Some Issues of Long-Term Investigations on Genetic Consequences by the Chernobyl Accident

Lazjuk G.I.[1], Satow Y.[2]

[1] – Belarus State Medical University, Minsk, Belarus

[2] – Hiroshima University, Hiroshima, Japan

1. SUMMARY

Results of almost 20-year investigations of possible consequences of the Chernobyl accident for Belarussian population, obtained by the National Research Institute for Hereditary and Inborn Diseases, provide grounds for the conclusion that at least four facts, related to genetic consequences, had connected to the Chernobyl accident: three could be defined as proven and one – as probable. The first three are:

- Significantly increased level of chromosome aberration in pregnant women and their newborn babies, who resided in 1986-1988 in zones with ^{137}Cs contamination of 555 kBq/m^2 and higher;
- Significant three-year increase of prevalence of embrional development defects in the same abovementioned zones and in the same years, which lead to the increase of prevalence of congenital anomalies in social abortuses, fetuses and newborns;
- January peak of babies with Down syndrome, born by women who were in the zones of maximum irradiation in the period 26-30 April 1986;

Of possible but not yet proven consequences we shall mention investigation in contaminated and control zones before and after the Chernobyl accident of the ratio of *de novo* structural chromosomal aberrations (SCA) to inherited SCA, which induce chromosomal diseases. This investigation revealed an increase of the share of *de novo* SCA in after-Chernobyl period. Etiology of observed changes is not uniform. While an increased level of chromosome mutations, including trisomies, is essentially induced by additional ionizing radiation, many congenital anomalies which determined increased frequencies of defects in 1987-1989, belong to the group of multifactoral defects, i.e. for their etiology important are radiation as well as nutritional problems, hormonal and immunological upheavals.

2. RESULTS OF INVESTIGATION OF CYTOGENETIC EFFECTS

Cytogenetic effects in pregnant women and their newborn babies who have lived in the regions with most severe contamination by Chernobyl fallouts (Gomel and Mogilev oblasts of Belarus) were investigated. Standard practices were used for cultivation of peripheral blood lymphocytes and registration of chromosome aberrations (G. Lazjuk et al., 1999). As could be seen from Table 1, all pregnant women and their fetuses received biologically effective doses of ionizing radiation, which resulted in increased numbers of dicentrics and ring chromosomes.

It appeared that mutagenic effect in evacuated pregnant women (group 1) was higher than in women who became pregnant 0.5-1 year after the disaster (group two) and women, who have lived more than 2 years on the territories with the soil contamination of 555 kBq/m^2 and higher. The level of dicentrics and ring chromosomes in newborns was also higher than in their mothers (0.38% and 0.32% in Gomel oblast, 0.21% and 0.19% in Mogilev oblast, respectively).

Table 1. Frequency of dicentrics and ring chromosomes in pregnant women and newborn babies in contaminated and non-contaminated regions of Belarus.

Regions	Investigated groups	Number of investigated metaphases	Frequency of rings and dicentrics
Gomel oblast	1st group*	14645	0.32
	Newborn babies of 1st group	9167	0.38
	2nd group**	7753	0.14
Mogilev oblast	3rd group***	7715	0.19
	Newborn babies of 3rd group	7486	0.21
CONTROL			
Novopolotsk	4th group****	4965	0.04
Minsk	Newborn babies	9670	0.04

- * - 1st group – pregnant women evacuated during May-June 1986 from districts of Gomel oblast with maximum contamination;
- ** - 2nd group - pregnant women who have lived more than a year in zones with ^{137}Cs contamination of 555 kBq/m^2 and higher;
- *** - 3rd group - pregnant women, who have lived more than two years in zones with ^{137}Cs contamination of 555 kBq/m^2 and higher;
- **** - 4th group – pregnant women of control group from the city of Novopolotsk

3. RESULTS OF INVESTIGATION OF THE PREVALENCE OF EMBRYONIC DEVELOPMENT DISRUPTIONS

Embryonic development disruptions were investigated by registration of congenital anomalies in social abortuses, infants and foetuses.

3.1. Results of investigation of social abortuses

By social abortion we understand the product of conception (embryo or fetus) was obtained after the termination of pregnancy on woman's request. Since the material was collected without sampling, it means that in fact this is a population investigation. Pregnancy terminations were carried out in specialized medical facilities by gynecologists, by curettage of uterine cavity when gestational age was 5 to 12 weeks. This material was investigated by embryologists of the National Research Institute for Hereditary and Inborn Diseases on non-formaline-fixed material with stereomicroscope and on histological sections. When necessary, cytogenetic investigations in cultivated tissues of embryos were carried out. The prevalence of embryonic development disruption was calculated for the number of investigated organs. The total number of investigated abortuses exceeded 31,000, of which 2,701 were received from the Chernobyl zone and the remaining were from Minsk, they were used as control. The frequency of embryonic development disruption in social abortuses from the contaminated areas was significantly higher than in Minsk during the same period (7.2% and 4.9%, respectively). Increase in malformations was observed in all systems of organs, while more significant prevalence was observed for cleft of lip and/or palate, dubling of kidneys and ureters, polydactyly and neural tube defects. The overwhelming majority of malformations in social abortuses are etiologically heterogenic and due to this reason an accurate estimate of the potential share of ionizing radiation in increased prevalences is impossible. Moreover, in our investigations we have not observed neither higher number of aneuploids, nor cluster death of cells during organogenesis, which are indicative for radiation effects.

3.2. Results of investigation of congenital anomalies prevalences

Prevalence of congenital anomalies was investigated in medical abortuses, obtained after termination of pregnancy due to genetic indications, in stillborns and newborns. Data of the national registry of congenital anomalies (collected since 1979 at the National Research Institute for Hereditary and Inborn Diseases) were used to this end. The details on the Belarus registry could be found in: G. Lazjuk et al., 2003 (7). In this paper we describe only the results of investigation of prevalence of strict registration congenital anomalies (SR CA). To this group belong anomalies which could be unambiguosly diagnosed in prenatal and neonatal periods. So, to SR CA group belong: anecephaly (Q00), spina bifida (Q05), cleft of lip and/or palate (Q35, Q37), polydactyly (Q69), limb reduction defects (Q71, Q73), digestive system atresia (Q39, Q39,2), anal atresia (Q42, Q42,3), Down syndrome (Q90), multiple congenital anomalies (Q86, Q87, Q89,7, Q91-93, Q96-99). Analysis of SR CA prevalence was carried out in 4 groups. The first group was represented by the material from 17 districts of Gomel and Mogilev oblasts, where there are territories with the surface density of soil contamination by ^{137}Cs from 555 kBq/m^2 and more, the 2nd group – control for the 1st group – 30 districts of Belarus in which the level of contamination by cesium was less than 37 kBq/m^2 , the 3rd group – all territory of Gomel and Mogilev oblasts (oblast centers excluded), not taking the level of radioactive contamination into account, the 4th group – control for the 3rd group - all territory of Minsk and Vitebsk oblasts (without the capital and oblast centre of Vitebsk oblast).

It is easy to see from Figure 1 that while the initial prevalences of anomalies in both groups are practically equal, during the first three years after the Chernobyl disaster the prevalence of SR CA is much higher in the first group than in the control.

Figure 1. Prevalence of SR CA in 17 contaminated (Zone of Strict Radiological Control, ZSRC) districts (N=982) and 30 control districts of Belarus (N=1876)

After these three years, the prevalence of anomalies in two compared regions was not statistically different, but there existed an annual smooth growth, and during 1990-2003 the level of prevalence of investigated anomalies in both groups reached the level that was observed in the most contaminated districts (Table 2) between 1987-1989.

Table 2. Prevalence of SR CA in ZSRC and control districts during three periods (1981-1986, 1987-1989, 1990-2003)

Districts	Contaminated districts of ZSRC (N=17)			Control districts (N=30)		
Years	**1981-1986**	**1987-1989**	**1990-2003**	**1981-1986**	**1987-1989**	**1990-2003**
Number of liveborns and stillborns	58128	23925	72143	98522	47877	153680
Total number of **SR CA**	237	187	558	430	239	1207
Prevalence (1:1000)	4.08	7.82*	7.73	4.36	4.99	7.85

When prevalence of SR CA is compared on regional (oblast) level in the groups 3 and 4, there could be seen a trend to increased prevalence of anomalies during the first 3 years, bur this rise was not as distinctive as in zones of maximum contamination.

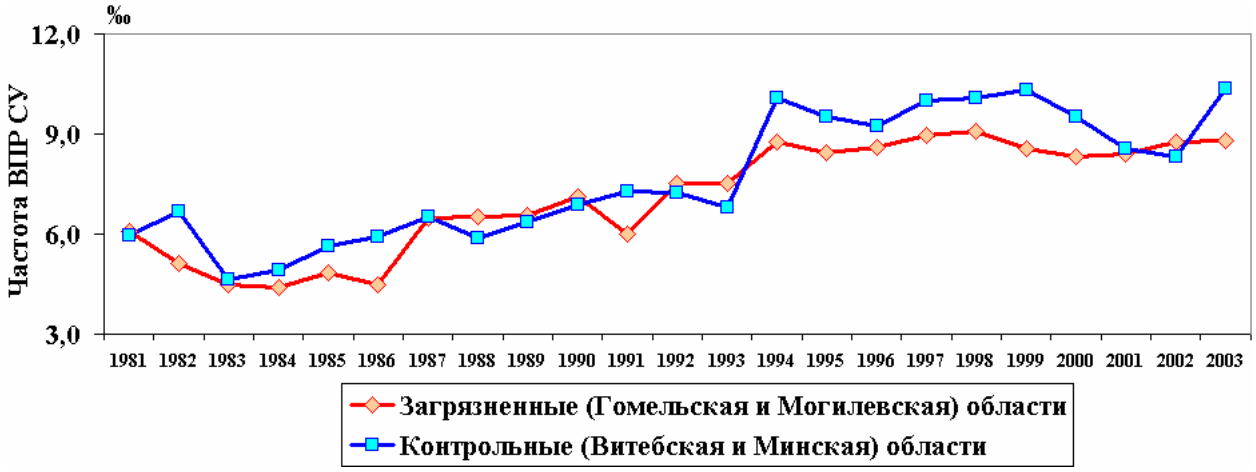

Figure 2 - Prevalence of SR CA in contaminated (Gomel, Mogilev; N=5692) and control (Vitebsk, Minsk; N=10008) oblasts.

The reason for a less clear 3-year rise of SR CA prevalence on oblast level as compared with the results obtained for districts, apparently is a "diluting" of the results of 17 districts severely contaminated by Chernobyl fallouts (with soil contamination by ^{137}Cs of 555 kBq/m^2 and more) by material obtained from other districts of the same oblasts where population lives on territories with low contamination or relatively clean areas. Moreover, there were territories in Minsk oblast (4th group) with soil contamination that exceeded 185 kBq/m^2.

Relative risk of SR CA for 17 most severely contaminated districts of Gomel and Mogilev oblasts increased from 0.9 in 1981-1985 to 1.6 in 1987-1989. After that (in 1990-2003) the relative risk fell to 1.0. The clearest increase, and hence the higher relative risk, was observed for polydactyly, limb reduction defects and multiple congenital anomalies. Significant input of dominant mutations, for which ionizing radiation is believed to be important, is characteristic for these anomalies. Summing up, in the most contaminated by Chernobyl fallouts regions of Belarus a three-year (1987-1989) increase in prevalence of CA was observed. In the following years the prevalence of CA increased in all areas indiscriminately to the level of contamination and there was practically no difference between "clean" and contaminated regions. Thus, if investigations of the prevalence of CA in Belarus were started 4-5 years after the Chernobyl disaster, as it happened in Japan after the nuclear bombardment, the difference between prevalence of CA in contaminated and non-contaminated zones would not have been detected.

4. RESULTS OF INVESTIGATION OF THE CONSEQUENCES OF CHROMOSOME AND GENOME MUTATIONS

As it was demonstrated by numerous investigations, the irradiation of population by radioactive fallouts of Chernobyl has induced an increase of mutations level in somatic cells (I.Eliseeva, 1991, M.Pilinskaya, S.Dybinskiy, 1992; G.Lazjuk et al,1995). It's not impossible that commeasurable changes did occur in gametal cells, but there are no investigations of this potential aftermath oft the Chernobyl disaster. Indirect assessment might be conducted by usage of the National Research Institute for Hereditary and Inborn Diseases results of investigation of prevalence of births with Down syndrome and prevalence of chromosomal diseases caused by non-balanced structural changes of chromosomes before and after Chernobyl period.

4.1. Investigations of prevalence of Down syndrome revealed that while variations of annual prevalence were in general low (about 1 case per 1000 births throughout the country and slightly higher variation in individual oblasts). In January 1987 a high increase (by the factor of 2.5) of births with Down syndrome was observed in Belarus. In Gomel oblast this factor reached its maximum – 3,6‰. These prevalences of Down syndrome were 2-3 times higher than expected (Table 3).

Table 3. – Territorial distribution of children with Down syndrome born in January 1987.

Region	Prevalence (‰)		O	E	O/E	95% CI	
	January 1987	1981-1989					
Belarus	2.5	1.0	31	13.9	2.2	1.5	3.2
Gomel oblast	3.6	1.1	8	2.6	3.1	1.4	6.2
Minsk oblast	3.1	1.1	6	2.2	2.8	1.0	6.0
Minsk city	2.7	1.1	6	2.6	2.3	0.9	5.1
Vitebsk oblast	2.2	1.0	4	1.8	2.1	0.6	5.7
Grodno oblast	1.7	0.9	3	1.6	1.9	0.2	4.6
Mogilev oblast	1.2	0.9	2	1.5	1.3	0.2	4.9
Brest oblast	1.0	0.8	2	1.8	1.1	0.1	4.0

Notes:
O – observed number of Down syndrome cases
E – expected number of Down syndrome cases
CI – confidence interval

Investigation of possible reasons for abrupt increase in the prevalence of Down syndrome (G.Lazjuk et al, 2002; G.Lazjuk et al, 2003) allowed for ruling out such factors as a change of mothers' age at birth, prenatal diagnostics and possible effect of increased (due to extraordinary situation) attention to this anomaly. After numerous discussions of these results it was defined that the only reason is an impact of short-term intensive irradiation of Belarus women's gametes. This conclusion is supported by the time period when the increase appeared (9 months after irradiation, when babies conceived in the period of the maximum increase of radiation were due to be born); by territorial distribution of numbers of such babies, which resembles a trajectory of air masses during first days after the accident; and by a known high radiosensitivity of mammals at the stage of ovogenesis preceding conception, which coincided with the maximum level of irradiation.

4.2. Investigation of frequencies of chromosomal diseases caused by sporadic structural changes of chromosomes

One of causes of chromosomal diseases is non-balanced sporadic structural changes of chromosomes (sporadic chromosomal aberrations - SCA). Such chromosomes could be found in gonads of parents (SCA de novo) or more remote ancestors (inherited SCA). By observing changes of the ratio SCA *de novo* to SCA inherited it is possible to estimate the pressure of mutagenic factors, including ionizing radiation, on hereditary structures.

Investigation of the ratio of SCA *de novo* to SCA inherited in the contaminated and the control zones before and after the Chernobyl disaster was carried out at the National Research Institute for Hereditary and Inborn Diseases of Belarus. For this purpose material of the Belarussian National Registry of congenital anomalies collected in 1979-1998 was used. Altogether, 209 families with various chromosomal diseases caused by SCA were examined. Of them, such children were born in 72 families before the Chernobyl disaster, and in 173 – after. As follows from Table 4, during the pre-Chernobyl period there was no statistical difference ($t=0.9$; $P=0.58$) between ratios of SCA *de novo* to SCA inherited in two compared regions. The ratio of inherited SCA to SCA *de novo* in families which had children with same diseases during 1987-1998 period shifted in favor of SCA *de novo* both in the contaminated and the "clean" zones, but the increase in the contaminated zones was more significant (89% and 68%, respectively, where $t=2.99$; $P<0.01$).

Table 4 – Comparison of sporadic (de novo) and inherited structural changes of chromosomes in children with chromosomal diseases in Belarus

Years of birth	Zones contaminated by radionuclides			Zones free from radionuclide contamination			Total
	de novo	inherited	total	de novo	inherited	total	
1979-1986	11(61%)	7	18	28(52%)	26	54	**72**
1987-1998	24(89%)	3	27	75(68%)	35	110	**137**
1979-1998	**35**	**10**	**45**	**103**	**61**	**164**	**209**

Thus, in spite of the fact that the presented data need more wide population investigations, already obtained data that the ratio of SCA *de novo* to SCA inherited has changed in favor of SCA *de novo* support the assumption that the pressure of mutagenic factors on hereditary structures increases, which is even more visible in zones with high contamination by Chernobyl fallouts.

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