0.1	71	0.4±0	97	0.5±0	51	14	6	-	-
C(n=20)	1863	-	-	-	-	-	-	-	-	-	-	-
1994												
1(n=15)	1707	28	1.6±0.3	-	-	-	-	-	-		-	-
C(n=15)	1339	-	-	-	-	-	-	-	-	-	-	-
2(n=25)	1331	90	6.7±0.6	84	6.3±0.6	87	5.5±0.6	81	3	-	-	-
C(n=18)	769	-	-	-		-		-	-	-	-	-

*:1 - the young age group of 1-7 years, 2 - the old age group of 7-15 years.

5. Significant differences found in the level and the spectrum of cytogenetic injuries of bi- and polynuclear lymphocytes in the children born before and after the Chernobyl catastrophe. It is necessary to stress that the significant increase of polynuclear lymphocytes in peripheral blood of the children in Bragin town is also connected with the factors of the radioecological situation.

From our point of view, *in vivo* cytogenetic analysis of lymphocytes with use of peripheral blood smears becomes an important part in the scheme of screening examination of the children in the polluted areas of Belarus. As it is seen from the results of our study, the most suffering part of the children population is those who were born before the catastrophe. So, they are just to be the principal object of investigation and rehabilitation activities.

2. *Ex vivo* study of mutation in lymphocytes of peripheral blood in the children of the Bragin district (1992)

The application of micronuclei analysis of peripheral blood lymphocytes under the condition of cytokinetic block during cell cultivation allows to distinguish clearly the cells which have not passed the mitosis and the cells after first and consequent mitoses [2, 12]. It is known that PHA reagent used for cell cultivation stimulates mainly small T-lymphocytes to cell division [13]. Therefore, by analysing effects on non-stimulated lymphocytes in cell culture, we can look into a heterogeneity of examined lymphocyte populations as compared with the results obtained by the smear method. Besides, the micronuclei registered in mononuclear T-lymphocytes in cell culture are also the result of their expression in process of lymphoid cell divisions *in vivo* [2].

In this connection, micronuclei frequencies in mononuclear and binuclear T-lymphocytes of peripheral blood were examined in order to reveal the effect of low doses of external and internal irradiation as well as of their combined effects, comparing the results of *in vivo* condition with those of *ex vivo* ones. Besides, such approach allowed us to follow to some degree the transgenerational somatic effect. Both the elimination of cells from circulating blood after the "acute irradiation" in the early post-accident period and the appearance of new ones containing unstable aberrations were registered in our experiments in a series of cell generations of lymphocytes. As a result of such analysis, we tried to get an answer to the question: "what is the trend of selection for micronucleus cells (plus- or minus-trend) during the process of their mitotic division?'.

In the same day of March of 1992, blood was taken from 2 children groups of 12-13 years old in Komarin town and Malejki village in the Bragin district of the Gomel region. The blood was transported to Minsk and analysed immediately [2, 12]. In accordance with the Law of Republic of Belarus of 12 November of 1991, the territories polluted as the result of Chernobyl accident were divided into zones in dependence of the density of soil pollution with radionuclides and the degree of radiation effect on man [1]. Both settlements belong to the zone of consequent resettlement, i.e. the zone on which the density of pollution is from 15 to 40 Ci/km^2 with Cs-137 or 2-3 Ci/km^2 with Sr-90, and the average annual effective equivalent dose can overreach 5 mSv per year (over the natural and technogenic background). The density of pollution of territory of Komarin town is 3.0 (Cs) and 2.8 Ci/km^2 (Sr), and that of Malejki village - 9.0 (Cs) and 2.8 Ci/km^2 (Sr) [14].

2.1. *Ex vivo* study of mutation in stimulated and non-stimulated T-lymphocytes of peripheral blood of the children in Komarin town

The results of micronuclei registered in mononuclear lymphocytes of the children in Komarin town are presented in Table 6. A significant inter-individual variation was observed in the frequency of micronucleus cells and the number of micronuclei in mononuclear cells. The individual cytogenetic examination showed that only in 3 children(No. 2, 4 and 9) significant increase of micronucleus cells and of micronuclei frequency per 100 analysed cells was observed. These indices did not differ from the control values in the rest of children. However, the group average indices of mononuclear cells with micronuclei and the indices of micronuclei per 100 cells in children of Komarin town overreached statistical significant level over the corresponding indices in the control (Minsk city).

It is substantial that cells with multiple micronuclei were found in the children of Komarin town, which appear in *in vitro* conditions under the quite high doses or the action of dense ionizing radiation. It is necessary to note also that the cells with chromatin pulverization were found in the half of examined children. The group average index of pulverization constituted 1.6±0.2%. Such phenomena were never observed in the control. This may be considered as confirmation of their elimination and appearance *de novo*.

Table 6. Individual analysis of *ex vivo* mutation in non-stimulated mononuclear lymphocytes of peripheral blood of the children in Komarin town (1992).

No.	Number of analysed cells	Number of cells with micronuclei		Number of micronuclei in mononuclear cells		Distribution of cells by the number of micronuclei				
		AN	x±Sx	AN	x±Sx	1	2	3	4	>4
1	101	1	0.99±0.98	1	0.99± 0.98	7	-	-	-	-
2	291	8	2.75 ±0.96	17	5.84 ±1.37	6	-	-	-	1(10)
3	500	8	1.60 ±0.56	10	2.00 ±0.63	12	2	-	-	-
4	500	13	2.60 ±0.71	16	3.20 ±0.79	6	-	-	1	-
5	500	6	1.20 ±0.49	6	1.20 ±0.49	6	-	-	-	-
6	304	4	1.32 ±0.65	5	1.64 ±0.73	3	1	-	-	-
7	55	-	-	-	-	-	-	-		-
8	292	3	1.03 ±0.59	3	1.03 ±0.59	3	-	-	-	-
9	286	6	2.10 ±0.85	6	2.10 ±0.85	6	-	-	-	-
10	349	3	0.86 ±0.49	4	1.15 ±0.57	2	1	-	-	-
11	153	4	2.61 ±1.29	4	2.61 ±1.29	4	-	-	-	-
12	351	4	1.14 ±0.57	4	1.14 ±0.57	4	-	-	-	-
Group total	3682	60	1.63 ±0.21	76	2.06 ±0.23	54	4		1	1
Control	3405	27	0.79 ±0.15	29	0.85 ±0.16	25	2	-	-	-

Table 7. Individual analysis of *ex vivo* mutation in binuclear lymphocytes of peripheral blood of the children in Komarin town (1992).

No.	Number of analysed cells	Number of cells with micronuclei		Number of micronuclei in binuclear cells		Distribution of cells by the number of micronuclei				
		AN	x±Sx	AN	x±Sx	1	2	3	4	>4
1	18	-	-	-	-	-	-	-		-
2	90	6	6.67± 2.63	6	6.67± 2.63	5	-	-	-	-
3	500	18	3.60± 0.83	19	3.80± 0.86	17	1	-	-	-
4	274	17	6.20± 1.46	23	8.39± 1.66	13	3	-	1	-
5	307	7	2.28± 0.85	7	2.28± 0.85	7	-	-	-	-
6	237	8	3.38± 1.17	9	3.80± 1.24	7	1	-	-	-
7	15	1	6.67± 6.44	1	6.67± 6.44	1	2	-	-	-
8	79	4	5.06± 2.47	4	5.06± 2.47	4	-	-	-	-
9	52	5	9.62± 4.09	8	15.38±5.0	4	-	-	1	-
10	288	11	3.82± 1.13	11	3.82± 1.13	11	-	-	-	-
11	52	5	9.62± 4.09	5	9.62± 4.09	5	-	-	-	-
12	328	4	1.22± 0.60	5	1.52± 0.68	3	1	1	-	-
13	117	6	5.13± 2.04	8	6.84 ±2.33	5	-	-	-	-
14	500	11	2.20± 0.66	15	3.00± 0.76	9	1	-	1	-
15	95	7	7.33± 2.68	8	8.42± 2.85	5	1		-	-
Group total	2952	110	3.73 ±0.35	129	4.37± 0.38	98	8	1	3	-
Control	16631	199	1.20± 0.08	217	1.30± 0.09	18	-	-	-	-

Table 8. Comparison of group average indices of *ex vivo* mutation in non-stimulated (mononuclear) and stimulated (binuclear) lymphocytes of peripheral blood of the children in Komarin town (1992).

Nuclearity of lymphocyte	Number of analysed cells	Number of cells with micronuclei		Number of micronuclei in binuclear cells		Distribution of cells by the number of micronuclei					Morphological changes of nucleus	
		AN	x±Sx	AN	x±Sx	1	2	3	4	>4	AN	x±Sx
Komarin												
mononuclear	3682	60	1.63± 0.21	76	2.05± 0.23	54	4	-	1	1(10)	66	1.79 ± 0.22
binuclear	2952	110	3.73±0.35	129	4.37± 0.38	98	8	1	3	-	66	2.24 ±0.27
Control												
mononuclear	3405	27	0.79± 0.15	29	0.85± 0.16	25	2	-	-	-	1	0.03
binuclear	16631	199	1.20 ±0.08	217	1.30± 0.09	181	18	-	-	-	-	-

The results of micronucleus analysis of binuclear lymphocytes of peripheral blood with use of cytocholasin B in cell culture of the same children [12] are adduced in Table 7. As shown in the Table, the group average indices of micronucleus in binuclear cells are approximately three times as high as the corresponding indices in the control (the difference is statistically significant). However, the results of individual cytogenetic examination indicate only a tendency of increase of mutation level in binuclear lymphocytes in the majority of children of this group. Two children of the experimental group (No. 5 and 12) did not differ in the mutation level from the control, and in one child (No. 9) the index of micronuclei rate per 100 analysed cells was significantly higher than the control.

Regretfully, the obtained material does not allow to carry out an exact quantitative assessment of transgenerational somatic effect and individual sensitivity because the proliferative activity of lymphocytes of the children in Komarin town turned out to be extremely low. However, the comparison of group average indices of the level of micronucleus cells indicate their significant increase in binuclear lymphocytes as compared with mononuclear ones, i.e. as a result of one cell division in culture (Table 8).

The fact of unusually large decrease of proliferative response of T-lymphocytes to PHA is worth attention. It was not observed in our experiments with the children in other settlements of the Bragin district with higher indices of territory pollution with cesium and strontium, such as Malejki village of the Bragin district. This lymphocyte reaction is apparently connected with the factors of radioecological situation of this settlement which is situated immediately close to the Chernobyl power station. Besides, the pulverization of genetic material was discovered and morphologically reproductive perishing of lymphocytes (1.5% of cells) was registered as well. Taking into account these high indices of mono- and binuclear cells perishing, we can suppose that a considerable share of micronuclei registered in binuclear cells arose *de novo*.

2.2. *Ex vivo* analysis of micronuclei in stimulated and non-stimulated T-lymphocytes of the children in Malejki village

At the cultivation of lymphocytes of peripheral blood of the children in Malejki village, the contrary picture was revealed referring to the proliferative indices as compared with cell cultures of the children in Komarin town. The proliferative response of lymphocytes to PHA was so high that we could analyse only about 50 mononuclear among bi- and polynuclear lymphocytes per each child. In this connection, we could assess only the group average indices, and the results are adduced in Table 9. However, we can complement the picture of mutation process in mononuclear lymphocytes of peripheral blood of the examined children with the data obtained by analysing this cell populations with *in vivo* smear method. As shown in Table 9, the level of micronucleus lymphocytes *in vivo* in the children in Malejki village is 5 times as high as the corresponding indices in the children in Minsk city, and that of micronuclei in them - more than one order. In both cases the differences are statistically significant.

The comparison of micronucleus frequency in *ex vivo* non-stimulated lymphocytes of the children of the experimental group with the control also showed significant increase. But it is only two times higher than the control in this case.

Taking into consideration the presence, among the examined *in vivo* and *ex vivo* lymphocyte populations, of cells with various morphological changes of their nuclei (apoptosis, necrosis, pulverization and others), in other words, the strong selection in minus-trend of the micronuclei frequency at mitotic divisions, we can suppose that the micronuclei analysed in binuclear cells, like in the respective experiments with lymphocytes of the children in Komarin town, were expressed *de novo*.

Table 10 shows the data of individual cytogenetic examination of 15 children in Malejki village. The results of cytogenetic examination allow to divide this group into 2 subgroups: the 1st subgroup consists of the children in which the frequency of binuclear cells with micronuclei and of micronuclei in binuclear cells are on the level of control, and the 2nd subgroup consists of the children in which the lymphocytes have the frequency of binuclear cells with micronuclei on the level of control whereas the second index - micronuclei number per 100 cells - is statistically higher than the control.

The 1st subgroup is also unequal by the spectrum of registered micronuclei. In children No. 3 and 4, the cells with 3-4 micronuclei were registered among 500 analysed cells, the child No. 10 had one cell with 15 micronuclei among 1000 analysed cells.

It is necessary to note a change observed in the results of cytogenetic examination of lymphocyte populations of peripheral blood in the children of the Bragin district. According to our *ex vivo* individual examination in 1986-1988, 100% of children showed significantly increased levels of mutation. In 1992, however, only *in vivo* analysis at the individual level allowed to register significant disturbances.

In theory of cytogenetic analysis, under the condition of uniform exposure, the distribution of chromosomal injuries in cells is considered to follow the Poisson distribution [15]. The preliminary analysis of our results showed that the hemopoietic tissue in the majority of the children in Komarin and Malejki received not uniform but non-uniform dose of radiation.

Table 9. Comparison of group average indices of *in vivo* and *ex vivo* mutation in lymphocytes of peripheral blood of the children in Malejki village (1992).

Nuclearity of lymphocytes	Number of analysed cells	Number of cells with micronuclei		Number of micronuclei in binuclear cells		Distribution of cells by the number of micronuclei					Morphological changes of nucleus	
		AN	x±Sx	AN	x±Sx	1	2	3	4	>4	AN	x±Sx
in vivo												
Malejki												
	1429	54	3.78± 0.50	156	10.91± 0.82	16	19	7	14		58	4.76± 0.56
Control												
Mononuclear	7945	57	6.72± 0.09	58	0.73± 0.10	56	1	-	-	-	-	-
ex vivo												
Malejki												
Mononuclear	1250	27	2.16± 0.41	34	2.72±0.46	24	2	-	-	1(6)	23	1.84 ±0.38
Binuclear	10000	126	1.26± 0.11	230	2.30± 0.15	103	12	2	2	7	23	0.23± 0.05
Control												
Mononuclear	3405	27	0.79± 0.15	29	0.98± 0.16	25	2	-	-	-	1	0.03
Binuclear	16631	199	1.20± 0.08	217	1.30± 0.09	181	18	-	-	-	-	-

Table 10. Individual analysis of *ex vivo* mutation in binuclear lymphocytes of peripheral blood of the children in Malejki village (1992).

No.	Number of analysed cells	Number of cells with micronuclei		Number of micronuclei in binuclear cells		Distribution of cells by the number of micronuclei				
		AN	x±Sx	AN	x±Sx	1	2	3	4	>4
1	500	10	2.00± 0.63	63	12.60±1.48	5	2	-	-	3(30)-1 c.
2	800	11	1.38± 0.41	13	1.62± 0.45	9	2	-	-	-
3	500	7	1.40± 0.52	10	2.00± 0.63	6	-	-	1	-
4	500	8	1.60± 0.56	12	2.40± 0.68	5	2	1	-	-
5	1000	10	1.00± 0.31	10	1.00± 0.31	10	-	-	-	-
6	700	11	1.57± 0.47	39	5.57± 0.87	9	-	-	-	2(15)
7	1000	6	0.60± 0.24	8	0.80± 0.28	4	2	-	-	-
8	800	9	1.12± 0.37	10	1.25± 0.39	8	1	-	-	-
9	500	6	1.20± 0.49	6	1.20± 0.49	6	-	-	-	-
10	1000	6	0.60± 0.24	20	2.00± 0.44	5	-	-	-	1(15)
11	500	11	2.20± 0.66	20	4.00± 0.88	8	-	1	1	1(5)
12	700	7	1.00± 0.38	7	1.00± 0.38	7	-	-	-	-
13	500	3	0.60± 0.34	3	0.60± 0.34	3	-	-	-	-
14	500	11	2.20± 0.66	13	2.60± 0.45	7	3	-	-	-
15	500	10	2.00± 0.63	13	2.60± 0.45	7	3	-	-	-
Total	10000	126	1.26± 0.11	245	2.45± 0.15	103	12	2	2	7
Control	7580	102	1.35± 0.13	109	1.44± 0.14	95	7	-	-	-

Thus, the results of cytogenetic analysis of the children in the Bragin district testify at present that, apparently, the leading dose-forming factor is internal irradiation with complex of radionuclides including α-, β- emitters.

3. Genetic examination of the children in Komarin and Malejki settlements of the Bragin district of the Gomel region

The ionizing radiation causes a whole range of genetic changes in somatic and sex human cells: gene mutations, chromosomal aberrations, and genome mutations. In this connection, the assessment of genetic risk of the children living on the radiocontaminated territories in Belarus depends on the completeness of study about genetic effects of chronic radiation at low doses. Therefore, beside of the individual and group cytogenetic monitoring, it is important to pay attention to the problems of mutagenesis connected with gene mutations.

The scheme of genetic examination of children, proposed and developed by the Institute of Genetics and Cytology of the Academy of Sciences of Belarus, is based on the Norman's method [16] which detects mutations in locus of hypoxanthine-guanine-phosphoribosil transferase (HPRT).

The short-term Norman's method was chosen for detecting mutant T-lymphocytes due to the next reasons: the potential possibility to apply it to the monitoring of populations because of its relative simplicity and lesser cost (as compared with other methods of gene mutations account), the possibility to

Table 11. Individual data of the frequency of TG-resistant lymphocytes in cells culture of the children in Komarin town of the Bragin district

Group/child No.	Number of TG-resistant cells
Control	6.1 * 10 - 6
Komarin	
1	260.2 * 10 - 6
2	211.2 * 10 - 6
3	485.9 *10 - 6
4	315.8 *10 - 6
5	214.3 *10 - 6
6	374.0 *10 - 6
7	410.7 *10 - 6
8	306.4 *10 - 6
Group average	314.4 *10 - 6

Table 12. Individual data of the frequency of TG-resistant lymphocytes in cells culture of the children in Malejki village of the Bragin district

Group/child No.	Number of TG-resistant cells
Control	6.1 * 10 - 6
Malejki	
1	2.2 *10 -5
2	4.2 *10 - 5
3	3.6 *10 - 4
4	8.0 *10 - 5
5	7.4 *10 - 5
6	1.6 *10 - 4
7	1.6 *10 - 4
8	1.8 *10 - 4
9	6.8 *10 - 5
10	5.8 *10 - 5
11	1.0 *10 - 3
12	3.9 *10 - 4
13	3.8 *10 - 4
14	1.6 *10 - 4
15	7.4 *10 - 5
Group average	2.2 *10 -4

analyse simultaneously the gene mutations and the micronuclei as chromosomal aberrations analog, the possibility to use a series of parallel cultures because of the difficulty to obtain from children big volumes of vein blood as well as the possibility of full automation of research.

Tables 11 and 12 present the results of TG (thioguanine) -resistant induction in lymphocytes in peripheral blood of the children in Komarin and Malejki settlements as compared with these indices in the control group of Minsk city.

As are shown in the results of Tables, the frequency of TG-resistant T-lymphocytes in the control group constituted 6.1×10^{-6} cells. In 100% of examined children from the given settlements, the level of mutant lymphocytes was significantly differing from the control. The significant quantitative inter-individual variations were also observed within the groups of examined children. So, the number of TG-resistant lymphocytes in the culture of cells of the children in Malejki village varied from 2.2×10^{-5} to 1.0×10^{-3}, that of the children in Komarin town - from 2.1×10^{-4} to 4.8×10^{-4}. The comparison of group average index of TG-resistant lymphocytes frequency in the children in radiocontaminated zones with the index in the control group of donors in Minsk city reveals substantial excess of mutant cells in the experimental group (approximately two degrees).

The obtained results conform the data by other authors of the examination of persons irradiated as a result of the accident in Goiania (Brazil) in September 1987. The conditions of irradiation in Goiania were quite similar to those of some population groups due to the Chernobyl catastrophe. In both cases, the people were subjected to the external and internal irradiation. The papers of Natarajan *et.al.* [17, 18] indicate the increase of TG-resistant lymphocytes in irradiated persons 10 to 100 times higher as compared with the control group.

Thus, the results of genetic examination of the children of 12-13 years old who live permanently on radiocontaminated territories of Komarin and Malejki settlements in the Bragin district of the Gomel region have shown a necessity and a possibility to evaluate radiation dose to individuals and groups after many years since the Chernobyl catastrophe, based on the frequency of gene mutations in HPRT locus.

Conclusion

The general conclusion of our study, which has to be addressed at first, is the seriousness of discovered genetic disturbances in the examined children in Bragin town and other settlements in the Bragin district of the Gomel region.

The results of seven-year monitoring of children with use of *in vivo* micronucleus analysis of lymphocytes have shown that the highest level of mutation was found in the children born before the Chernobyl catastrophe. Consequently, the principle of radiation protection according to the level of average annual radiation dose is not acceptable to protect the children in the Bragin district because it does not take into account the total radiation dose since 1986 which conditions the radiation consequences for children health.

The analysis of the results of 1988-1994 indicates that, under the chronic action of ionizing radiation, complicated interactions between mutation pressure and selective process against cells with genetic injuries have been taking place in lymphocyte populations of the children in the Bragin district. Substantial differences between the examined children and the control were found in the level of mutations registered in peripheral blood lymphocytes both *in vivo* and *ex vivo*. The micronuclei level in lymphocyte populations *in vivo* did not decrease during 1988-1994. On the contrary, it increased approximately one order, whereas one mitotic division *ex vivo* in cell culture indicated substantial changes in different trends.

The cells with gene mutations capable to continue their life activity, apparently, undergo the selection in minus-trend to some extent but, probably, also contribute to the plus-trend selection both *in vivo* and *ex vivo*. As a result, in the last years we observe in *ex vivo* examination the high level of gene mutations against the background of relatively low level of chromosomal injuries.

The results of genetic monitoring of the children subjected to the long-term non-controlled radiation action with different intensity and duration in the Bragin district have shown that the frequency of gene mutations in HPRT locus can be a highly efficient bioindicator to evaluate individual and group irradiation. The obtained results confirm a principal necessity to carry out genetic monitoring of Belarus children with obligatory use of this method to account gene mutation . Simultaneous registration of mutation spectrum and frequency obtained with *in vivo* and *ex vivo* examination of peripheral blood lymphocytes in children will give more reliability to the bioindicator of chronic radiation effects.

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