Early and Late Cytogenetic Effects Study in Groups Exposed Due to the Chernobyl Accident Using Conventional Chromosome Analysis and Fluorescence In-Situ Hybridisation (FISH) Technique

Nataliya A. Maznyk
Institute of Medical Radiology named by S.P Grigiriev, Academy of Medical Science of Ukraine
Pushkinskaya St., 82, Kharkiv, 61024 Ukraine: lrcg.imr@mail.ru

Introduction

During last two decades a large body of scientific data have been accumulated by observations targeted to assessing and quantifying various biomedical effects in humans exposed to ionizing radiation due to the catastrophe at the Chernobyl nuclear power plant (Ukraine, former USSR) in 1986. It was commonly believed that Chernobyl-related radiation doses for the majority of affected individuals were clinically low, particularly not exceeding 1 Gy of equivalently acute low-LET rradiation, and that was confirmed by the absence of stable deterministic sindroms. But whatever low, those doses must be taken into account when late stochastic effect risk is calculated for the exposed populations, which are generally subdivided into three main categories: (i) the population evacuated from 30-km exclusive zone around the Chernobyl NPP and some nearby regions heavily contaminated with radionuclides, (ii) clean-up workers ("liquidators") who were irradiated during their duties in the Chernobyl zone and (iii) people who continue to live in areas with increased levels of radioactivity [1].

The cytogenetic analysis based on chromosomal aberration scoring in cultured human peripheral lymphocytes appeared to be one of the most demanded techniques for monitoring the Chernobyl critical groups. Firstly, post-Chernobyl cytogenetic research provided fundamental radiobiological data about spectrum and magnitude of genetic damage caused in human somatic cells by Chernobyl genotoxic factors, amongst which ionizing radiation dominated, but chemical agents also played quite a noticeable role [2-5]. But more importantly, the cytogenetic method was applied so intensively because of its ability to serve as the most powerful tool for biological dosimetry [6]. It should be fairly noted that initial steps of chromosomal dosimetry in the post-Chernobyl critical groups sometimes suffered from limitations due to specific irradiation scenarios (chronic or protracted exposure) and low number of cells scored per person when huge number of cases required rapid cytogenetic screening. With time pass after the accident a natural process of elimination of lymphocytes carrying unstable chromosomal aberrations comprised another big problem for conventional chromosomal biodosimetry, and stable chromosome aberration analysis using fluorescence *in situ* hybridization (FISH) technique also didn't provide an ideal alternative at the beginning of its application into real biodosimetry practice. It took about 20 years to develop proper methodological approaches which allow satisfactory overcoming the majority of listed problems [4, 7-10].

Regarding cytogenetics, evacuees from the 30-km Chernobyl exclusive zone have remained less investigated in compare with liquidators and inhabitants of radioactively contaminated areas. That could be a result of the lack of specialized cytogenetic laboratories in research institutes and hospitals, where initial examinations of those persons took place. Nevertheless cytogenetic data in evacuees were obtained by research groups from Minsk [11], St. Pitersburg [12], Moscow [2, 13] and Kharkiv (Ukraine) [3]. But only the Radiation Cytogenetics Laboratory of Kharkiv Institute for Medical Radiology started the cytogenetic survey in this group nearly immediately after the accident (since 28th April 1986). We carried out our study during long period of time, with several time-effect points gathered in randomly sampled cohort between the first few days and 14 years after evacuation. The summary results of these investigations performed by conventional cytogenetic analysis and additional data concerning the

possibilities of retrospective biological dosimetry using FISH technique in Chernobyl evacuees and chronically exposed residents of radioactively contaminated areas are presented in this paper.

Materials and Methods

Study groups

In total 112 evacuees had been investigated by the conventional chromosome analysis. They were 50 adult males and 52 adult females, age ranged from 23 to 66 years, and also 10 children 4 to 17 years old. Blood samplings for cytogenetic assay were done in the time range from 2 days to 14.8 years after the departure from the Chernobyl exclusive zone. Amongst them there were 18 individuals (7 males and 11 females, age ranged from 16 to 55 years), for whom FISH analysis was also carried out in period 12.8-14.8 years after evacuation. The studied evacuees group did not contain cases of acute radiation syndrome, local skin and soft tissue injuries or cancer.

Another examined group consisted of 21 residents of radioactively contaminated regions of Belarus (6 males and 15 females). They were children at the time of the Chernobyl accident and continued to live in areas with increased levels of radionuclide deposition. Their age varied from 15 to 26 years at the time of blood sampling which was performed 12.8-14.8 years after the Chernobyl accident. This group was investigated by FISH technique only. Blood samples from the Belorussian residents group were collected at the Institute of Genetics and Cytology (Minsk, Belarus) and passed to the National Radiological Protection Board of the United Kingdom (NRPB, currently HPA-RPD) for cell culturing, and then coded metaphase preparations were transferred to the KhIMR for further FISH analysis.

Control group established for conventional chromosomal assay consisted of 50 healthy persons (19 males and 31 females), unexposed inhabitants of Kharkiv region aged from 19 to 58 years (mean 33 years). Amongst them a subgroup of controls for FISH study was formed, comprising 5 males and 7 females, aged from 19 to 58 years, randomly selected in trying to cover the age ranges in both exposed groups.

Cell culturing, conventional analysis, FISH painting and aberration scoring

The details of techniques and aberration scoring criteria used at KhIMR Radiation Cytogenetic Laboratory during investigations in post-Chernobyl human cohorts were published earlier [3-5, 8-10]. Briefly, throughout all the period of investigations the unified method of peripheral blood lymphocyte culturing was used with PHA-stimulated lymphocyte cultures set up for 48-50 h, metaphases harvesting after 4 h colchicin treatment and fixing in methanol/acetic acid mixture, that well corresponds to the standard technique described in IAEA manual [6]. From each sample replicated slides were prepared, coded and either stained by Giemsa for conventional analysis or processed by FISH technique according to the protocol [14].

For conventional assay all cytogenetic abnormalities recognised without special karyotyping were recorded, i.e. dicentrics and centric rings, both accompanied by acentric, excess acentric fragments, chromatid breaks and exchanges (combined below into total chromatid aberrations), hyper- and polyploids (combined below into total genomic abnormalities; all polyploids found didn't contain replicated aberrations; virtually all hyperploids represented threesomics, i.e. 47 chromosomes).

For FISH assay slides were FITC painted, highlighting chromosome combinations 1, 2 and 4 (Cambio), other chromosomes were counterstained with DAPI (diamidino-2-phenylindole), pancentromere probes (Oncor) that fluoresced red were also applied. Slides were examined under fluorescence microscopes (Nikon, Zeiss) equipped with filter sets for FITC, DAPI and all three fluorochromes vizualizing. Aberrations were counted in cells containing 46 centromeres and diploid amount of painted material from FITC-highlighted chromosomes. Translocations were recorded using the modified hybrid of conventional/PAINT descriptive nomenclature as complete t_{comp} or incomplete t_{inc}Ab and t_{inc}Ba. The latter were subdivided into three subgroups: involving an unshorterned painted

chromosome – t_{inc}Ba*, accompanied by a fragment from the painted chromosome – t_{inc}Ba+ac, involving a markedly shorterned chromosome with no missing painted fragment present somewhere in the cell – t_{inc}BaMP ("missing part"). Insertions of Aba and Bab-types were pooled into one category. Each exchange, either complete or incomplete, was accounted as an entity. Deleted painted chromosomes with a segment absent, dicentrics and centric rings accompanied by fragment and excess acentrics in painted chromosomes were also recorded for data completeness.

Statistical analysis

From 50 to 1200 metaphases were analyzed per person. Numbers of actual cells scored by FISH assay were converted into genome equivalents by monocolour version of Lucas' formula for the sum of DNA content in highlighted chromosomes [15, 16].

When individual data were pooled, the randomness of the individual aberration yield distribution within the group was checked and weighted mean yields of cytogenetic damage were estimated. Standard errors for the mean were calculated from the observed dispersion of the cytogenetic damage yields amongst individuals that nearly always was close to Poisson statistics. For intergroup data comparison Student's *t*-test was applied.

Results and discussion

The yield of aberrations measured by conventional analysis: general characteristics and time course

Kharkiv region remained non-polluted by the Chernobyl fallout and therefore comprised one of the main destinations for the routs of evacuation of citizens from town Pripiat' and nearby villages located within 30-km exclusive zone around the Chernobyl NPP. Thus initial decontamination procedures and biomedical examinations of exposed persons were carried out by specialised departments and laboratories of the Kharkiv Institute for Medical Radiology (KhIMR). Chernobyl-related cytogenetic research started at KhIMR 28th April 1986, i.e. just 2 days after the catastrophe, and continued for the following 15 years.

Individual cytogenetic data in evacuees were combined depending on time gap between departure from the Chernobyl zone and investigation (Table 1). Within each group the individual aberration yields appeared at random. The individual values of total aberration frequency varied from 1.2 to 12.0 per 100 cells in groups studied up to 1 year after exposure; later that range narrowed to 0.0-5.0 per 100 cells. Dicentrics and centric rings accompanied by fragment represented a significant proportion amongst chromosome type aberrations: 30-40 % at the beginning of the observation, 18-29 % later, in compare with 13.4 % in the control group. Cells with more than 1 chromosome exchange were not found, thus aberration-per-cell distribution in that cohort was in a good agreement with Poisson statistics.

Table 1. Total cytogenetic damage yields in evacuees sampled at various times after their departure from the 30-km Chernobyl exclusive zone

Time after evacuation		Number of persons	Cells scored	Mean cytogenetic damage yield per 100 cells ±SE			
range	mean ± SE			Aberrant cells	Chromosome aberrations	Chromatid aberrations	Genomic abnormalities
1-9 days	4.25±0.65 d.	20	2316	4.66±0.54	2.94±0.36	1.77±0.34	0.48±0.15
0.2-1.0 y.	0.73±0.03 y.	40	4221	5.19±0.39	3.61±0.29	1.85±0.21	0.31±0.08
1.4-3.7 y.	2.13±0.11 y.	20	2065	4.65±0.60	2.52±0.40	2.61±0.51	0.34±0.13
4.6-10.7 y.	7.99±0.87 y.	14	3603	2.66±0.38	1.72±0.26	1.17±0.22	0.17±0.10
12.8-14.8 y.	14.32±0.15 y.	18	9314	1.48±0.21	0.64±0.14	0.91±0.12	0.14±0.04
Controls		50	19289	1.24±0.11	0.62±0.09	0.66±0.07	0.08±0.02

Here and in other tables: SE is a standard error for the mean; chromosome aberrations don't include abnormal monocentrics resulted from translocations and insertions; genomic abnormalities comprise the sum of hyper- and polyploids.

Up to 2 years after exposure the mean frequency of aberrant cells remained on the plateau of the 4-fold excess above control, the mean yields of chromosome type aberrations and genomic abnormalities were 4-6 times higher and chromatid type aberrations – 3-4 times higher than the respective spontaneous values ($p<0.001$ for all end-points). Later an elimination of lymphocytes carrying chromosome aberrations took place, and the difference between evacuees and controls became statistically insignificant for genomic abnormalities approximately 8 years after exposure, for chromosomal rearrangements – 14 years after departure from the Chernobyl zone.

According to well known mechanisms of cytogenetic damage formation in mature human lymphocytes, the over-spontaneous excess of chromosome type aberrations, particularly dicentrics and centric rings in evacuees can be attributed to ionizing radiation exposure, but elevated yield of chromatid aberrations, polyploids and aneuploids should be considered as fingerprints of chemical genotoxic action or non-specific, stress-related endogenous mutagenesis (for details see [7]). In our opinion, the most possible source of chemical genotoxic action could be various compounds released from the reactor and also some reagents used during firefighting and decontamination procedures. The conclusion about appearance of those chemical factors in the environment due to the the Chernobyl accident is confirmed by the finding of the same spectrum and very similar quantitative outcome of cytogenetic damage inspecific to radiation in Chernobyl clean-up workers [17, 18].

The time course for dicentric and centric rings yield in evacuees is presented in Fig. 1. The general tendency for this end-point was gradual disappearence. However, the phenomenon of an initial rise of the dicentrics plus rings frequencies over one year was found, that can be explained by continuing exposures from short-lived radionuclides. After that aberration yield declined quite rapidly and in 14 years after irradiation it nearly reached the control level.

Dependence on age, gender and departure time

Regarding these results, the following analysis of cytogenetic data in relevance to age and gender was concentrated on 60 persons examined within 1 year after evacuation, when the dicentrics plus centric rings yield didn't start a decrease (Table 2). In control donors subdivided into appropriate subgroups no difference for cytogenetic parameters was found between males and females, and the same occurred for non-exposed individuals of different age, with the only exception of excess chromosome acentric

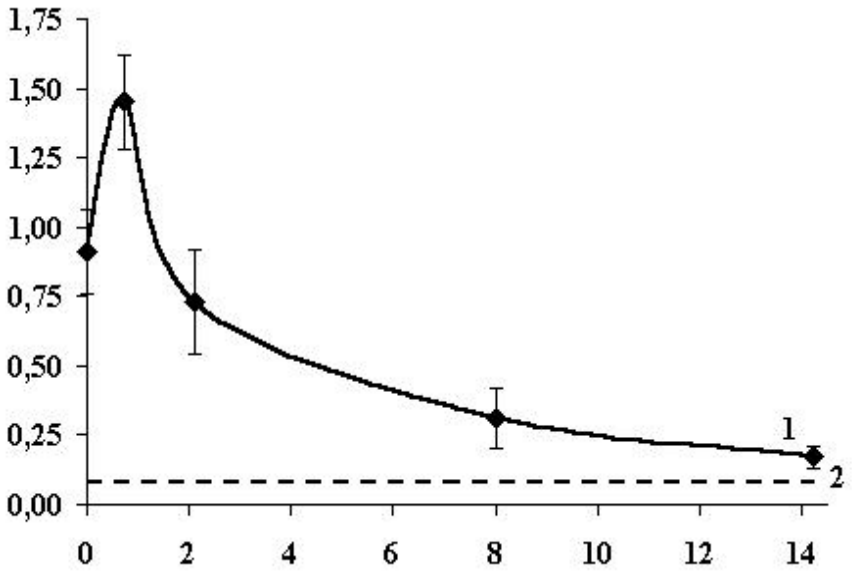

Fig. 1. Time effect relationship for the mean dicentrics plus centric rings yield in evacuees from the 30-km Chernobyl zone (1) in compare with controls (2). Y axis – aberration frequency per 100 cells; X axis – time after departure, years. Vertical bars represent standard errors of the mean.

Table 2. Early cytogenetic damage levels in evacuees depending on age and gender

Group formation parameter	Group	Number of persons	Mean cytogenetic damage yield per 100 cells ±SE			
			Dic+CR fr	Excess acentrics	Chromatid aberrations	Genomic abnormalities
Age	4-17 years	10	0.92±0.26	1.30±0.32[a]	1.53±0.28	0.31±0.19
	23-35 years	30	1.31±0.20	2.18±0.20[b]	1.96±0.30	0.50±0.11
	36-66 years	20	1.39±0.21	2.52±0.35[c]	1.78±0.29	0.20±0.11
Gender	Females	28	1.26±0.19	2.22±0.24	1.89±0.29	0.50±0.13
	Males	32	1.25±0.18	2.02±0.23	1.76±0.22	0.26±0.08

Dic+CR fr are dicentrics and centric rings accompanied by fragment; a, b & c – the spontaneous level of excess acentrics in the respective age subgroups in control, that were a = 0.09±0.09; b = 0.37±0.11; c = 0.91±0.22 per 100 cells.

fragments yield (for acentrics see footnote for Table 2; for other cytogenetic damage data not shown, as they didn't differ from the mean control values presented in Table 1 and Fig. 1). No changes in chromatid aberrations and genomic abnormalities levels were observed with evacuees' age. The difference for dicentrics plus centric rings yield between the youngest and the oldest age subgroups didn't reach the statistical significance ($p>0.05$), but the tendency for lower yield of radiation-specific chromosome exchanges in children may reflect some attempts to minimize the radiation load for youth, probably by limiting their outdoors activity between the accident and evacuation. The positive age dependence for excess acentric yield in evacuees was obviously related to the similar tendency for the spontaneous level in control donors. The statistical difference between respective evacuees and controls age subgroups for each cytogenetic end-point was equally highly significant ($p<0.001$).

No obvious influence of gender on cytogenetic parameters was initially found in evacuees, but when children were excluded from the data analysis a statistically higher yield of chromatid exchanges was observed in young adult males in compare with young adult females ($p<0.05$), and the latters also had an increased outcome of poliploids in compare with three other age/gender subgroups (Fig. 2). The possible reasons for those differencies, particularly active smoking status in young males and higher intensity of endogeneous mutagenesis resulted from more reactive neuro-humoral response to psichological stress in young females, were discussed earlier [5].

The initial phase of the time course for unstable aberrations yields was compared between

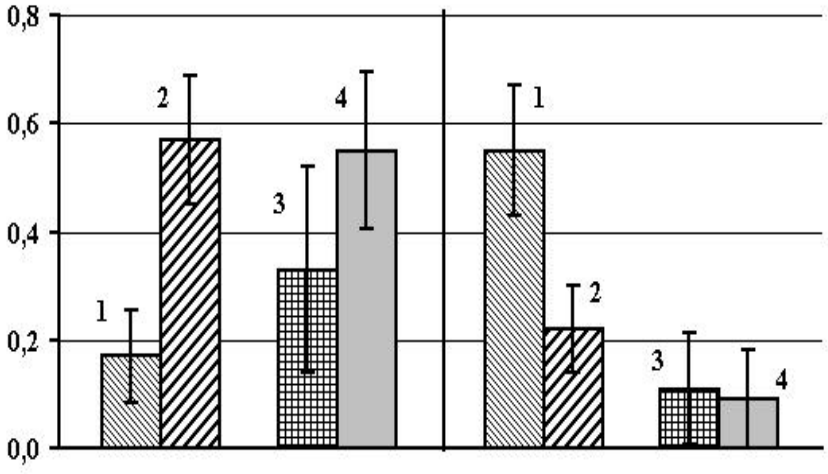

Fig. 2. The yields of chromatid exchanges and poliploids in lymphocytes of adult evacuees samples within 1 year after departure from the Chernobyl zone: 15 females (1) and 15 males (2) aged 23-35 years, 10 females (3) and 10 males (4) aged 36-66 років years. Y axis – cytogenetic damage frequency per 100 cells. Vertical bars represent standard errors of the mean.

Table 3. Chromosomal rearrangements in evacuees with different time of departure from the Chernobyl zone

Time between explosion and evacuation	Time between evacuation and blood sampling	Number of persons	Mean aberration yield per 100 cells ±SE		
			Dic+CR fr	Excess acentrics	Chromatid breaks
2 days	4.2±0.8 d	13	0.87±0.16	2.60±0.36	1.60±0.42
	0.79±0.03 y	22	1.30±0.24	1.95±0.22	1.18±0.19
	2.20±0.14 y	10	0.58±0.24	1.75±0.61	1.36±0.62
3-11 days	5.7±1.2 d	7	0.98±0.35	0.98±0.29 [a]	1.48±0.41
	0.66±0.05 y	18	1.65±0.33	2.45±0.36[b]	1.82±0.31
	2.07±0.17 y	13	0.87±0.29	1.83±0.40	3.47±0.57 [a,b]

The difference is statistically significant ($p<0.05$): a – between subgroups "2 days" and "3-11 days" at similar time after exposure; b – in compare with the first time point inside the subgroup "3-11 days".

evacuees with different time of departure from the Chernobyl zone (Table 3). During the first two years after exposure chromatid break yield slightly declined in persons evacuated in 2 days after the accident but increased in those who left the Chernobyl zone later. These changes appeared to be associated with chromosome acentrics behaviour that was expessed as elimination in the former evacuees' subgroup and stability in the latter. The initial increase of dicentrics plus centric rings yields during 1 year after irradiation appeared to be a common feature for all evacuees but was more pronounced in persons who were evacuated from 3 to 11 days after the accident compared with those who left sooner; just 2 days after the explosion, and this tendency could be traced during 2 years after exposure. As the duration of exposure in evacuees had such a remarkable influence on the aberration outcome, it was judged as a main factor to be considered during biological dose estimating, in addition to the known role of exposure protraction in deriving an appropriate dose-response equation from a standard acute calibration curve for chromosomal dosimetry [6].

The observation of significantly increased level of chromatid aberrations in evacuees, apart from detection of chemical impact, provided a serious implication for biodosimetry: the list of end-points suitable for dose reconstruction must be restricted to dicentrics and rings only, with all excess acentrics excluded, because the latter could be caused with quite high probability by chemical mutagens either directly or as derived from chromatid breaks in lymphocyte precursors.

Biological dose estimations based on conventinal analysis data

To carry out the radiation dose estimation the individual dicentrics plus centric rings yields from evacuees investigated during non-elimination phase of those aberrations dynamics (i.e. within 1 year post-exposure) were pooled in two subgroups depending on when they left the exclusion zone – 2 days or 3-11 days (Table 4). In both groups, chromosome aberrations appeared at random amongst individuals, and the parameters of distribution were close to Poisson statistics that indicated an absence of persons significantly overexposed in compare with others inside the groups. Thus, carrying out group biodosimetry was methodologically correct.

According to the IAEA Chromosomal Biodosimetry Manual [6], mean doses for the evacuees groups were calculated by referring their mean dicentrics plus centric rings yields to intralaboratory *in vitro* dose response curve constructed for acute γ-exposure within low dose range [7], with quadratic coefficient reduced by the Lea & Catchiside G-function, which takes into account the exposure duration. Thus the dose response equation $Y = c + \alpha \bullet D + \beta \bullet G \bullet D^2$ was solved using the following parameters: the spontaneous level of aberrations c=0.08 per 100 cells and linear term $\alpha = 2.98$ per 100 cells per Gy for both groups; initial acute quadratic term $\beta = 8.05$ per 100 cells per Gy^2 was reduced to 1.5 per 100 cells per Gy^2 for persons evacuated in 2 days after the accident and to 0.52 per 100 cells per Gy^2 for those who left later (average departure time was 7 days after the explosion).

Table 4. Individual dicentrics plus centric rings yields distributions and mean biological dose estimations in former citizens of t. Pripiat' and 30-km Chernobyl exclusive zone depending on time of their evacuation

Departure after the accident (number of persons)	Individual Dic+CR yield per 100 cells					Mean Dic+CR yield per 100 cells	Biological dose estimation, mGy
	0	1	2	3	4-5		
	Number of individuals						
2 days (35)	9	15	9	2	0	1.13±0.17	300±29
3-11 days (25)	5	10	7	2	1	1.44±0.20	420±39
Total (60)	14	25	16	4	1	1.25±0.13	360±16

Table 5. Aberration levels measured by FISH technique in Chernobyl groups in compare with controls

Group (number of persons)	Genome equivalents scored	Aberration frequencies ±SE per 100 genome equivalents (actual numbers are given in parenthesis)							
		Dicentrics +Rings	Acentric fragments	t_{comp} (Ab+Ba)	t_{inc} (Ab)	t_{inc} (Ba*), (Ba+ac)[a]	t_{inc} (BaMP)	Insertions	Deleted chromosomes
Controls (12)	4088	0.10±0.04 (4)	0.66±0.11 (27)	0.37±0.05 (15)	0.42±0.06 (17)	0.07±0.03 (3)	0.29±0.06 (12)	0.07±0.04 (3)	1.44±0.12 (59)
Evacuees (18)	5282	0.17±0.05 (9)	0.98±0.13 (52)	0.57±0.07 (30)	0.63±0.10 (33)	0.15±0.07 (8)	0.44±0.10 (23)	0.13±0.06 (7)	2.08±0.27 (110)
Residents of RCA (21)	7916	0.10±0.03 (8)	0.71±0.12 (56)	0.29±0.05 (23)	0.44±0.07 (35)	0.10±0.03 (8)	0.21±0.05 (17)	0.09±0.03 (7)	1.20±0.14 (95)
Spontaneous levels for inhabitants (mean age 21 yrs)[b]		0.10±0.04	0.54±0.16	0.22±0.02	0.27±0.02	0.00	0.18±0.02	0.02±0.01	1.17±0.04

RCA – radioactively conraminated territories. [a] – one t_{inc} (Ba+ac) was detected in the control group, one in evacuees group and six in residents group. [b] – applying age-effect regressions for complete translocations, insertions and deleted chromosomes and real yields of acentrics and incomplete translocations observed in a subgroup of young control donors [6].

The resulted dose estimate of protracted irradiation appeared to be 1.4 times higher in those persons who were evacuated during the period 3-11 days after the accident that was well in line with irradiation scenario. To the best of our knowledge, these are the only results of direct (i.e. early dicentric) biodosimetry obtained for the Ukrainian Chernobyl evacuees in accordance with methodological requirements of IAEA Manual [6]. A group of Belorussian children evacuated from contaminated areas soon after the reactor explosion and examined cytogenetically in short time after exposure by authors [11] showed quite similar dicentric yields and similar resultant dose estimates to that of in our study.

However, any biodosimetry data limited to group mean dose only, whatever useful, seems to be not informative enough for epidemiologists, who also need a picture of individual dose distribution within exposed cohort. It turned to be a real challenge due to very large statistical uncertainty of individual dose assessments occurred with low number of aberrations (up to 5) found in limited number of cells (typically, 100) scored per person in early post-accident period. Moreover, the significant proportion of all cases were those with zero dicentrics observed, so according to classic biodosimetry the zero should be set as mean radiation dose for such individuals, but statistics showed that with zero dicentrics in 100 cells we can not exclude doses up to 0.5 Gy.

To solve this problem we applied a probabilistic approach, namely Bayesian analysis, that allows looking at aberrations as stochastic events, which occur with some probability and moreover can be detected (observed) also with some probability. The Poisson distribution of aberrations amongst cells seen in all exposed individuals in our study provided an opportunity to apply this function as a main part of *a priori – a posteriori* equation, which links the probability of existence of "true" number of aberrations in the sample in case when any particular number of aberrations is observed. The "true" number of aberrations divided by number of cells scored results into the "true" yield of aberrations, and that can be converted into absorbed radiation dose using an appropriate dose response curve. Initially we applied this approach and accordant mathematical algorithm to the group of Chernobyl clean-up workers that showed very satisfactory results [4, 10].

The probability distributions of absorbed dose were constructed for every evacuee in our study. It was done in two variants: using dose response relationship for protracted exposure but also for acute exposure. The later provided dose values which can be used in risk assessment without necessity of correcting data for relative biological effectiveness of protracted irradiation. All individual distributions were pooled together by combining the probability density in short dose intervals – 50 mGy, and in this

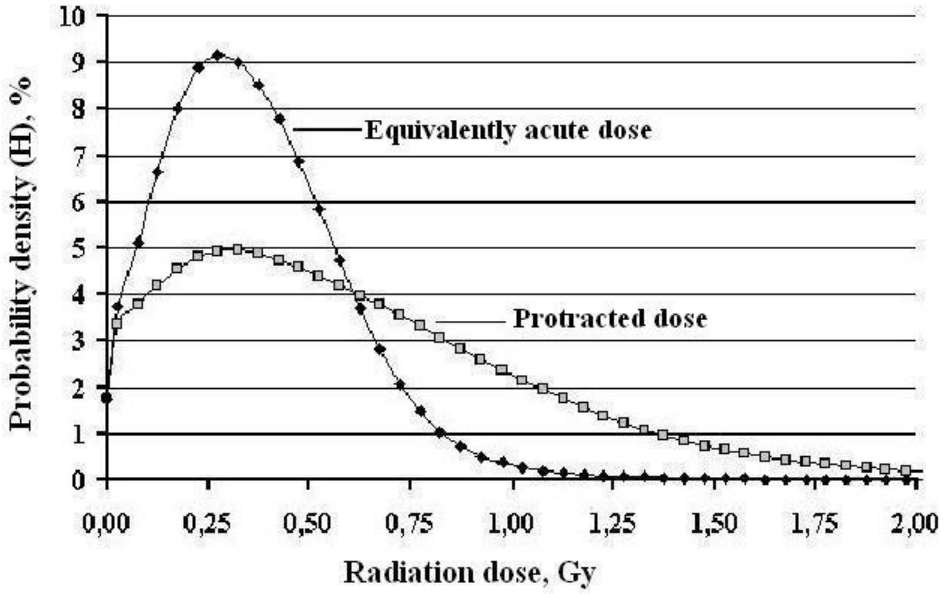

Fig. 3. Probability density distributions of protracted and equivalently acute doses of γ-irradiation estimated using Bayesian analysis applied to cytogenetic data in evacuees from t. Pripiat' and 30-km Chernobyl exclusive zone.

manner the total probabilistic distribution of protracted and equivalently acute radiation doses were finally obtained (Fig.3).

Mean dose of protracted γ-irradiation estimated from the total probability density distribution appered to be equal to that of obtained by classic biodosimetry data treatment. Modal protracted doses in evacuees fall within 200-400 mGy. Probability density up to 1 Gy of protracted exposure was the main part of total dose range, but about 20 % of protracted doses in evacuees exceeded this 1 Gy limit. The distribution of probability density for equivalently acute radiation doses within the interval from zero to 750 mGy appeared to be nearly symmetrical around modal values (tha same 200-400 mGy, as for protracted doses), and 99.8 % of all probability density of acute doses was contained by the dose range up to 1 Gy. Such data were in total agreement with clinical observations in those persons, particularly with absence of acute radiation syndromes.

It should be noted, that physical dose calculations for citizens of t. Pripiat', which represented the basis for "official" opinion about radiation doses to that population, resulted into estimations of average effective dose about 11.5 mSv, the maximum of individual dose about 114 mSv and 0.75 % of Pripiat' population exceeded the accidental permissible limit 50 mSv for public [19]. Obviously, these results were obtained by modeling involved many assumptions concerning irradiation conditions, and some factors seem to be not taken into account (e.g. remarkable external radioactive contamination of those evacuees), therefore the mentioned dose values were in controversy to our results of cytogenetic dosimetry carried out in the group of evacuees from t. Pripiat'. However, there is another set of physical dose calculations for this category of persons affected by the Chernobyl [20], where the estimations of accumulated doses appeared to be up to 1 Gy for inhabitants of certain villages around the reactor, and that was well in excess of estimates in publication [19], but in a good agreement with our data presented here.

Cytogenetic data and biodosimetry estimations resulted from the FISH assay

Regarding the debates around true radiation doses to evacuees from Chernobyl, who (in contrast to clean-up workers) were non-monitored by physical dosimetry, and for whom modeling dosimetry data appeared to be unsure, the necessity occurred for additional verification of chromosomal biodosimetry results. From the studies of time course of conventionally scored aberrations (see Fig. 1) it became obvious, that any attempts of applying the conventional cytogenetics based on unstable aberrations analysis several years after the accident wouldn't give sufficient results for biological dosimetry due to elimination of cells carrying dicentrics from the circulating lymphocyte pool [3, 6, 8]. Therefore the alternative approach based on stable chromosome rearrangements quantification should be used for estimating the yield of cytogenetic damage in lymphocytes of exposed persons. To solve this task, the last group of evacuees amongst five mentioned in Table 1 (12.8-14.8 years after exposure) was examined by both conventional cytogenetic analysis and FISH technique, specifically applied for stable aberrations visualizing. Simultaneously, a group of young residents of radioactively contaminated areas of Belarus' was also surveyed by FISH to check whether this method can serve as biodosimetry tool for quantifying low doses of not past, but long lasted chronic exposure.

Actual numbers of unstable and stable chromosome aberrations and their yields per 100 genome equivalents in studied groups are shown in Table 5. Cytogenetic parameters in evacuees were compared directly to those of in total control group due to similarity of mean age values. The spontaneous levels of translocations, insertions, acentrics and deleted chromosomes for inhabitants were calculated using age-effect regressions generated for our control group earlier [21]. Metaphases with complex rearrangements were rare and all aberrations from the complexes (apart from insertions) were included into appropriate columns of Table 5. Individual aberration yields were randomly distributed in consistence with Poisson statistics within both groups.

The average levels of dicentrics plus centric rings and acentric fragments in evacuees were slightly increased above control, but no statistical difference was observed ($p>0.05$). That was in a very

good agreement with cytogenetic effects outcome measured by conventional method, confirming again the fact of elimination of lymphocytes with unstable aberrations from the circulating pool during years post-irradiation. The level of unstable aberrations in residents was even more close to spontaneous values than that of in evacuees that probably reflected very low dose rates during their living in contaminated areas.

In contrast to unstable aberrations, the average yields of stable rearrangements were markedly increased in exposed groups above control. However that was related mainly to exchanges, but not to deleted chromosomes. This type of chromosomal abnormalities was rarely reported to be measured in exposed persons, because they obviously resulted from the lost of chromosome acentrics or chromatid breaks during mitosis of the lymphocyte precursors, where fragments were initially induced. Both types of unstable aberrations have no exclusivity to radiation, so a low sensitivity of deleted chromosomes as an exposure marker after protracted or chronic irradiation to low doses could be concluded. Unlike deleted chromosomes, the stable chromosome exchange yields were increased above control, and the meaningful difference with spontaneous level occurred for t_{comp} in evacuees ($p<0.05$), $t_{inc}Ab$, $t_{inc}Ba*$ plus $t_{inc}Ba+ac$ and insertions in inhabitants ($p<0.05$-0.01). The total level of incomplete translocations was statistically elevated in both exposed groups ($p<0.01$-0.001). Noteworthy, the sum of incomplete translocations was 1.9-2.6 times higher than the level of complete ones. The ratio of $t_{inc}Ab$ to total $t_{inc}Ba$ translocations had similar values in evacuees (1 : 0.9) and residents (1 : 0.7).

The cytogenetic parameter applied for biological dosimetry was the yield of stable exchanges with actual or assumed full presence of chromosomal material in "stable" cells. That represented a combination of complete translocations, insertions, incomplete translocations $t_{inc}Ba*$ and virtual proportion of $t_{inc}Ab$ involving unshortrened chromosome – $t_{inc}Ab*$. The reasons of choosing this particular combination of parameters instead of total translocation level for practical purposes of retrospective biodosimetry were fully explained earlier [7].

Particularly, it was suggested that incomplete translocations with "missing part" had no exclusivity to ionising radiation and may occur as a result of segregations of chemically-induced balanced chromatid exchanges in dividing lymphocyte precursors [22]. The presence of increased level of chromatid exchanges in post-Chernobyl critical groups (including residents of contaminated areas) was reported in several independent studies [17, 18], and particularly for evacuees it can be seen in early conventional analysis results presented here. Thus for preciseness of radiation exposure detection the data analysis in Chernobyl groups has to be restricted to chromosome exchanges in cells with full presence of chromosomal material. Additionally, incomplete translocations accompanied by acentric fragments were also withdrawn due to instability of their wholeness during mitotic divisions of lymphocyte precursors. Elimination of acentrics would result in a lack of genetic material following by cell death or arising of incomplete translocations with "missing part" in daughter lymphocytes. It should be noted, that amongst three exposed groups a significant number of $t_{inc}Ba+ac$ was detected only in inhabitants of radioactively contaminated areas that obviously reflected the radiation induction of this type exchanges directly in mature cells during lymphocyte lifetime. Therefore, the cytogenetic assessment of radiation doses accumulated years ago or during long term chronic exposure has to be based on the yield of chromosome exchanges formed without accompanying acentrics. Amongst incomplete translocations these were unshortened chromosomes with joined counterstained material that probably represented reciprocal exchanges involving a small telomeric region beyond the limits of visual resolution by FISH. Aassuming the identity of the mechanisms of tAb and tBa exchanges formation, the yield of $t_{inc}Ab*$ was calculated by multiplying the number of total $t_{inc}Ab$ by the respective fraction of $t_{inc}Ba*$ within total $t_{inc}Ba$ in studied groups. The obtained values were rounded off to the integer numbers and applied for deriving the full-genome yields of $t_{inc}Ab*$, which was used for calculating the total yield of stable chromosome exchanges as described above (the most full version of this approach was patented [23]).

The calibration dose-response curve for the mentioned end-point was constructed *in vitro* within a low dose range (up to 1 Gy) and fitted to a linear-quadratic model [7]. Taking into account the protracted exposure conditions in both Chernobyl groups the biological dose assessment was performed using only the initial linear slope of the curve, that was expressed by equation $Y = c + \alpha \bullet D$, where α=1.401 per 100 genome equivalents per Gy, and the background incidence of aberrations for evacuees was established in the total control group (c=0.55±0.09 per 100 genome equivalents), and that for young residents was calculated from the empirically generated regression $Y_{Sp}=0.11+2.68 \bullet 10^{-4} \bullet A^2$, where A is age in years (for A=21 years c=0.23±0.11 per 100 genome equivalents) [7, 21].

The mean yields of stable exchanges of 0.97±0.14 per 100 genome equivalents in evacuees and 0.44±0.06 per 100 genome equivalents in residents corresponded to protracted dose estimations about 300±130 mGy and 150±90 mGy, respectively (errors for mean doses were calculated applying Poisson standard errors for the excess of aberration level above control).

The results of retrospective FISH biodosimetry in evacuees were in a good agreement with early dose estimates based on conventional aberration scoring (see Table 4). In residents of contaminated areas the yield of dicentrics measured by FISH technique appeared to be unsuitable even for distinguishing exposed group from the control. It is well known that chromosomal biodosimetry utilizing unstable aberrations in chronically irradiated persons is a highly challenging task, which particularly can be solved using an elegant approach suggested by M. Sasaki [24]. We were happy to succeed with FISH-detected stable chromosome exchange yield, which provided another tool for identification of the exposed population and quantitative measurement of the low radiation dose.

From data present in literature concerning dose estimations by FISH analysis in Chernobyl cohorts one can see that biodosimetry performed by other authors were based on measuring the yield of either complete translocations alone or total translocations (including those with missing part of chromosomal material) [25-33]. Our approach with splitting translocations into “full presense of chromosomal material” and “missing part” categories seems to be unique in application to biodosimetry *in vivo*. Therefore our data have to be better compared with other authors’ results regarding total translocation levels or, even better, overspontaneous excess for this end-point in Chernobyl groups, rather than for dose estimations made by other laboratories. The literature analysis showed that in general our results of measuring the translocation yields were in a good agreement with other data presented for similar categories of persons exposed to ionizing radiation due to the Chernobyl accident [25, 28, 32, 33]. Thus, in total, retrospective FISH biodosimetry confirmed our early assessments of radiation doses accumulated by common population due to the Chernobyl NPP accident.

Conclusions

The cytogenetic survey carried out in population exposed to ionising radiation due to the catastrophe at the Chernobyl NPP showed the significantly increased level of unstable chromosomal aberrations in blood lymphocytes of individuals sampled early after the accident, and also the presence of markedly increased yield of stable chromosomal rearrangements was detected by FISH technique late time after irradiation. The time-course changes of chromatid type aberrations, chromosome type fragments, hyperploidy and polyploidy levels in evacuees were displayed as a gradual decline of chromosomal rearrangements and genome abnormality frequencies from the statistically elevated level in the first 1-2 years after the accident to the subcontrol meanings at the end of the 14-years period. The increased level of these cytogenetic damages indicated the role of the combination of mutagenic factors acted in the accidental situation at Chernobyl zone.

In Ukrainian evacuees from the Chernobyl zone both early dicentric assay and late FISH translocation measurement resulted in very similar mean dose estimates in range 300-400 mGy of protracted γ-irradiation. The yield of stable chromosome exchanges in Belorussian inhabitants of radioactively contaminated areas corresponded to doses of chronic exposure about 150 mGy.

According to our data, both evacuees from the 30-km Chernobyl exclusive zone and residents of radioactively contaminated areas should be considered as a category of exposed persons, who's radiation doses (especially if expressed in acute exposure equivalents) were below the threshold of induction of deterministic radiation syndromes, but high enough for expecting an increased risk for late effects occurrence on population level. Thus, development of advanced technologies in chromosomal analysis and, more importantly, gradual improvement of methodology of cytogenetic data interpretation allows obtaining meaningful practical results for biological dosimetry of past and chronic radiation exposure to low doses. Therefore medico-biological observations in Chernobyl groups have to be continued and early data can also be retreated and discussed using knowledge and experience obtained during post accident period by various scientific groups throughout the world.

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