Analysis of Chromosome Aberrations in Human Lymphocytes after Accidental Exposure to Ionizing Radiation

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Abstract

Aim: Analysis of the results of cytogenetic examination and reconstruction of irradiation doses by the frequency of chromosome aberrations in the liquidators of the consequences of the Chernobyl NPP accident.

Materials and methods: Over 1500 people who worked in the zone of the accident predominantly in 1986 and in 1987 have been examined since 1986. All of them were examined by means of the classical cytogenetic method which makes it possible to assess the level of unstable chromosome aberrations. 64 patients (including 12 professional workers of the Kurchatov Institute) were examined by the FISH method which permits the estimation of the level of symmetrical translocations.

Results: The cytogenetic examination performed in 1986 revealed a high level of cells with dicentrics (exceeding 16-fold the control level) in the group of liquidators. In succeeding years this level significantly decreased. However even in 15 years after the works in the zone of the accident were completed the frequency of cells with dicentrics in the group of liquidators significantly exceeds the control level. Using the frequency of cells with dicentrics and the calibration dose-response curve, the dose of irradiation was determined for the group of liquidators examined in 1986. The average dose of irradiation made up 0.14 Gy. The frequency of translocations was used to estimate the exposure dose for the group of 52 liquidators. The average dose for the whole group was estimated to be 0.16 Gy. For 18 patients in whom the frequency of translocations significantly differed from the control level the individual doses of exposure were determined. The scatter in the doses was from 0.22 to 1.0 Gy.

In 1996, 22 workers of the Kurchatov Institute were examined. In most of them (13 patients) the frequency of dicentrics in peripheral blood lymphocytes was significantly higher than the control values. In five patients, cells with multiple chromosome aberrations were discovered. Three examined workers were exposed to super-high doses. The frequency of dicentrics in peripheral blood lymphocytes of these patients was 100 and even 1000 times higher than the control level. The doses of irradiation estimated for five workers of the Kurchatov Institute by the frequency of translocations using the calibration curve were in the range between 0.21 and 2.51 Gy. These doses were calculated without correction coefficients, i.e. without taking into account the fact that those workers were for several years exposed to multiple irradiations at different dose rates. Consideration of these conditions will lead to an increase of the estimated absorbed doses.

Conclusion: The cytogenetic methods are sufficiently sensitive for assessing the condition of the cell hereditary structures. Analysis of unstable chromosome aberrations is a decisive method in monitoring of large groups of people exposed to radiation as a result of nuclear accidents. The data of cytogenetic examination can be one of the criteria in the formation of groups with an increased risk of development of different diseases. One of the most promising cytogenetic methods of biological dosimetry is the analysis of stable translocations by the FISH method. The lower limit of dose estimation by the frequency of translocations is 20-25 cGy.

1. Introduction

It is already over several decades that the analysis of chromosome aberrations in peripheral blood lymphocytes has been successfully used to examine people exposed to radiation during various accidents [1-9]. The results of cytogenetic analysis serve as a basis for estimating absorbed radiation doses and for predicting possible negative effects of irradiation.

The traditional procedure in biodosimetric studies is the analysis of the frequency of chromosome aberrations, namely, dicentrics or symmetrical translocations. The radiobiological basis for applying cytogenetic indices in dosimetric studies is the existence of a relationship between the dose and the yield of chromosome aberrations as well as the coincidence of dose-effect curves upon irradiation of blood cells in vitro and in vivo. It is customary to assume (and it was corroborated in some investigations) [10,11] that the frequencies of dicentrics and translocations immediately after irradiation are practically the same.

Cells with dicentrics are good indicators of irradiation within short periods after radiation exposure. With post exposure time such cells are eliminated from peripheral blood because their passage through mitosis is impeded [12]. In connection with this, the analysis of the frequency of dicentrics is of low efficiency for retrospective estimation of radiation doses. After a long time following the radiation exposure as well as under conditions of long-term chronic or prolonged irradiation the accumulated dose is estimated using the analysis of stable chromosome aberrations – symmetrical translocations. In the opinion of some authors [5,6,13-15] the frequency of cells with translocations remains constant for a long time after irradiation or, as other researchers [16,17] suppose, it may decrease, but with a considerably slower tempo as compared to cells with dicentrics. The latter fact imposes some limits on the assessment of radiation doses by the frequency of translocations but the importance of using cytogenetic methods in biodosimetric studies does not become less. In emergency situations, when reliable methods of physical dosimetry are often absent, the analysis of chromosome aberrations permits the degree of radiation damage of the organism to be determined taking into account its specific individual features and, in the first turn, its individual radiosensitivity. Information on “biological dose” is of great prognostic value for assessing the remote effects of irradiation and for choosing adequate methods of therapy and prophylaxis.

In the case of large-scale cytogenetic monitoring in groups of people exposed to radiation as a results of radiation accidents the analysis of unstable chromosome aberrations assumes particular importance. It allows sufficiently large contingents of exposed people to be examined without special financial expenditures and without employing sophisticated techniques. The results of such monitoring make it possible to obtain information on the effects of the radiation factors on the organism, on the degree of genome damage, and finally they can be used as criteria in the formation of groups of people with a high risk of occurrence of different diseases, including oncological ones [18,19].

2. Subjects, materials and methods

2.1. Case reports of examined patients

The Chernobyl nuclear power plant accident is the most dramatic ecological disaster of the 20th century. One of the largest categories of the population that has suffered most as a result of this catastrophe are the clean-up workers (liquidators). Beginning in 1986, the Russian Research Center of Roentgeno-Radiology and N.I. Vavilov Institute of General Genetics have been carrying out joint cytogenetic examinations of the liquidators [20,21]. Over 1500 persons have been examined by the present time. All examined liquidators participated in the restoration works within the 30 km zone in 1986 and 1987. Some of the liquidators came to work in Chernobyl in succeeding years, too, up to 1995. The total term of working in the zone of the accident was from 2 weeks to 13 months. The works made by the liquidators were highly diversified. Among the examined patients there were specialists in the field of dosimetry and decontamination, scientific workers, physicians, drivers, erectors, “sarcophagus” builders, helicopter pilots and unskilled labourers. Most of the liquidators were exposed to doses up to 1 Gy.

Official data on irradiation doses are available for approximately 60% of the examined patients. It should be noted that during the work in the zone of the accident the liquidators were exposed to additional factors of the non-radiation nature, including acute and chronic psychoemotional overloads, changes in the habitual stereotype of life and in the dietary regimen determined by the forced staying on the contaminated territory [22].

All people who participated in the restoration works were examined by the classical cytogenetic method permitting us to estimate the level of unstable chromosome [23]. Part of the patients (52 persons) was cytogenetically analyzed by the FISH method which allowed estimating the level of stable chromosome aberrations [24].

The obtained cytogenetic data were compared with the results of examination of the control group composed of quite healthy people of analogous age having no occupational contacts with ionizing radiation sources (118 persons for classical analysis and 15 persons for FISH analysis).

2.2. Lymphocyte culture and slide preparation

The venous blood was collected in sterile test-tubes with lithium heparinate. Lymphocyte enriched plasma was obtained by centrifugation of whole blood for 40-50 min at 1000 rpm. After that, 0.5 ml of the blood plasma was mixed with 4.5 ml of RPMI-164 medium containing 15% fetal calf serum. 2,5% of phytohaemagglutinin, 2 mM glutamine, antibiotics and 10 mM BrdU. The cultures were incubated at 37^0C for 48 hours. Chromosome preparations were obtained according to standard procedures [23].

Slides used for analysis of unstable chromosome aberrations were kept for 5 days at room temperature and then subjected to special fluorescence plus Giemsa (FPG-) staining. For analysis of stable chromosome aberrations the slides were kept in a nitrogen atmosphere at -20^0C until the moment of further treatment. A cocktail of probes was used for investigation with the FISH method: biotin-labelled DNA probes for chromosome 1, 4 and 12 in combination with a digoxigenin-labelled pancentromeric probe. DNA probes for our work were kindly provided by the Laboratory of Cytogenetics of the Institute of Radiobiology (GSF, Munich). Hybridization in situ with DNA probes was carried out by the method of [24]. Probes for chromosomes were identified using fluorescein isothiocyanate (FITC) – labelled streptavidin and pancentromeric probes and 7-amino-4-methylcoumarin-3-acetic acid (AMCA) – labelled antibodies. Propidium iodide (PI) was used as a counterstain in antifade solution. The analysis was performed using a set of filters permitting a simultaneous observation of the fluorescence of FITC and PI as well as AMCA and PI.

Genomic frequencies (F_G) for symmetrical translocations were calculated from the frequencies of painted translocations (F_P) for target chromosomes by inversion of the equation $F_P=2.05\times f_P(1- f_P)\times F_G$, where $f_P=0.192$ is the fraction of the DNA contained in the painted chromosomes.

2.3. Dose-response curves

The doses of irradiation received by the liquidators in the course of the restoration works in the zone of the accident were determined, depending on the situation, either by the frequency of dicentrics or by the frequency of translocations using the calibration dose-response curves obtained by us earlier [25].

An extensive experimental material was analyzed to construct calibration dose-response curves – blood samples from 5 donors were examined in the dose range from 0 to 4 Gy (dose rate – 0.1 Gy/min). 55007 cells were analyzed by the classical method (dicentrics) and 15561 cells were analyzed by the FISH method (translocations). The calibration curve for the frequency of stable chromosome aberrations was based on the frequency of translocations involving chromosomes 1, 4 and 12. Complete and incomplete translocations were taken into account.

The data of cytogenetic analysis of dicentrics and translocations served as a basis for regression equations characterized by a high degree of significance. α and β coefficients for dicentrics made up 0.015

± 0.04 Gy^{-1} and 0.063 ± 0.003 Gy^{-2}, respectively, and for translocations – 0.007 ± 0.003 Gy^{-1} and 0.017 ± 0.002 Gy^{-2}, respectively.

3. Results

3.1. Analysis of chromosome aberrations in the group of liquidators

3.1.1. Dicentrics

Table 1 presents the results of analysis of the frequency of unstable chromosome aberrations (dicentrics) in the group of liquidators for the period from 1986 to 2001. In the first year of examination the average frequency of cells with dicentrics in the group of liquidators was 0.33 per 100 cells and exceeded 16-fold the analogous index in the control group (control – 0.02 per 100 cells). In a year the frequency of cells with dicentrics in the examined group of liquidators decreased more than 2-fold and constituted 0.14 per 100 cells. Further on, the frequency of cells with dicentrics continued decreasing but not so sharply as in the first year after the accident. Through the whole period of observation, this index remained to be significantly higher as compared to the control level. The frequency of cells with dicentrics varied from 0.06 to 0.18 per 100 cells throughout the whole period of examination. Even 15 years after the Chernobyl accident the level of this index exceeds the control. Thus, despite a very long period after the end of the clean-up works in the zone of the accident, we can observe a radiation-induced effect which is most likely determined by the radiation damage of hematopoietic tissue stem cells leading to a gradual introduction in peripheral blood of lymphocytes carrying chromosome aberrations.

For the group of liquidators cytogenetically examined in 1986 the dose of irradiation was estimated using the calibration dose-response curve for dicentrics. The average dose for the group made up 0.14 Gy.

3.1.2. Translocations

Table 2 presents the results of cytogenetic examination of 52 liquidators. They were examined between November 1992 and July 1995. The average frequency of translocations (1.2 ± 0.16 per 100 cells) in the group of liquidators significantly (2.5-fold) exceeds the control level (0.47 ± 0.09 per 100 cells). In the subgroup of liquidators who worked several times in Chernobyl in the period from 1986 to 1995 the frequency of translocations exceeds 2-fold that in the subgroup of liquidators who worked only in 1986. The differences between these subgroups of examined liquidators are statistically significant. There were no differences in the frequency of translocations between the subgroup of liquidators (35 patients) for whom we had official data on the irradiation dose (1.2 ± 0.19 per 100 cells) and the subgroup

Table 1. Cytogenetic results (classical method), pooled data for groups of liquidators.

Year of examination	Number of subjects	Number of cells scored	dic per 100 cells ± SEM
1986	443	41927	0.33 ± 0.03
1987	280	44268	0.14 ± 0.02
1990	23	4268	0.10 ± 0.05
1991	110	20077	0.09 ± 0.02
1992	136	32000	0.14 ± 0.02
1993	75	18581	0.09 ± 0.02
1994	69	20879	0.16 ± 0.03
1995	110	30012	0.06 ± 0.01
1996	53	17960	0.07 ± 0.02
1997	126	63462	0.09 ± 0.01
1998	77	39426	0.09 ± 0.01
1999	41	22524	0.12 ± 0.02
2000-2001	28	14000	0.09 ± 0.02
Controls	118	48124	0.02 ± 0.01

dic, dicentric chromosomes; SEM, standard error of the mean.

Table 2. Cytogenetic results (FISH method), pooled data for the total group and for subgroups of liquidators.

Group	Number of subjects	Number of cells scored	Translocations $F_G \pm$ SEM per 100 cells	Estimated dose (95% c.l.), Gy
Total	52	44283	1.20 ± 0.16	0.16 (0.11-0.21)
Working only in 1986	35	28767	0.86 ± 0.13	0.06 (0 – 0.14)
Working 1986-1995 (several times)	17	15516	1.81 ± 0.35	0.29 (0.25-0.34)
With documented dose	35	29614	1.20 ± 0.19	0.16 (0.11-0.21)
Without documented dose	17	14669	1.20 ± 0.31	0.16 (0.11-0.21)
Controls	15	21953	0.47 ± 0.09	

F_G, genomic translocation frequency; SEM, standard error of the mean; c.l., confidence limits.

of liquidators (17 patients) for whom such data were missing (1.2 ± 0.31 per 100 cells).

Using the frequency of translocations and the dose-response calibration curve, we calculated the irradiation doses for the group of examined liquidators. The average value of the calculated dose for the whole examined group constituted 0.16 Gy. For the subgroup of liquidators who worked only in 1986 the value of the "biological dose" made up 0.06 Gy, and for the liquidators who worked in Chernobyl several times for the period from 1986 to 1995 the value of the "biological dose" was 5 times higher – 0.29 Gy. It is important to note that the obtained dose values are equivalent to the dose of a single acute exposure.

In 18 out of 52 examined liquidators the frequency of translocations significantly differed from the control level. The individual data of cytogenetic analysis for these liquidators are presented in Table 3. It also demonstrates the individual doses of irradiation calculated using the calibration dose-response curve. For three examined liquidators the dose values were rather high. The dose calculated for patient L31 was 1 Gy. The dose recorded for this patient in the documents was 1.36 Sv. Patient L53 did not have official data on the irradiation dose although he worked in Chernobyl several times during 1986-1995. The "biological" dose of irradiation for this patient made up 0.68 Gy. The dose of 0.5 Gy was also calculated for liquidator L51 who worked at the reactor in 1986. Official data on the dose absorbed by him were also

Table 3. Cytogenetic results and biodosimetry estimates for 18 liquidators.

Case no.	Blood sampling	Number of cells scored	St per 100 cells $F_G \pm$ SEM	Estimated dose (95% c.l.), Gy	Documented dose, Sv	Working time (months)		
						1986	1987	>1987
L17	March, 1994	1297	1.9 ± 0.7	0.31 (0.16-0.46)	0.14	1.3	-	-
L19	May, 1995	570	1.7 ± 1.0	0.26 (0.05-0.47)	0.02	2.0	-	-
L20	March, 1994	1314	2.4 ± 0.8	0.43 (0.29-0.57)	0.17	1.3	-	-
L23	May, 1995	857	2.2 ± 0.9	0.36 (0.17-0.54)	0.29	1.5	2.5	-
L25	April, 1994	1161	2.4 ± 0.8	0.40 (0.26-0.54)	0.11	-	2.5	-
L27	June, 1995	1688	2.0 ± 0.6	0.34 (0.20-0.48)	0.80	4.5	2.0	0.5
L28	June, 1995	395	2.4 ± 1.4	0.39 (0.14-0.64)	0.25	4.8	1.0	3.0
L30	June, 1996	797	1.6 ± 0.8	0.25 (0.04-0.46)	0.50	1.0	1.0	3.0
L31	April, 1994	473	7.3 ± 2.2	1.00 (0.40-1.40)	1.36	1.0	1.2	3.0
L33	June, 1995	1157	1.6 ± 0.7	0.26 (0.11-0.41)	0.60	1.9	-	6.5
L34	July, 1995	1097	1.4 ± 0.6	0.22 (0.07-0.37)	0.26	2.0	-	6.0
L41	March, 1994	756	1.2 ± 0.7	0.17 (0.00-0.40)	-	1.0	-	-
L45	April, 1993	410	2.3 ± 1.3	0.38 (0.13-0.64)	-	2.0	-	-
L46	May, 1995	1459	1.5 ± 0.6	0.23 (0.08-0.38)	-	0.3	-	-
L47	February, 1993	859	1.8 ± 0.8	0.30 (0.10-0.50)	-	1.4	-	-
L51	January, 1994	583	3.2 ± 1.3	0.50 (0.31-0.69)	-	1.0	-	-
L52	June, 1995	1209	1.8 ± 0.7	0.29 (0.14-0.44)	-	1.5	1.5	-
L53	February, 1994	1016	4.6 ± 1.2	0.68 (0.55-0.81)	-	3.0	3.0	2.5

St; symmetrical translocation, F_G; genomic translocation frequency,
SEM; standard error of the mean, c.l.; confidence limits.

missing.

For most of the examined liquidators (15 patients) the individual doses were estimated in the range from 0.22 to 0.43 Gy. For those liquidators whose irradiation dose was recorded in official documents the "biological doses" were in agreement to the data of physical dosimetry (L23, L28, L34). However, it should be kept in mind that in certain cases the obtained doses might be slightly underestimated (L27, L30, L31, L33) because the doses were calculated on the basis of the calibration curve generated for irradiation at a dose rate of 0.1 Gy/min under conditions of an acute single exposure. In fact, many of the clean-up workers could be exposed to prolonged irradiation at a lower dose rate. An illustrative example is liquidator L27 who himself is a specialist in the field of dosimetry and used an individual dosimeter for the whole period of working in Chernobyl. By his measurements, his irradiation dose is 0.83 Sv. The dose calculated by the frequency of translocations made up 0.34 Gy.

The results obtained clearly demonstrate that the analysis of translocations by means of the FISH method is a sufficiently sensitive technique for retrospective assessment of the radiation effect in the liquidators participating in the clean-up works after the Chernobyl accident. Unfortunately, it is difficult to find a scientific meaning in comparing the data of physical and biological dosimetry since it is known that in many cases the data of physical dosimetry (from the point of view of official dosimetry) did not correspond to the real doses that might be received by the liquidators during their work in the zone of the accident. In connection with this, the results obtained by means of biological dosimetry acquire particular importance and, namely, they help in assessing possible unfavorable impacts of radiation on the human organism.

3.2. Cytogenetic examination of the workers of I.V.Kurchatov Institute of Atomic Power participating in the liquidation of the consequences of the Chernobyl NPP accident

In 1996 a group of 22 workers of the Kurchatov Institute was subjected to cytogenetic examination. In the first days after the accident they began research work in the zone of the accident. The main tasks of this work were to establish the causes of the accident, to examine the condition of the reactor, nuclear fuel, structural constructions of the 4th block and to study the radioactive conditions in the zone of the Chernobyl NPP and on the controlled territory. All of the examined patients were exposed to low-dose chronic irradiation in the course of their professional activity due to contacts with different sources of radiation. Most of them started working under unhealthy occupational conditions in the early 60ies. The official annual dose up to 1986 did not exceed 0.05 Gy. 18 persons from the examined group worked in Chernobyl in 1986 and in 1987. Four out of the examined patients carried out research works at the nuclear plant till the middle 90ies. For 19 persons the official doses of irradiation ranged from 0.08 to 0.52

Table 4. Personal data for the examined group from the Kurchatov Institute.

Case No.	Number of cells scored	Working time at Chernobyl	Documented dose (cSv)*
K_3	65	1986; 1981-1991	8.57
K_4	57	May, 1986	24.85
K_6	56	November, 1986	27.27
K_8	49	June, 1986	24.45
K_9	44	September, 1986	12.60
K_{12}	48	October, 1986	10.71
K_{13}	64	July, 1986	1.55
K_{14}	51	October, 1986	4.63
K_{15}	56	October, 1986	41.69
K_{20}	49	1986-1996	1710**
K_{21}	63	1986-1994	360**
K_{28}	44	1986-1996	1160**

* Doses accumulated during working in Chernobyl.

** This dose was obtained from the paper of A.V. Sevan'kaev et.al., 1995.

Sv. The values of these doses were obtained from the dosimetric register of the Kurchatov Institute. Three persons (K20, K21, K28) from the examined group were exposed to super-high doses. The individual characteristics of the examined group are presented in Table 4. The publication about these persons was in the journal Radiation Protection Dosimetry [26].

Table 5 presents the individual data of cytogenetic examination by means of the classical method (analysis of the frequency of unstable chromosome aberrations). The values of the frequencies of dicentrics and centric rings (markers of radiation exposure) in 13 examined workers of the Kurchatov Institute significantly exceed the control level. In 5 patients (K4, K5, K20, K21, K28) the blood contained cells with multiple chromosome aberrations (with dicentric chromosomes). Taking into consideration that the examined persons could be irradiated from different radiation sources, it can be suggested that the appearance of such cells in their blood is the result of the action on the organism of high-LET ionizing radiation

In three patients from the examined group who were exposed to super-high doses (K20, K21, K28) the frequency of dicentrics and centric rings was 100 and even 1000 times higher than the control level. These workers were subjected to cytogenetic examination several times in the period from 1996 to 2001. Here we present only the results of cytogenetic analysis obtained in 1996. It should be noted that by the moment of examination there were no health complaints from these patients.

Table 6 presents the individual results of cytogenetic analysis performed with the use of the FISH

Table 5. Cytogenetic results (classical method) for the workers from the Kurchatov Institute.

Case No.	Number of cells scored	dic+ Rc per 100 cells ± SEM	Cdr per 100 cells ± SEM
K_2	871	0.69 ± 0.28 *	0.69 ± 0.28 *
K_3	1300	0.08	0.08
K_4	1200	0.42 ± 0.28 *	0.25 ± 0.14 *
K_5	600	0.33 ± 0.24 *	0.33 ± 0.24 *
K_6	1004	0.40 ± 0.24 *	0.30 ± 0.17 *
K_7	500	0	0
K_8	1300	0.08	0.08
K_9	550	0	0
K_{10}	1300	0.08	0.08
K_{12}	800	0.38 ± 0.22 *	0.38 ± 0.22 *
K_{13}	800	0	0
K_{14}	1000	0.20 ± 0.14 *	0.20 ± 0.14 *
K_{15}	1000	0.30 ± 0.17 *	0.30 ± 0.17 *
K_{16}	1000	0	0
K_{17}	1000	0.20 ± 0.14 *	0.20 ± 0.14 *
K_{18}	1000	0.30 ± 0.17 *	0.30 ± 0.17 *
K_{22}	1133	0	0
K_{25}	1000	0.50 ± 0.22 *	0.50 ± 0.22 *
K_{27}	1000	0.10	0.10
K_{20}	279	20.50 ± 2.70 *	18.00 ± 2.50 *
K_{21}	830	2.80 ± 0.60 *	2.80 ± 0.60 *
K_{28}	500	20.40 ± 2.00 *	19.20 ± 1.90 *
Control	48124	0.02 ± 0.01 *	0.02 ± 0.01 *

dic; dicentric chromosomes, Rc; centric rings, Cdr; cells containing dic+Rc,
SEM; standard error of the mean (only for cases with more than one aberration),
* significantly different from control ($p<0.05$).

method among 12 workers of the Kurchatov Institute. The frequency of symmetrical translocations in five cases significantly exceeds the control level (0.47 per 100 cells). The table also gives the values of individual doses calculated on the basis of cytogenetic indices. The dose values for patients K4 and K14 turned out to be higher than those presented in Table 4. One of the causes seems to be the fact that before starting working in the zone of the accident at the Chernobyl NPP these persons worked for a long time under unhealthy occupational conditions. For instance, patient K4 worked with the sources of ionizing radiation since 1962. By 1986 his total accumulated dose constituted 29 cSv. Patient K14 worked with fluorine-containing substances for more than 10 years.

For three workers of the Kurchatov Institute (K20, K21, K28) exposed to super-high doses of ionizing radiation the biological doses calculated by the frequency of translocations significantly differ from those presented in Table 4. The causes of such discrepancy can be summarized as follows. First, the doses were determined using the calibration curve obtained under conditions of acute single irradiation. In reality, these specialists worked in the zone of the accident for several months almost every year over 10 years. In addition, they were repeatedly exposed to high-dose radiation. The total irradiation dose for these patients was accumulated under conditions of fractionated exposure. Taking into account the character of irradiation, it is more desirable to use the linear dose-response relationship for dose calculations [8]. In this case the irradiation dose can be determined by the following formula:

$$D = (Y - Y_0) / \alpha,$$

where Y is the frequency of measured translocations, Y_0 – the frequency of spontaneous translocations, α - the linear coefficient.

The doses calculated according to this formula for the examined patients K20, K21 and K28 made up 10.3, 3.1 and 19.1 Gy, respectively. The values of these doses are already quite comparable with the doses presented in Table 4. It should be noted, however, that such estimates are rather rough. A more precise assessment of doses needs a detailed analysis of the history of irradiation of these persons during their work in the zone of the accident at the Chernobyl nuclear power plant.

Another important point to be mentioned is that the cytogenetic examination was carried out 10 years after the beginning of exposure. During that time two opposite processes took place in the blood of the irradiated patients: gradual elimination of cells with unstable chromosome aberrations (dicentrics, centric rings) in which stable translocations were also detected, and a simultaneous accumulation in peripheral blood of cells with chromosome aberrations due to additional irradiation. There is no doubt that this fact

Table 6. Cytogenetic results (FISH method) for the workers from the Kurchatov Institute.

Case No.	Number of cells scored	$F_G \pm$ SEM (ST per 100 cells)	Estimated dose (95% c.l.), Gy
K3	1135	0.57 ± 0.38	NS
K4	1134	3.32 ± 0.94*	0.54 (0.40-0.68)
K6	1043	0.91 ± 0.53	NS
K8	1062	0.88 ± 0.50	NS
K9	843	1.13 ± 0.66	NS
K12	1271	0.50 ± 0.35	NS
K13	1000	0.33	NS
K14	1350	1.38 ± 0.57*	0.21 (0.06-0.36)
K15	1571	1.00 ± 0.44	NS
K20	2169	22.20 ± 1.00*	1.78 (1.69-1.87)
K21	2031	6.96 ± 0.60*	0.88 (0.78-0.98)
K28	950	40.90 ± 2.10*	2.51 (2.38-2.64)
Control	21953	0.47 ± 0.09	

F_G; genomic translocation frequency, ST; symmetrical translocations,
SEM; standard error of the mean (only for cases with more than one aberration),
c.l.; confidence limits, * significantly different from control ($p<0.05$).

considerably impedes obtaining reliable information on irradiation doses. However, a rough estimation of absorbed radiation doses, and, what is more important, with regard to the individual peculiarities of an irradiated organism, can be made.

The data obtained by the frequency of translocations (Table 6) permitted us to analyze the dependence between the level of individual values and the documented dose for the examined persons. These data are presented in Fig. 1. The results of examination of patients K20, K21 and K28 are omitted here. The dotted line indicates the linear-quadratic curve and the unbroken line indicates its linear component. It should be noted that for all patients (except one) a good correlation between the frequency of translocations and the officially registered irradiation dose is observed. The values of translocation frequencies for two patients lie higher than the linear-quadratic calibration curve. It can thus be suggested that those workers could be exposed to higher doses than those recorded in the documents. In three of the examined workers with documented dose larger than 0.2 Sv, the value of the translocation frequency lies considerably below our linear-quadratic calibration curve but in the range of its linear part. Assuming that the official dose in this worker is close to the real one, this fact, as has been mentioned above, shows an evidence that irradiation with rather low dose rates or fractionated exposures produce cytogenetic damages less effective than single acute irradiation.

It is important to mention that the dependence of the translocation frequency on the exposure dose is also observed for persons in whom the dose values were not determined because of insignificant differences between the cytogenetic indices and the control. With a low level of translocations in peripheral blood lymphocytes an analysis of extensive material (a large number of cells) is required in order to obtain reliable data. Unfortunately, it is often impossible in connection with large expenses. The lower limit of dose estimation by the frequency of translocations, as shown by our studies, is 20-25 cGy with 1000 – 1500 cells analyzed. To estimate a dose below this threshold it is necessary to significantly increase the number of cells to be analyzed. The data presented in Table 6 and in Fig. 1 support this conclusion.

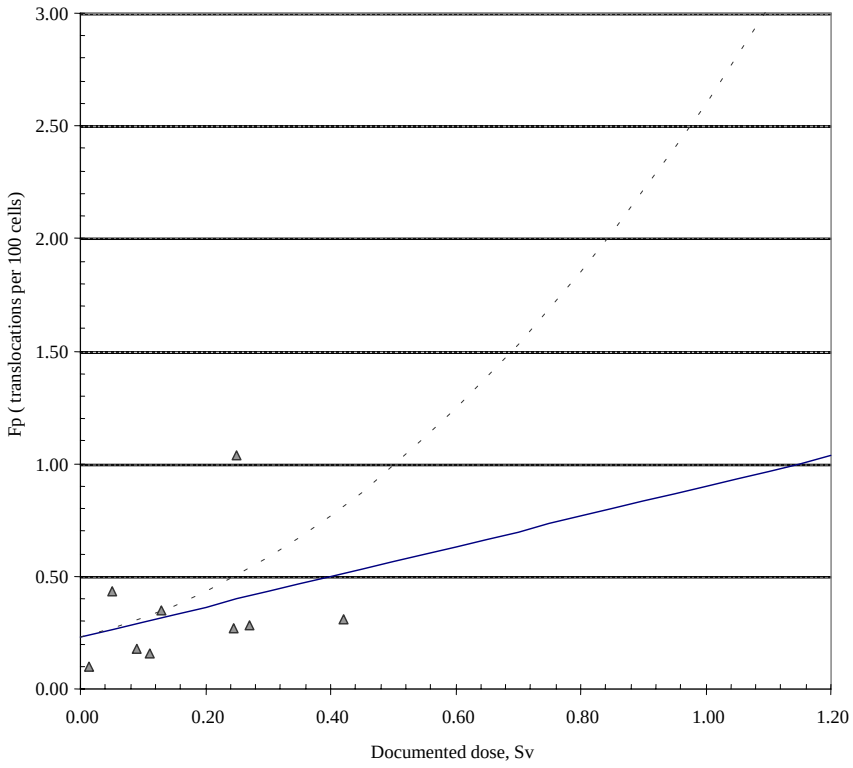

Figure 1. Dose-response relationship of translocation frequencies (Fp) with documented doses (explanatory notes are in the text).

4. Conclusion

The results of cytogenetic examination of the “liquidators” permit the following conclusions to be made.

- The cytogenetic methods (classical and FISH methods) are sufficiently sensitive for assessing the condition of the cell hereditary structures.
- It was found that the level of cells with dicentrics in the examined liquidators in the first year after the work in the zone of the accident significantly exceeded the control level. With time, years after the radiation exposure, this level gradually decreased but it did not reach the control level even within 15 years after the accident. The average frequency of cells with dicentrics in the recently examined group of liquidators made up 0.09 % exceeding 5-fold the control values.
- Using the frequency of cells with dicentrics, an average dose for the group of liquidators examined in 1986 was determined. The calculated dose constituted 0.14 Gy. Retrospective estimation of irradiation doses (both individual and for the groups) on the basis of analysis of cells with dicentrics does not seem possible. It is first of all due to the elimination of such cells from the peripheral blood of the examined patients.
- However, in the course of large-scale monitoring in groups of people who suffered after accidents or emergency situations, analysis of unstable chromosome aberrations by means of the classical cytogenetic method is decisive as it does not require large funds and technical resources. During retrospective examination of the liquidators the analysis of cells with unstable chromosome aberrations (dicentrics and centric rings) can be used predominantly for bioindication of the mutagenic action of ionizing radiation. The results of such analysis can be considered as a criterion in the formation of groups at risk with regard to the development of different diseases and primarily oncological ones
- The FISH method is the most promising cytogenetic method in biological dosimetry. It allows one to analyze the frequency of cells with stable chromosome aberrations – symmetrical translocations. The data presented here suggest that this method can be used rather extensively for estimating radiation doses. In most cases the doses calculated by the frequency of translocations are in good agreement with the data of physical dosimetry. Some underestimation of biological doses is primarily due to the fact that the calibration dose response curves for the frequency of translocations were obtained under definite conditions – single acute irradiation. Under real conditions the liquidators were, as a rule, exposed to chronic or prolonged radiation. In connection with this, in determining irradiation doses it is necessary to introduce the correction coefficient which makes it possible to take into account the real conditions of exposure.
- The results of cytogenetic examination by means of the FISH method presented in this work suggest the existence of irradiation levels below which the dose estimation by the frequency of translocations seems to be unlikely. The causes of such limitation can be quite diverse: interindividual variability of the translocation frequency, ability to repair radiation damage, the volume of analyzed material (the number of analyzed cells). According to our data, the real lower threshold of dose estimation by the frequency of translocations is about 20-25 cGy. It seems reasonable in future to carry out cytogenetic examinations among the liquidators of the accident at the Chernobyl nuclear power plant using not only the classical and FISH methods but also new molecular-genetic methods permitting the estimation of remote genetic effects of radiation and their comparison with detected structural damage of the cell genome.

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