KAPITEL 8 / CHAPTER 8 [8]
RADIATION-INDUCED INSTABILITY OF THE HUMAN GENOME DURING LOW-DOSE IRRADIATION

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Introduction.

The action of ionising radiation (IR) on the human body is associated with natural (space and solar energy, radon sources) and anthropogenic (medical research, nuclear power, nuclear power plant incidents) sources. Therefore, the problem of assessing biological effects in low-dose ionising radiation exposure is one of the central and practically important for medical and environmental monitoring and risk assessment, primarily carcinogenic [1-3]. It is well known that about 80% of radiation exposure of the world population occurs under the influence of natural sources of IR and only 20% - from artificial sources used mainly in medicine [4]. Protection from the influence of IR in medicine is currently the key in the world practice of ensuring radiation safety of the population. The use of IR sources in medicine is one of the main factors of population exposure, ranking second in terms of contribution to the collective dose after natural sources and first among anthropogenic sources [2-4]. Patients undergoing diagnostic and therapeutic procedures, practically healthy individuals undergoing preventive radiological (e.g., fluorography) examinations, and medical personnel are exposed to medical radiation. It is important to note that more people are exposed to radiation for medical purposes than in any other industry using IR sources; individual doses in medicine are higher compared to other applications of man-made IR sources [5]. Exposure to IR during computed tomography (CT) is a source of potential carcinogenic risk due to repeated examinations. The COVID-19 pandemic, during which CT was an indispensable method for diagnosing respiratory lesions, has been an additional source of increased CT examinations [6]. Highly radiosensitive individuals require special attention. For example, about 10% of CT examinations are performed on children and adolescents under 18 years of age [7]. In Germany and the USA, this figure is 13% and 20%, respectively [8]. Further research in this direction is the

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prospect of obtaining new scientific knowledge about radiation risk in the field of low doses of diagnostic radiation, including radiation-induced genome instability, which often precedes tumour formation [9]. It is important to note that as a consequence of the Chernobyl accident, radiation hazard issues have become the focus of much of the world's population. The reduction of the annual dose limit for professionals to 20 m3v (100 m3v in consecutive 5 years) was associated with the emergence of carcinogenic effects as an indicator of harm to the individual and to society as a whole. Introduction of modern highly informative methods of radiation diagnostics of cancer along with increase of diagnostic efficiency may cause increase of stochastic (carcinogenic) effects. Radiation risks are studied all over the world. The main criterion of radiation safety assessment is stochastic effects of low IR doses, primarily radiation-associated tumours. When performing medical radiological procedures, it is necessary to compare the benefit of diagnostic examination with the potential radiation damage to health. In this regard, the main task of radiation safety standardisation for both patients and personnel is to analyse two alternative categories - "benefit" and "harm" - and to determine the exposure conditions under which the benefit significantly prevails over the harm (ALARA principle - As Low As Reasonable Achievable). Exposure to IR is potentially oncogenic and therefore the best way to protect oneself is to keep exposure levels "as low as reasonably achievable" [10]. The issue of the dose dependence of tumour occurrence and its threshold remains complex and debated. As indicated above, the increased frequency of medical radiological procedures is predominantly due to the increased use of computed tomography (CT). Single CT examinations have a low carcinogenic risk, but the carcinogenic risk increases with repeated CT examinations. Development and introduction of new technologies, improvement of medical diagnostic equipment reduces radiation exposure of patients. However, the collective dose of medical radiation exposure increases due to the use of new highly informative high-dose examination methods. It should be taken into account that in most cases the benefit of CT procedures may significantly exceed the radiation risk. For the population of Ukraine in the conditions of long radioecological crisis after the Chernobyl catastrophe the relevance of such diagnostic examinations is obvious. The problem of

radiation-induced carcinogenesis has become particularly relevant due to the distant negative consequences of the Chernobyl disaster. According to modern ideas, the genome instability of irradiated persons plays a determining role in initiation of radiation carcinogenesis. Genome instability is a phenomenon in which multiple changes accumulate in cells, contributing to the transition of a stable genome of normal cells into an unstable genome. It refers to untargeted effects of IR action on humans and manifests itself as hidden, delayed or transmissible chromosomal instability.

This article presents the results of genome research in representatives of various categories of the Ukrainian population exposed for various reasons in the range of low doses of IR. These are participants in the liquidation of the consequences of the accident (PLCA) at the Chernobyl nuclear power plant; professionals whose activities are related to the use of IR sources, etc. They have given informed consent for the use of their blood samples. In accordance with the principles of bioethics, they gave informed consent for the use of their blood samples for research purposes in accordance with the provisions of the Declaration of Helsinki of the World Medical Association (2008). The best radiobiological basis for performing this kind of research is the human peripheral blood lymphocyte (PBL) culture test system with metaphase analysis of chromosome aberrations.

Justification of the use of PBL culture test-system in accordance with the set goal. PBL are the most radiosensitive somatic cells of the human organism, allowing to register a reliable increase of radiation-induced level of chromosome aberrations over the population average in a wide range of doses, including low doses of IR. The same yield of chromosomal aberrations during PBL irradiation under in vitro and in vivo conditions means that the lymphocyte responds to irradiation as an autonomous biological system. The high mobility of lymphocytes in the bloodstream, the distribution of lymph nodes throughout the body, the ability of these cells to accumulate chromosome rearrangements and the low spontaneous level of chromosome aberrations make it possible to assess the radiosensitivity of the human organism as a whole. Being part of the circulating blood pool, T-lymphocytes actively interact with various cells of the human body, including tumour cells, inevitably entering the zone

of therapeutic irradiation. Being in the resting stage (G_0), they are a synchronised population of cells. But under the influence of mitogen (phytohaemagglutinin) in vitro blasttransformation of T-lymphocytes begins, which allows to determine their radiosensitivity under different irradiation conditions, as well as the spontaneous level of chromosome aberrations compared to the population average. The test system based on human PBL and their cytogenetic analysis is recognised by international organisations IAEA, ICRP, WHO, UNSCEAR as a "gold standard" for biodosimetry/bioindication of human exposure. The above evidence is in favour of using T-lymphocytes as an adequate model for determining the radiosensitivity of the human genome under different irradiation scenarios.

PBL were cultured according to the international standard protocol [13] for 52 hours at 37^0C in a CO_2 incubator. Phytohaemagglutinin T-lymphocyte mitogen (Gibko, USA) was used. Colcemid (Sigma, USA) was used to accumulate metaphase plates of chromosomes at 49 h of cultivation. Metaphase plates were selected according to the international standard [13]. An average of 200-300 metaphases were analysed per cytogenetic observation. We determined the total frequency of chromosome aberrations and aberrations of different types: chromosomal and chromatid aberrations, fragments and exchanges.

Impact of the Chernobyl radiation factor on the genome of affected individuals. The radiation factor of the Chernobyl catastrophe in the form of external exposure, incorporation of radionuclides and radiation contamination of territories continues to have a negative impact on the health of the population of Ukraine. The result of large-scale radiation epidemiological studies performed by us was the conclusion about the increased frequency of oncological diseases in the post-Chernobyl period in representatives of various contingents of the Ukrainian population, exposed mainly in the range of low doses [14, 15].

If deterministic (general systemic diseases) effects of the Chernobyl accident are realised to the level of decompensation during 25 years of the post-accident period, stochastic (carcinogenic) effects have no statute of limitations.

In recent years the discussion on the development of stochastic effects - radiation

carcinogenesis - in the low dose range has intensified. The issue of dose dependence of the occurrence of radiogenic cancer remains extremely complex and topical [16].

Special attention should be paid to the studies of D. Hoffman [17], in which the author thoroughly analysed the radiation consequences of the Chernobyl catastrophe, including their dose character, relying in his predictions on the works of Japanese authors who studied the distant consequences for the victims of the atomic bombing of Hiroshima and Nagasaki. However, the author limited himself to a discussion of planetary effects rather than a prediction for specific PLCA groups because he did not have data on the specifics of the work performed in the accident area. For example, the author estimated the radionuclide content in the cloud trace based on data from Finnish researchers rather than on observations made directly in the 30 km accident zone.

We performed a large-scale (17698 PLCA) radiation epidemiological study to identify the causal relationship of diseases with work in the Chernobyl accident zone [4]. In this PLCA cohort, 1680 cancer patients were identified between 1990 and 1996, representing 9.5% of the total number of referrals to the commission. In all cases, the diagnosis of cancer was verified by a morphologist. In 1990 this class of diseases took the fourth rank in the structure of morbidity (135 patients per 1000 people), and in 2002 - already the second rank.

In order to determine the nature of dependence of cancer incidence on radiation dose, each age subgroup (up to 40 and after 40 years) was divided into 9 subgroups according to the range of documented doses: 1-3; 3-5; 5-10; 10-15; 15-20; 20-25; 25-50; 50-75; 75-100 cGy. For each dose subgroup, the mean dose and tumour incidence were calculated. A first-order linear regression scheme was used for statistical processing [18]. In addition, the values of upper and lower confidence intervals were calculated, which correspond to the 5% significance level (Figs. 1, 2).

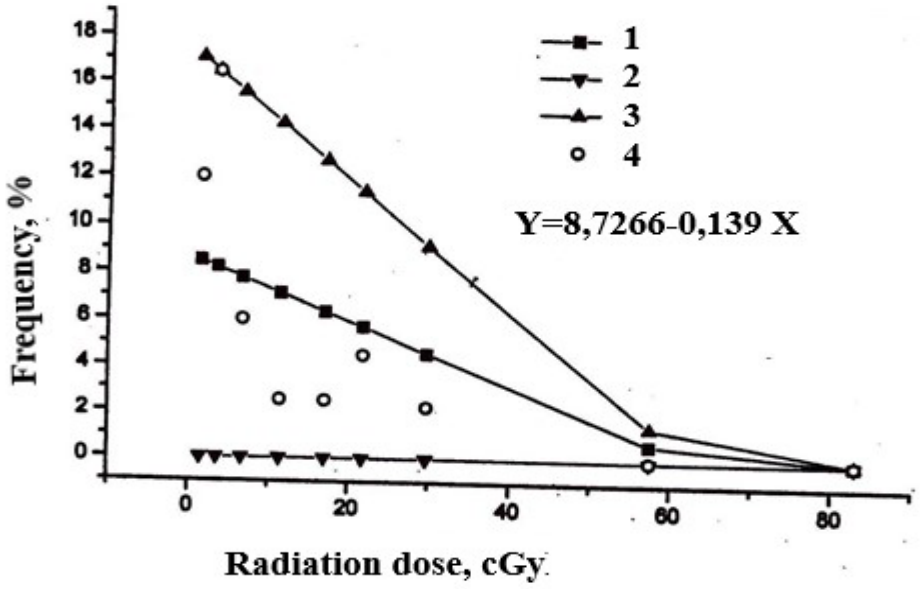

Figure 1 - Linear regression dependence of tumour incidence on radiation dose in the PLCA age group up to 40 years. Here and in Fig. 2. : 1 - calculation results, 2 - lower confidence interval, 3 - upper confidence interval, 4 - observation results.

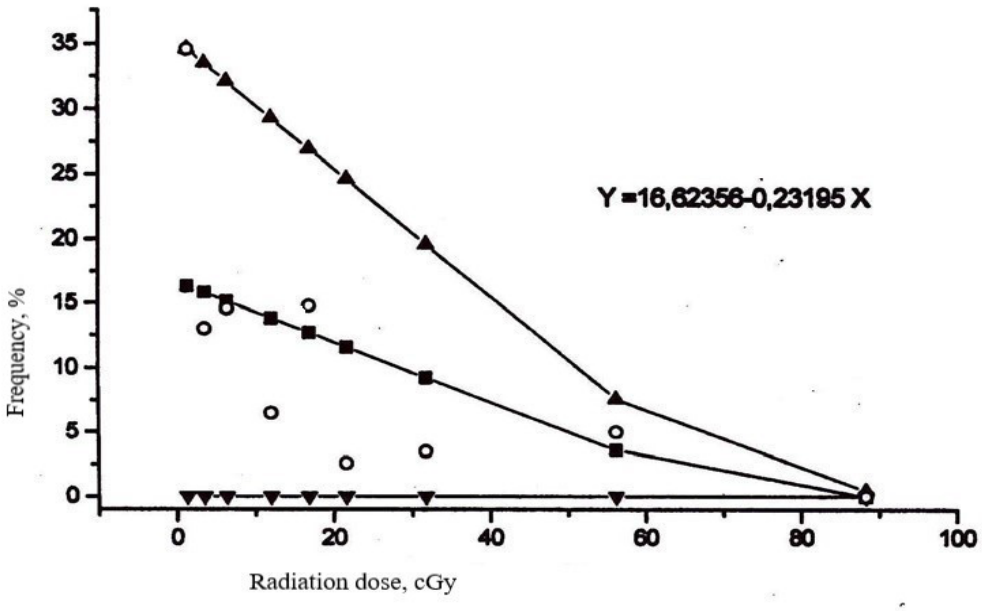

Figure 2 - Linear regression dependence of tumour incidence on radiation dose in the PLCA age group after 40 years.

The data presented in Figures 1, 2 show a tendency for tumour incidence to decrease with increasing absorbed radiation dose for both PLCA age groups.

Table 1 - Correlation coefficients of cytogenetic parameters with PLCA radiation dose at the Chernobyl nuclear power plant and diseases of different classes

Cytogenetic index per 100 cells	Correlation coefficients disease classes*				
	1	2	3	4	5
Frequency of cells with aberrations, %	0,442	0,198	0,290	0,172	0,220
Total frequency of aberrations	0,412	0,166	0,174	0,176	0,146
Frequency of chromatid aberrations	-0,097	0,191	0,194	-0,149	0,078
Frequency of chromosomal aberrations	0,572	-0,027	0,262	0,206	0,153
Frequency of paired fragments	0,224	-0,004	0,209	0,159	0,107
Frequency of acentric rings	0,385	-0,168	0,023	-0,010	-0,072
Centrifugal ring frequency	0,561	-0,215	-0028	-0,010	-0,105
Frequency of dicentrics	0,594	0,152	0,094	0,100	0,110
Frequency of abnormal monocentrics	-0,188	-0,020	0,375	0,167	0,158

*Note. * Classes of diseases: 1 -cancer; 2 - diseases of the nervous system; 3 - diseases of the circulatory system; 4 - diseases of the digestive organs; 5 - other diseases.*

It was established that despite the distant terms of cytogenetic examination and the associated elimination of a significant part of radiation markers (including dicentrics, centric rings) from the circulating pool of peripheral blood, only in the PLCA group with oncological pathology the "dose-effect" dependence for relevant indicators of radiation-induced genome instability is preserved - the correlation coefficients of dicentric chromosomes and centric rings with radiation dose are 0.59 and 0.56, respectively (Table 1). This significantly exceeds the values of the corresponding coefficients in PLCA groups with diseases of non-oncological nature. Thus, the presented data on the state of the genome of the persons irradiated as a result of the Chernobyl accident indicate the radiogenic nature of the developed cancer [19]. Thus, disturbance of stability and balance of the human genome as a result of irradiation can lead to abnormal differentiation and malignant transformation of cells. It is the radiation-induced instability of somatic cell genome and the appearance of chromosomal aberrations in cell populations that can initiate uninterrupted and self-sustaining variability, which is a potential factor in the occurrence of prognosis carcinogenesis.

Radiation-induced genome instability of professionals working in the sphere of ionizing radiation action. The problem of radiation-associated cancer (RAC) is without exaggeration the most complicated among occupational diseases due to the multifactorial nature of its etiology. The key problem is the lack of control of individual radiosensitivity (IRS) of professionals, primarily radiation diagnosticians, radiation oncologists, and personnel of nuclear facilities. According to modern concepts, the accumulation of chromosomal mutations in the cell population is considered potentially oncogenic, and low (super background) doses of IR are recognised as carcinogenically dangerous [16]. Radiation-initiated sublethal and potentially lethal damage can persist in cells for a long time until subsequent promoter action. In our study, a comparative analysis of the IRS of chromosomes of blood lymphocytes of the examined professionals (radiologists and radiologists) showed that specific IRS indicators are the frequency of chromatid deletions, and its variability depends on the length of employment in the radiation sphere. In most cases no correlation between individual values of spontaneous level of chromosome aberrations (G0-assay) and chromosome IRS (G2-radiation sensitivity assay) was found.

The first group of cytogenetic examination consisted of 23 radiologists whose work experience in the field of radiation did not exceed 1.5 years (Fig.3).

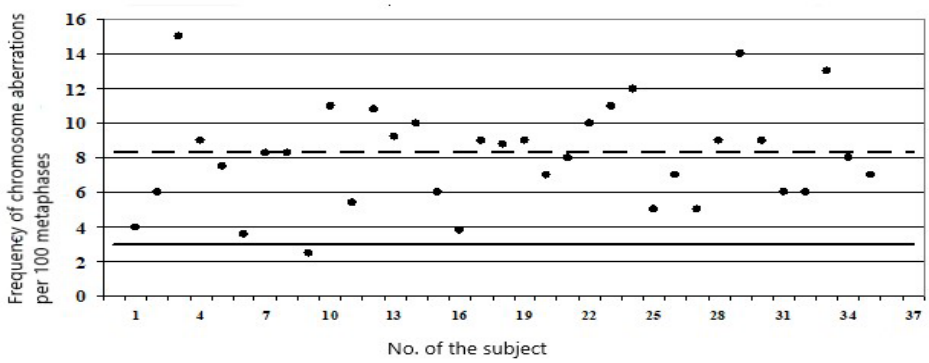

Fig. 3 - Total radiosensitivity of blood lymphocytes of professionals of the first group (chromosome test G_0). Straight line - population-average level of chromosome aberrations; dotted line - group-average frequency of aberrations in blood lymphocytes of professionals.

The group average frequency of spontaneous chromosome aberrations in PBL of

professionals of this group according to chromosome G0-test was 3,8±0,3 aberrations/100 metaphases (from 1 to 13 aberrations/100 metaphases). It slightly exceeds the value of population average index (3 aberrations/100 metaphases), which is a standard for estimation of spontaneous and induced level of genetic damages in human radiation cytogenetics. But in 40% of cases the individual frequency of chromosome aberrations in lymphocytes of the examined radiologists was 1.5-2.0 times higher than the value of the population average index. The spectrum of chromosomal rearrangements is mainly dominated by chromatid type aberrations, namely deletions, indicating the instability of the genome of the examined individuals. Based on the paradigms of radiation carcinogenesis, additional occupational exposure of these individuals will aggravate the instability of their genome and potentially contribute to increased carcinogenic risk. This interpretation is supported by the data of cytogenetic examination of radiologists of the second group.

The second group of the survey consisted of 35 radiologists whose work experience in the sphere of ionising radiation exceeded 1.5 years (Fig. 4).

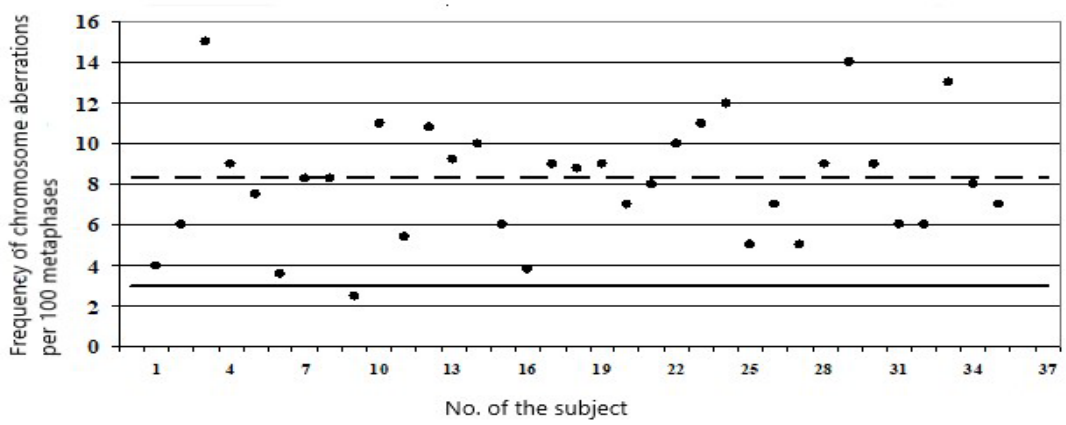

Fig. 4 - Total radiosensitivity of blood lymphocytes of professionals of the second group (chromosome test G_0). Denotation: see Fig. 3.

In contrast to the first group, at cytogenetic examination of radiologists with long work experience, the group average frequency of spontaneous aberrations by chromosome G_0-test was already 8.3±0.6/100 metaphases. The value of the studied index is more than 2 times higher than the value of the population average, as well as the value of spontaneous aberrations determined by the first group radiologists. It was

found that in ~ 90% of cases an individual increased level of chromosome aberrations is registered. Radiation markers (dicentric and abnormal chromosomes) from 0.5 to 6.0/100 metaphases were observed in 38% of the examined individuals in the spectrum of genetic damages, which indicates radiation load of the genome of the examined professionals. Figs. 5 and 6 show examples of the variability of the IRS chromosomes of blood lymphocytes of radiologists with different years of work experience in the field of IR. These data were obtained using the chromosome G_2-test, the key step of which is "provocative" irradiation of cell culture in the late G_2-period of the mitotic cycle (irradiation of cells at 46 hours of their incubation).

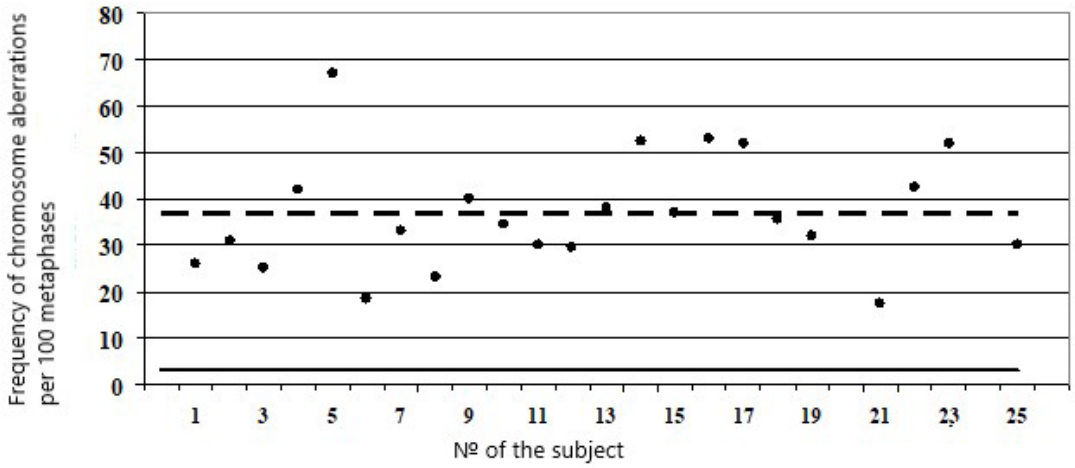

Figure 5 - Individual radiosensitivity of blood lymphocytes of professionals of the first group (chromosome G2-test). Denotation: see Fig. 3

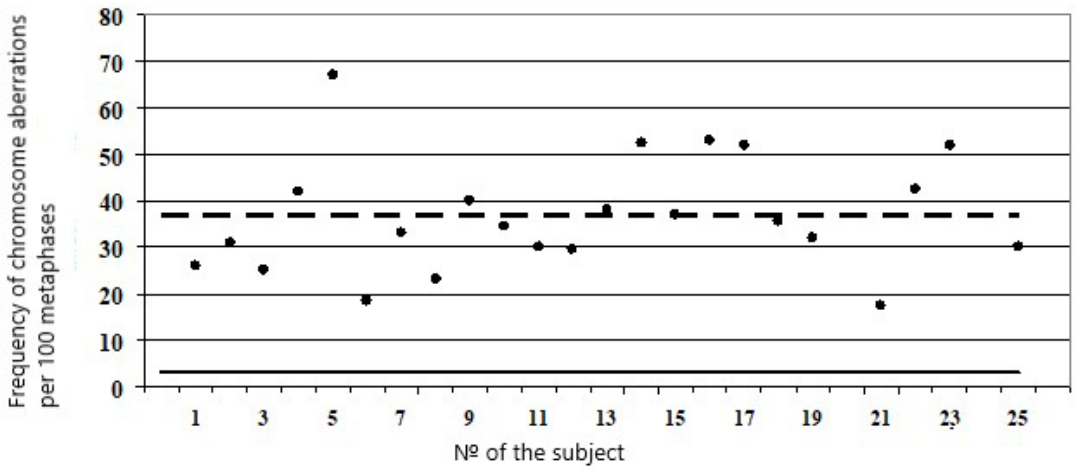

Figure 6 - Individual radiosensitivity of blood lymphocytes of the second group specialists (chromosome G2-test). Denotation: see Fig. 3.

The group-average frequency of aberrations determined on the basis of the G2-test in the professionals of the first group was 36.6 aberrations/100 metaphases. The range of variability of values of the total number of radiation-induced structural rearrangements of chromosomes in blood lymphocytes of professionals of this group ranged from 19 to 67 aberrations/100 metaphases (Fig. 5). In most cases, induced aberrations were registered in the range of 25 to 40 chromosomal rearrangements for every 100 cells analysed. Analysis of the spectrum of radiation-induced rearrangements showed that a significant contribution to the formation of genetic instability is made by chromatid-type aberrations, namely deletions. The frequency of chromatid breaks represented as deletions in metaphase cells under test irradiation in the G2-period of the mitotic cycle reflects the level of unrepaired double DNA breaks, and individual differences in the radiosensitivity of chromosomes are formed due to the genetically determined system of repair of radiation-induced damage.

In contrast to the cytogenetic data obtained during the examination of the professionals of the first group, the average group frequency of aberrations was 80.2 aberrations/100 metaphases, i.e. 2 times higher in professionals with a long work experience in the field of radiation exposure. The range of variability of the total frequency of chromosomal rearrangements was from 45 to 140 aberrations/100 metaphases (Fig. 6). Almost 90% of observations registered radiation-induced aberrations in the range of 50 to 100 rearrangements for every 100 cells analysed. Along with registration of chromatid deletions, the peculiarity of the spectrum of induced genetic changes in blood lymphocytes of representatives of this group is the formation of exchanges of both chromatid and chromosomal types. This significantly complicates the instability of the genome of the examined individuals and potentially increases the risk of radiogenic cancer (RC).

The comparative analysis of (RC) of lymphocytes blood chromosomes of the examined professionals has shown that specific indicators of RC are the frequency of chromatid deletions, and its variability depends on the length of service in the field of IS action. The group of increased occupational radiation risk was composed mainly of industry veterans. The study revealed that in most cases there was no correlation

between individual values of the spontaneous level of chromosome aberrations (G_0-test) and chromosome IRS (G_2-test).

To assess human IRS, researchers traditionally use chromosomal G_0-test - analysis of spontaneous level of chromosome aberrations and search for radiation markers in its spectrum. Such method of human IRS assessment is an integral indicator of the impact of mutagens and carcinogens of different nature on the organism. In contrast, the chromosomal G_2-test evaluates genetically determined sensitivity of an individual specifically to ionising radiation, which environmental factors can modify under certain circumstances.

Based on these results and the paradigms of clinical radiobiology, we believe that when an increased spontaneous level of chromosomal rearrangements coincides with a high IRS, the greatest risk of RC should be expected.

Genetic instability of healthy cells from the tumor environment. Radiation therapy, in addition to decreasing the probability of cancer recurrence, increases the risk of post-radiation complications [20]. This is due to the fact that healthy cells from the tumour surroundings invariably fall into the zone of therapeutic irradiation. Let us briefly present the results of cytogenetic examination of gynaecological cancer patients (endometrial and cervical cancer). We found that the average group frequency of spontaneous chromosome aberrations in PBL patients is 7.82±0.33 per 100 metaphases, which is almost 6 times higher than the value of this index in the control group of conditionally healthy individuals (1.33±0.37). In the spectrum of the registered chromosomal rearrangements chromatid type aberrations, namely deletions and exchanges, prevail, making up about 66% of the total number of chromosome aberrations. The predominance of chromatid-type aberrations in the spectrum of chromosomal abnormalities in T-lymphocytes of primary patients indicates that by the beginning of radiation therapy genetic instability is formed in healthy cells (the model of which is PBL). The genetic instability observed by us may be a consequence of oncogenesis and low efficiency of repair processes in non-malignant cells surrounding the tumour.

According to modern ideas, chromosomal instability in somatic cells in contact

with the tumour may also be associated with humoral factors freely circulating in the blood of patients, i.e. with "bystander-effect". It should be noted that the total frequency of radiation markers, namely dicentric chromosomes, in the group of cancer patients by the beginning of therapy is 0.12±0.08, while in the blood of conditionally healthy donors this type of chromosome aberrations was not registered. The obtained data unambiguously testify to the fact that T-lymphocytes of blood of endometrial cancer patients are "compromised" before the beginning of treatment due to the existing genetic instability.

Spontaneous level of chromosome aberrations in PBL of cervical cancer (CC) patients before radiotherapy. The study of the background frequency of chromosome aberrations in PBL culture of CC patients showed the following: the average frequency of cells with chromosome aberrations in blood lymphocytes of the examined patients was 6.98±0.84% and exceeded almost 6 times the value of this index in the control group and more than twice. - upper limit of the average population level. The total frequency of chromosome aberrations in the group of RSM patients was 7.44±0.95/100 cells, i.e. 1.07 aberrations per aberrant cell, which exceeded the value of this index in the control group.

The ratio of the frequency of chromatid and chromosomal type aberrations was 2.2:1. Chromatid type aberrations were mainly (90.6%) represented by chromatid fragments, the level of which was 4.52 ± 0.75/100 cells, which significantly ($p \geq 0.05$) exceeded the value of this index in the donor group on average more than 5 times.

Thus, the increased level of spontaneous chromosome aberrations in T-lymphocytes of primary gynaecological cancer patients and the predominance of chromatid-type aberrations in the spectrum of registered chromosomal rearrangements indicate that genetic instability is formed in healthy cells by the beginning of the course of radiation therapy.

Conclusion.

The generalised and analysed results of our cytogenetic examinations of representatives of various categories of the Ukrainian population [14, 16, 19, 21], who are exposed to low (above background) radiation doses, prove the concept of modern radiobiology - low radiation doses are unsafe carcinogenic factors. The radioecological crisis as a consequence of the Chernobyl catastrophe, as well as the nuclear threat from the aggressor Russia, forces urgent development of preventive measures using radio-modifiers that increase the radioresistance of the human body [21].