Monitoring of Cytogenetic Damages in Peripheral Lymphocytes of Children Living in Radiocontaminated Areas of Belarus

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The complicated radiation situation in Belarus conditioned by the large scale and irregularity of radioactive precipitation as a result of the Chernobyl APS catastrophe, resulted in a number of different sources of irradiation to children.

In the first period after the catastrophe, short-lived isotopes, mainly I-131, made the principal contribution to the formation of irradiation to children. Dose values for thyroid in children turned out 3-10 times as high as in adults /1-2/. The situation having arisen after the Chernobyl disaster is characterized by different duration of contact with radiation and by diversity of pollution comprising irradiation from external and incorporated radionuclides. An intensive radionuclide mixture discharge continued till 10th of May. Their large quantity was released into the environment from the destroyed reactor for 2 weeks more.

Regretfully, information on genetic important dose in children population based on the data of physical dosimetry is practically absent. Counts of chromosome aberrations in peripheral blood lymphocytes (PBL) of the people who live in particular radioecological conditions are at present the most objective biological indicator of radiation effects. Chromosome injuries are the earliest and the most significant among intracellular reactions to ionizing radiation; they can be calculated precisely. Therefore, the WHO, IAEA and UNSCEAR documents /3-5/ recommend to use cytogenetic methods in order to estimate the value of dose absorbed by organism.

The necessity of using methods of biological dosimetry and, in particular, traditional cytogenetic analysis of unstable chromosome aberrations - such as dicentrics and ring chromosomes - was confirmed repeatedly in the studies of the consequences of people's overexposure in different nuclear catastrophes /6/. However, it is considered that the use of unstable type aberrations in biological dosimetry is not possible in remote times after overexposure /7/. It is connected with the fact that the frequency of cells having dicentric and ring chromosomes is decreases with the course of time after irradiation.

The cytogenetic monitoring of Belarussian children was started by the Human Radiation Cytogenetics Group of the Institute of Genetics and Cytology of the Academy of Sciences of Belarus in June 1986. Groups of children for cytogenetic examination were formed on the basis of administrative division of the territory (Table 1).

The aims of the present work is to analyse the data of 1986 as a practical case of cytogenetic method in the assessment of absorbed dose in children from Gomel region affected by the Chernobyl APS catastrophe, and to compare the data of cytogenetic examination of 1986 with the results obtained in the following years.

In this connection, we examined children evacuated from 10 villages within the 30-km zone of Bragin district, Gomel region, exposed to short-time (2 weeks) radiation, mainly due to fallout of short-lived radionuclides, and children residing in settlements in Bragin and Khoiniki district, Gomel region. The data of radiation monitoring of the radiocontaminated areas are given as of July 1, 1989 (7).

The results of cytogenetic examination of children in Minsk, and Braslav of Vitebsk region, were used as a control. Selection of the children was random. Significance of the differences was calculated by t-Students test.

Conventional methods (9-10) were used for preparing cytogenetic specimens. Blood samples were taken and transferred to the laboratory for cultivating within the same day. Fetal calf serum (10%, Minsk), phytohemagglutinin Difco P, 100 IU penicillin/ml in 0.5 ml of blood were added to the culture medium, consisting of Eagle's minimal essential medium (Moscow). For each individual 2-3 cultures were set up. 200- 300 cells per child were examined for asymmetrical chromosomal aberrations (dicentrics, centric rings, and excess acentric fragments) and chromatid aberrations (breaks, exchanges).

In Table 2 are presented group-average data on chromosome aberration yield for 60 children from 10 villages in the 30-km zone of Bragin district. The children were evacuated on May 7-8, 1986 to a "clean" zone in Minsk region (two weeks after the

Table 1. Districts from which the study cohorts were sampled and their contamination levels

Village	Radionuclide contamination (Ci/km^2)		
	Cs-137	Sr-90	Pu-239
30-km zone of Bragin district, Gomel region			
v. Nudichi	18.38	3.02	0.0503
v. Ilichi	22.03	3.44	0.0140
v. Glukhovichi	13.26	1.81	0.0201
v. Refalov	20.48	3.35	?
v. Kozeluzhtsy	20.43	3.60	0.0106
v. Jasmentsy	9.00	3.69	?
v. Krug-Rutka		3.80	?
v. Gorodistche	6.00	0.97	0.0030
v. St. Urkovichi		1.40	
v. Krivichi	3.45	0.98	0.0188
Bragin district			
t. Bragin	19.23	1.79	0.0418
v. Burki	11.0	0.7	?
v. Mikulichi	17.0	0.95	?
Khoinik district			
t. Khoinik	6.0	0.93	?
v. Novoselki	19.1	1.97	?
v. Rudnoe	14.0	3.0	?
v. Rudakovo	10.0	1.83	?
Narovlya district			
t.Naovlya	17.0	0.9	?
Control zone			
c. Minsk	0.04	0.02	0.0016
t. Braslav	0.04	0.02	0.0016

Table 2. Cytogenetic damages in peripheral blood lymphocyte of children evacuated from the 30-km zone of Bragin district and residing in Bragin town examined in 1986.

Settlement	No. of children examined	No. of cells scored	No. of Cells with aberr. (mean per 100 cells±SE)	No. of dic/ring per cell
30-km zone (10 villages)	60	18000	6.8±0.2	0.0095
t. Bragin	15	3900	7.7±0.4	0.0053
Control	18	3000	1.4±0.2	0.0006

Chernobyl nuclear accident). Cytogenetic examinations were conducted in August, 1986.

A statistically significant increase of the level of aberrant metaphases, aberrations per 100 cells, including markers of radiation effect, namely, dicentrics and centric rings, was revealed for all the groups examined. Thus, the level of chromosome-type exchanges in the spectrum of chromosome aberrations in children's lymphocytes varied from 0.0065 per cell to 0.0133 in different villages. The population average number of dicentrics/rings per cell in the children from the evacuation zone was 0.0095, being 0.0006 in the control.

Because of poor organization of radiation monitoring in the first months after the Chernobyl accident, the data of biological dosimetry have become more and more important. Biodosimetry

Table 3. Whole-body dose of liquidators and of a group of children evacuated from the 30-km zone of Bragin district

Liquidators (1)		Children from 30-km zone (our own data)	
Whole-body dose (rem)	% of examined	Whole-body dose (rem)	% of examined
5-10	30	1-20	10
10-25	47	20-30	26
		30-40	32
25-50	7.3	40-50	32

Table 4. Distribution of children by Iodine-131 content in thyroid and liver according to the γ-radiation exposure dose rate measured on 7-8 May 1986

γ-radiation exposure rate (μR/h)	% of examined with exposure rate	
	in area of neck	in area of liver
10-49	-	16.1
50-99	27.5	45.2
100-200	45	35.5
200-350	27.5	-
700	-	1.6

Table 5. Radionuclides in organisms of children from Gomel region

Radionuclides	Concentration of minimum/maximum value, Ci/l	
	Blood	Urine
Cesium-134	$0.127x10^{-9}$ - $1.17x10^{-9}$	$0.199x10^{-9}$ - $9.751x10^{-9}$
Cesium-137	$0.13\ x10^{-9}$ - $6.86x10^{-9}$	$0.57\ x10^{-9}$ - $2.5\ \ x10^{-9}$
Potassium-40	$2.073x10^{-9}$ - $6.70x10^{-8}$	$1.586x10^{-8}$ - $6.7\ \ x10^{-8}$
Zirconium-95	$0.97\ x10^{-9}$ - $1.46x10^{-9}$	$0.973x10^{-9}$ - $0.34\ x10^{-9}$
Antimony-125	$2.45\ x10^{-9}$ - $0.21x10^{-9}$	-
Silver-110	$6.7\ x10^{-10}$ - $5.6x10^{-11}$	-
Ruthenium-106	-	$0.648x10^{-9}$ - $2.162x10^{-9}$
Cerium-144	-	$0.648x10^{-8}$ - $2.23\ x10^{-9}$
Zinc-65	-	$1.189x10^{-9}$ - $1.1\ \ x10^{-10}$

carried out on the basis of our findings by using different standard curves (4,12-13) to evaluate absorbed dose has shown that the recorded level of chromosome aberrations in children evacuated from the 30-km zone of Bragin District and residing in Bragin town corresponds to the dose of 200-500 mSv.

According to the data of the Institute of Radiological Medicine (Belarus) based on the methods of physical dosimetry, radiation doses received by residents of those districts for 1986 to 1988 did not exceed 155 mSv. The reasons of the discrepancies in the estimated doses require further thorough investigation .

According to the estimations of EC/IAEA/WHO /1, 11/ the highest doses were obtained by the liquidators of the consequences of the Chernobyl APS catastrophe. The dose of whole-body irradiation spreads as follows (Table 3): 30% of studied people obtained 5-10 rem; the majority of liquidators - namely, 47% - obtained 10-25 rem, and only 7.3% - 25-50 rem /1/. Our data of biological dosimetry of children from the 30-km zone show the shift of dose distribution to the side of higher dose. Consequently, the resettled children and the children living in Bragin district and born before the catastrophe represent the same groups of high genetic risk as the liquidators do.

Based on the data of measurement of radioactive Iodine-131 content (7-8 May 1986) with portable γ-irradiation dosemeters, only 27% of examined people had the indices of exposure dose rate from 50 to 100 μR/h, the rest - from 100 and higher (Table 4).

The comparison of distribution of children by the whole-body irradiation dose (on the basis of cytogenetic examination) with internal dose for thyroid and liver by intake of radioactive iodine isotopes into the organism for the same period, showed the qualitative correspondence of both distributions, i. e. with the shift to the side of higher doses.

In order to confirm the presence of radionuclides in children organisms, the direct analysis was carried out of γ-radiating radionuclides in their biological media. The radionuclides in blood and urine of 37 children from the age of 4 to 15 from controlled areas were studied at the Institute of Nuclear Energy of the Academy of Sciences of Belarus by means of γ-spectrometer "ADKAM-300" of "ORTEC" firm (USA). It was established that the next radionuclides

Table 6. Sample of the cohorts and observed yields of unstable chromosomal aberrations in 1986

Settlement	Average age (y)	No. of children examined	No. of cells scored	No. of Cells with aberr. (mean per 100 cells±SE)	No. of dic/ring per cell
Gomel region					
Bragin district					
v. Burki	7-14	6	1500	5.7±0.6	0.0055
v. Mikulichi	7-14	17	4250	6.8±0.4	0.0063
Narovlya district					
t. Narovlya	12	7	1750	4.9±0.5	0.0041
Minsk					
Control 1985		18	3000	1.4±0.2	0.0006
Control 1986		17	4250	1.5±0.2	0.0006

Table 7. Sample of the cohorts and observed yields of unstable chromosomal aberrations in 1987

Settlement	Average age (y)	No. of children examined	No. of cells scored	No. of Cells with aberr. (mean per 100 cells±SE)	No. of dic/ring per cell
Gomel region					
Khoiniki district					
t. Khoiniki	1-6	5	652	3.7±0.7	0.0092
v. Novoselki	1-6	6	700	7.6±1.0	0.0143
v. Rudnoe	1-6	10	2050	9.8±0.6	0.0098
Narovlya district					
t. Narovlya		5	1250	6.4±0.7	0.0064
Control		17	4250	1.5±0.19	0.0006

were present in blood: Cesium-134, Cesium-137, Potassium-40, Zirconium-95, Antimony-125, Silver-110. In the urine, the next radionuclides were found besides above-mentioned: Carbon-14 (June 1986), Ruthenium-106, Cerium-144, Zinc-65, Zirconium-95 (1986-1987-1988). There were found in the excrement: Cesium-134, Cesium-137, Potassium-40 (Table 5).

Table 6 shows the principal results of cytogenetic examination of children residing in Burki and Mikulichi villages of Bragin district and Narovlya town of Narovlya district of Gomel region. In all groups, the number of cells with unstable aberrations - dicentrics and centric rings - differed significantly from the control one. The dose values calculated on the basis of cytogenetic analysis of unstable aberrations are proximate to the doses obtained by the children of Bragin town shown in Table 2.

Thus acute and prolonged irradiation obtained by the children in Bragin district and Bragin town were considerable in the first period (first weeks and months after the accident) and, obviously, conditioned not only by short-lived isotopes (molybdenum, technetium, lanthanum, barium), inert gases (xenon, krypton) and I-131, but also by long-lived isotopes of cesium, strontium and plutonium /1/. They are comparable (and even higher) with the dose obtained by the liquidators. Regretfully, these dose commitments were not taken into account before, and still are not considered in recent assessment of obtained doses values.

In the second stage (1987-1991), prolonged external and internal irradiation for account of long-lived radionuclides of cesium, strontium, plutonium and others is the particularity of dose formation of the children living permanently on radiocontaminated territories, in addition to the early formed dose during the 1st stage including the thyroid irradiation. It should be noted that in most cases the same children were not examined in the samples.

The cytogenetic effect specific for radiation action was also revealed in children in Bragin town examined in 1988. A similar picture was observed in a group of children in Bragin, examined in 1991 by using the micronuclei method under cytokinetic block (10). The results confirmed the fact that an increased level of chromosome aberrations remained in lymphocytes of the children. The number of detected binuclear cells with micronuclei was 5.3 times higher than the number in the control. Such situation was arisen, obviously, as a result of relatively balanced state between formation of mutation and disappearance both on the level of blood-producing cells and on that of peripheral blood.

However, the more complicated picture of mutation process was discovered in lymphocytes of younger children (age from 1 to 6). In the spring of 1987 clinical and cytogenetic examination was carried out of 21 children, aged 1-6 years, residing permanently in the settlement of Khoiniki and in the villages of Novoselki and Rudnoe, Khoiniki district, Gomel region. The data obtained indicated an increased level of chromosome aberrations in cultures of peripheral blood lymphocytes of these children's groups: 3.7±0.7; 7.6±1.0; 9.8±0.5 % of aberrant metaphases, respectively, as compared to 1.5-0.5% in the control (Table 7). In all examined groups chromosomal aberrations of exchange type were found - dicentrics and centric rings - the frequency of which was over one degree as high as that of control group. The highest frequency of dicentrics and rings as well as of cells containing such type of aberrations was revealed in the children of Novoselki and Rudnoe villages.

In spring of 1987 and 1988 cytogenetic examination of the same children of Bragin and Khoiniki districts was conducted in dynamics. The first examination was carried out 12 months after the Chernobyl nuclear accident. An interval between the first and the second examinations was also 12 months. The increase of chromosome type aberrations from 5.2±0.5% in 1987 to 8.7±0.6% in 1987 ($p<0.001$) was observed. A significant increase in the cell number with 2-4 aberrations (from 16.4±3.3% in 1987 to 27.0±3.4% in 1988, $p<0.01$) was observed in the same children. Cells with 2 and more aberrations were not found in the control.

It was shown experimentally that the external irradiation dose of population in radiocontaminated areas of Belarus had stabilized by 1989-1990; meantime, strontium radionuclides content in organisms of the people had increased 2.5-5 times as compared with pre-accident period /1/. Probably, the increase of the level of chromosomal aberrations with the course of time and appearance of multiple injuries in cells of small children are connected with this situation.

Some "rogue" cells, which were named by Awa and Neel (15) , were found by the present authors in lymphocytes of the children in Khoiniki and Bragin districts almost a year after the Chernobyl accident. Single "rogue" cells were detected by other researchers in various groups of children living in the radiocontaminated areas of Belarus (16). However, those authors do not ascribe emergence of "rogue" cells to radiation exposure, though we consider the question doubtful.

More frequent occurrence of cells with 3 or 4 aberrations with increase of the time of children staying in the radiocontaminated areas of Khoiniki and Bragin districts seems to be associated with the time change of the dose structure due to increase of internal contribution to the total radiation dose.

According to available data, Cs-137 and Cs-134 contents in the examined children's organisms in Khoiniki and Bragin districts, Gomel region, in 1987-1988 varied between 0.18 and 9.14 μCi (the data were obtained by a whole body counter). However, no correlation was revealed between the total γ-activity of the organism and the yield of chromosome aberrations. In this connection, the decrease of dose rate from external (1, 17-18) and incorporated (1,18) γ-sources on one side, and the increase of accumulation of α- (1, 19) and β-sources (1,20), on the other side, deserve attention.

The present results have shown that the level of cytogenetic damages in human cells was higher in all radiocontaminated regions than in the control. Thus, In the 2nd period the high tempo of mutation process is characteristic in peripheral blood lymphocytes of all (100%) examined groups of children in Gomel region, as well as the appearance of multiple aberrations in cells of considerable part of children. The task of following surveys is the detailed characterization of peculiarities in the 2nd and the 3rd periods of mutation process dynamics in populations of somatic cells of children in Belarus.

Conclusion

The presented data of cytogenetic investigation of different groups of children affected by ionizing radiation as a result of the Chernobyl APS catastrophe allow to make the following conclusions:

- The use of cytogenetic method in the investigation of children born before the Chernobyl accident made possible to reveal significant increase of unstable chromosomal aberrations as compared with control groups.
- The absorbed dose of 200-500 mGy was evaluated for the children from the 30-km zone of Bragin district and Bragin town with the use of materials of cytogenetic investigation conducted in 1986.
- It is shown that the level of unstable type aberration in young children is rather increasing with the course of time than decreasing. The appearance of multiaberrant cells in children several years after the Chernobyl APS catastrophe, probably, testifies the effect of dense ionizing radiation of plutonium and its fission products on children.

REFERENCES

1. "Consequences of the Chernobyl APS Catastrophe for the Republic of Belarus", Chernobyl Catastrophe: Causes and Consequences, Minsk 1992, 207 p. (in Russian).
2. "Chernobyl Catastrophe", Kiev 1995, 547 p. (in Russian).

3. United Nations. Ionizing radiation. Sources and biological effects. UNSCEAR 1982, report to the General assembly with annexes. UN sales publication.T.82.1982. United Nations,NY,1982.
4. Biological Dosimetry: Chromosomal aberration analysis for dose assessment. Technical reports series N 260.IAEA, Vienna, 1986. p. 1-69.
5. Method of human chromosome aberration analysis. Edit. by K. Bacon, H. Evens. WHO, Geneva, 1976. 64 p.
6. Shevchenko V. A., Snigireva G. P. "Cytogenetical Effects of Ionizing Radiations on Human Populations", Consequences of the Chernobyl Catastrophe: Human Health, Moscow 1996, p 24-49 (in Russian).
7. Awa A.,Sofuni T., Honda T. et al. // J.of Rad.res. 1978 V.19.- P. 126-140
8. USSR State Committee for Hydrometeorology. (1989). The data on 137Cs and 90Sr Radioactive Contamination of Belarus Settlements (as of June 1989) - Minsk, Polymya.- 176p.
9. Moorhead P.C., Nowell P.C., Mellman W.J., Battips D.M., Hungerford D.A. Chromosome Preparations of Leukocytes Cultured from Human Peripheral Blood. Exptl. Cell Res. 1960.- 20, P. 613-618.
10. Fenech M., Morley A.A.(1985). Measurement of Micronuclei in Lymphocytes. Mutat. Res. 1985.V. 147, P. 29-36.
11. Highlights of Conclusions from the International Conference "One Decade after Chernobyl". Vienna, 8-12 April 1996. P. 1-11.
12. de Campos I.M.A., dos Santos O.R., Mesquita C.H. (1990). Dose Estimation by Cytogenetic Analysis in a Radiation Accident with 137Cs in Goiania (Brazil): Evaluation of Probable Exposure Doses Range. Radiat. Protect. Dos.1990, 30, P. 33-36.
13. Sevankaev A.V., Nasonov A.P. Standard Dose Curves of Chromosome Aberrations in Human Lymphocytes. Meditsinskaya Radiologia, 1978 23, P. 26-33.
14. Matyukhin V.A. Radiation and Ecological Situation and Medicobiological Aspects after the Chernobyl Accident in Belarus. Zh. Zdravookhranenie Belarusi, 1990.- P.8-11.
15. Awa A.A., Neel J.V. (1986). Cytogenetic "rogue" Cells: What is Their Frequency, Origin and Evolutionary Significance? Proc. Natl.Acad. Sci., USA, 83, 1021-1025.
16. Sevankaev A.V., Tsyb A.F., Lloyd D.C., Zhloba A.A., Moiseenko V.V., Skryabin A.M., Klimov V.M. (1993)."Rogue" Cells Observed in Children Exposed to Radiation from the Chernobyl Accident. Int. J. Radiat. Biol.63, 361-367.
17. Matyukhin V.A.(1992). Problems of the Chernobyl Accident. Proc.: An Intern. Symp. on Chernobyl Catastrophe.Minsk, 6-9.
18. Kazakov V.S., Matyukhin V.A., Kenigsberg Ya.Z., Minenko V.F., Ispenkov E.A., Moshchik K.V.(1991). Radioecological Situation in Belarus after the Chernobyl Accident, Medicobiological Consequences and Scientific Basis of Measures for Radiological Protection of People. In: Scientific _and Practical Aspects of Health Safety of People Exposed to Radiation as a Result of the Chernobyl Accident. BSSR, Ministry of Health. Minsk, 3-6.
19. Khokhryakov V.F., Suslova K.K., Skryabin A.M. (1992). Content of Pu Isotopes in Organisms of Residents in Gomel Region. In: Scientific and Practical Aspects of Health Safety of People Exposed to Radiation as a Result of the Chernobyl Accident. BSSR, Ministry of Health, Gomel, 10-11.
20 .Loseva L.P.(1991). Estimation of 90Sr Content in Human Organism In: Scientific and Practical Aspects of Health Safety of People Exposed to Radiation as a Result of the Chernobyl Accident BSSR, Ministry of Health, Minsk, 16-17.
21. Mikhalevich L.S., Savchenko V.K.,Pavlova M.P. et al. Chromosome Aberrations Yield under Additional Irradiation of Children Lymphocytes in Gomel Region .In: Proc. of Republican Scientific and practical Conference for Radiobiology and Radioecology. Mn.,1988.