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COMPREHENSIVE CHROMOSOMAL ABERRATION TEST IN THE POPULATION OF SEMEY REGION OF KAZAKHSTAN

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The objects of the study was a population of Abay and Beskaragai regions of the East Kazakhstan region, living in the area covered by the former Semipalatinsk nuclear test site.

Purpose - a comprehensive cytogenetic analysis of the population living in radiation-contaminated areas.

In the process, were selected group of persons who are descendants (second, third generation) of the population living in the period of atmospheric, surface and underground explosions at designated areas. They held the fence biological material (peripheral blood) and cytogenetic studies performed to identify chromosomal aberrations routine and FISH (in situ hybridization) methods.

As a result of our work, we defined and studied the frequency and types of chromosomal aberrations in generations of descendants of persons living in radiation-contaminated areas. A comprehensive analysis and the evaluation of the identified chromosomal abnormalities in peripheral blood lymphocytes and calculated dose irradiation incorporated in the descendants living in the study area.

Keywords. Semipalatinsk nuclear test site, chromosomal aberration, FISH- methods.

КОМПЛЕКСНЫЙ ЦИТОГЕНЕТИЧЕСКИЙ АНАЛИЗ ХРОМОСОМНЫХ НАРУШЕНИЙ У НАСЕЛЕНИЯ СЕМЕЙСКОГО РЕГИОНА КАЗАХСТАНА

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Объектами исследования явилось население Абайского и Бескарагайского районов Восточно-Казахстанской области Казахстана, проживающее в регионе действия бывшего Семипалатинского ядерного полигона.

Цель работы - провести комплексный цитогенетический анализ у населения, проживающего на радиационно-загрязненных территориях.

В процессе работы были отобраны группы лиц, являющихся потомками (второго, третьего поколения) населения, проживающего в период проведения атмосферных, наземных и подземных взрывов на обозначенных территориях. У них был проведен забор биологического материала (периферическая кровь) и выполнены цитогенетические исследования на предмет выявления хромосомных аберраций рутинным и FISH (in situ гибридизация) методами.

В результате работы нами определены и изучены частоты и виды хромосомных аберраций у поколений потомков лиц, проживающих на радиационно-загрязненных территориях. Проведен комплексный анализ и дана оценка выявленным хромосомным нарушениям в лимфоцитах периферической крови, а также подсчитана доза инкорпорированного облучения у потомков, проживающих на изучаемой территории.

Ключевые слова. Семипалатинский ядерный полигон, хромосомные аберрации, FISH-метод.

ҚАЗАҚСТАН РЕСПУБЛИКАСЫ СЕМЕЙ АЙМАҚТА ТҰРАТЫН ТҰРҒЫНДАРДЫҢ ХРОМОСОМДЫ БҰЗЫЛЫСТАРДЫҢ ЖИЫНТЫҚТЫ ЦИТОГЕНЕТИКАЛЫҚ ЗЕРТТЕУІ

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Зерттеу объектісі бұрыңғы Семей ядролық полигон іс әрекетінің Қазақстан аймағында тұрған Шығыс-Қазақстан облысы Абай және Бесқарағай аудандарының тұрғындар болып табылады.

Жұмыстың мақсаты: радиациалы ластанған аймақта тұратын тұрғындардың жиынтықты цитогенетикалық зерттеуі.

Жұмыс үдерісінде белгіленген аймақта атмосфералық, жер үстіндегі, астындағы халықтың (екінші, үшінші ұрпақтың) жарылыстардың жүргізген уақытта тұрған ұрпақтары болып табылатын тұлға топтары іріктілген. Олардан биологиялық материалдарды (перифириялық қан) алу жүргізілді FISH (in situ гибридизация) және рутинді тәсілдермен хромосомды аберрацияны анықтауға цитогенетикалық зерттеу орындалды.

Жұмыс қорытындысында біз радициялы ластанған аймақта тұрған ұрпақтың хромосомдық аберрация түрлері мен жиілілігін зерттеп анықтадық. Жиынтықты зерттеу жүргізілді және перифириялық қанның лимфоциттерінде анықталған хромосомды бұзылуларға қорытынды берілді, зерттелген аймақта тұрған ұрпақтың инкорпорирленген сәуленің мөлшері саналды.

Негізгі сөздер. Семей ядролық полигоны, хромосомдық аберрациялары, FISH- тәсілі.

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Introduction. Environmental pollution is the cause of increasing the rate of mutation process and the amount of genetic load in human populations, as evidenced by the growing number of hereditary and multifactorial diseases, birth defects and malformations, especially pronounced in ecologically disadvantaged regions.

In order to predict the severity of radiation damage to the body, in time to provide effective assistance, as well as evaluate the possible effects of radiation, it is necessary to have reliable information on the dose received ionizing radiation. In such cases, special significance biological markers of radiation exposure. To date, it is generally accepted that the most informative and sensitive cytogenetic indicators are, namely chromosomal aberrations in peripheral blood lymphocytes [1]. Principles of cytogenetic methods of dosimetry and radiation exposure indicating convincingly substantiated in many domestic and foreign research, the results of which served as the basis for recommendations of WHO, IAEA and UNSCEAR on practical use of the analysis of chromosomal aberrations in peripheral blood lymphocytes as a test system for quantitative evaluation of mutagenic agents Radiation nature (UNSCEAR, 1986). Information about the "biological" dose obtained by

cytogenetic methods wider than its physical significance, since it reflects not only the result of radiation exposure, but also its individual radiosensitivity, which allows more correctly predict early and late effects of radiation. Analysis of chromosomal aberrations in peripheral blood lymphocytes have found wide application in molecular epidemiological study of people exposed to radiation. It is known that the genetic apparatus of the cell damage that can occur at the level of chromosomal rearrangements in symmetric translocations in some cases underlies radiation carcinogenesis [2]. However, the outstanding issues related to the role of somatic mutations in the development of other illnesses. Elevated levels of chromosomal aberrations in peripheral blood lymphocytes may precede the development of pathological processes, or simply be an indicator of trouble in the human body. Available data on the relationship of chromosomal aberrations with somatic diseases in those exposed as a result of living in radiation-contaminated areas are not unique and highlight the need for systematic research in this area for more information. In most cases, we are talking about irradiation in small doses. Therefore, the main concern is the effects of radiation exposure at low doses, a feature of the biological effect of which is still the subject of active debate [3,4,5]. At the same time quantification of small doses, as well as the possible consequences of exposure in the population of the Semipalatinsk region of East Kazakhstan remain problems facing serious scientific and methodological difficulties. In this regard, one of the urgent problems of radiation medicine is the development of sensitive criteria by which to judge objectively about the dangers of radiation exposure, especially in small doses on the human body. Naturally, this problem can be successfully solved only on the basis of cytogenetic monitoring of people exposed to radiation as a result of the operation of the Semipalatinsk nuclear test site (SNTS), the consequences of activities which are felt at the moment.

The aim of this study is a comprehensive cytogenetic analysis of the population living within the area of the former Semipalatinsk nuclear test site.

The objects of the study was the population of the East Kazakhstan region (EKR) - Abay, Beskaragai areas, the control group - Kokpekty area. Samples of heparinized peripheral blood of 135 people. The study was conducted in two stages.

Materials and methods. Material for cytogenetic examination was the culture of peripheral blood lymphocytes of persons and their descendants living in the study area. Sampling of biological material (blood) for cytogenetic analysis was carried out with the consent of the persons selected for the study, and for children, with their parents' consent, joining scheduled blood sampling in hospitals. For each person completed a questionnaire that included data on age, radiation route, bad habits (smoking, alcohol), as well as diseases, medication, X-ray procedures in the six months preceding the cytogenetic examination.

The choice was dictated by the research groups of the availability of published data on the display and verification of oncology and general diseases in groups of persons exposed to radiation in the dose range of 250 mSv or more [6].

Just the first stage of the study involved 80 people, of which one generation - 35 people; from age 61 to 84 years; 2nd generation - 37 people; age 30 to 49 years; 3rd Generation - 8 persons; age from 5 to 13 years (Table 1). Quantitative and qualitative assessment of chromosomal aberrations routine method conducted on 24,000 metaphases that was - 300 on each test.

Table 1.

General characteristics of the surveyed population.

	Number of people	Number of cells	The average number of metaphases per person	The total number of identified aberrations
5-13	8	2400	300	-
30-49	37	11100	300	190
61-84	35	10500	300	260
in total	80	24000	300	450

At the second stage of the research was the analysis of stable chromosome aberrations by FISH in the second generation descendants of persons residing in the v. Sarzhal Abai area and v. Dolon Beskaragai area. We proceeded from the assumption that in some cases we can talk about the inheritance of deterministic effects of parents as a result of the formation of radiation-

induced genomic instability. On the other hand, living in areas exposed to radioactive contamination could create a situation in which the ultra-low doses of radiation exposure suffered by persons born after 1963. Of the 55 people the main group and 10 controls were examined 27 men and 28 women from the main group, whose average age was 40-50 years. The control group – the residents of Kokpekty area. All descendants were born and live in the v.Sarzhal and v.Dolon. Their parents are directly exposed to the radiation factor in the period of the surface and underground tests, and at the time of blood sampling were apparently healthy. Preparation of drugs for chromosome FISH studies done in stages under the following conditions: blood sampling from the cubital vein (9 mL) was carried out in vacuum tubes with heparin. Transport of specimens from the collection site was carried out in a cold container (8^0 C -10^0 C). Tubes of blood are transported in Semey that takes 7 to 10 hours (time allowed hospital transport is 16-24 hours). FISH analysis was performed using a cocktail mixture of probes for the 1st, 2nd and 4th chromosomes (MetaSystemsGmbH): 1 chromosome labeled with FITC (green), chromosome 2 - Spectrum orange (orange), chromosome 4 -2 dye (FITC and Spectrum orange).

Results and discussion. In the analyzed 24,000 metaphase plates revealed 450 chromosomal aberrations, which amounted to 1.87%. According to published data [7], the spontaneous frequency is within 1.0% - 1.5%. Previously, it was shown [8] that the ratio of the frequency of chromosomal aberrations in chromatid has predictive value for determination of genetic risk in the offspring. Thus, the proportion of chromosomal aberrations was 49% - 51% of the chromatid type - for those 1st generation, 2nd generation in individuals (offspring) is equal to the ratio - 33% and 67%, respectively (Table 2).

Table 2.

Frequency of chromosomal aberrations in population EKR (routine method).

Age (generation)	The frequency of chromosomal aberrations		Chromosome type		Chromatid type		Ratio
	n	on 100 cells	n	on 100 cells	n	on 100 cells	
5-13 (3)	-	-	-	-	-	-	-
30-49 (2)	190	1,71	63	0,57	27	1,14	1:2
61-84 (1)	260	2,47	128	1,21	32	1,25	1:1

Thus, the analysis routine cytogenetic studies suggests that the descendants of persons residing in the test areas have chromosomal abnormalities, mainly chromatid type, which are now, along with chromosomal aberrations, regarded as one of the effects of prolonged radiation exposure. Increasing the frequency of aberrations in elderly may indicate a developing age-chromosome instability and, therefore, their greater sensitivity to genotoxic effects of the environment. However, we can not exclude at the same time the influence of radiation and medical and household factors.

In the next step was carried out cytogenetic analysis of unstable chromosomal aberrations in the second generation descendants living in the v. Sarzhal Abay area and the v.Dolon Beskaragai area EKR using routine staining of chromosomes. To assess the level of unstable chromosome aberrations drugs metaphase plates were analyzed for the presence of dicentric, atsentrikov, rings, deletions, chromosomal breaks and exchanges. Routine cytogenetic methods were analyzed 5500 metaphase cells, 100 metaphases for 1 person. Table 3 shows the results of cytogenetic analysis.

Table 3.

Analysis of unstable chromosome aberrations using routine staining of chromosomes in the off spring of the v.Sarzhal and v.Dolon.

Populated locality	Aberrant cells (%)	All aberrations (%)	Chromosome type (%)	Chromatid type (%)
Sarzhal	0,956±0,22	1,244±0,25	0,926±0,21*	0,318±0,12
Dolon	0,829±0,15	0,829±0,15	0,457±0,11*	0,371±0,1
Average	0,893±0,126	1,037±0,138	0,692±0,11*	0,345±0,08
Control	0,87 ± 0,1	0,87 ± 0,1	0,19±0,05	0,68±0,09
* p=0,001;				

As can be seen from the data presented in people from the village Sarzhal frequency of chromosomal abnormalities was (1,244±0,25)% that almost does not exceed the control level (0,87 ± 0,1)%.

Analysis of the spectrum of cytogenetic disturbances persons surveyed indicated that as chromosomal aberrations occurred - 0,926 ± 0,21%, and chromatid type - 0,318±0,12%, the absence of these types of rearrangements as dicentric. Chromosomal aberrations were presented double break and fragments, chromatid type - single breaks and fragments.

People from the village of Dolon frequency of chromosome aberrations was 0,829 ± 0,15%, which is slightly lower than that of the surveyed villages Sarzhal and do not differ from those of the control group, but nevertheless conducted a comparative analysis of the types of aberrations also showed a significant excess chromosomal aberrations.

Generalizing the data obtained by cytogenetic analysis of both groups were established: the spectrum encountered aberrations of chromosome aberrations was submitted chromatid type and substantially equal ratio which in turn was represented by single or double break and fragments.

As noted earlier, cytogenetic markers of radiation damage are structural rearrangements of chromosomes and, above all, stable aberrations (translocations, chromosome breaks), and then presents the results of the frequency of chromosomal aberrations in 55 descendants of persons in the second and third generation living in the East Kazakhstan region, determined by FISH.

In addition to the stable chromosome aberrations (deletions, duplications and translocations) in the analysis of FISH-stained preparations drew attention to the presence of unstable chromosome aberrations: the presence of chromosomal breaks and chromatid type, acentric fragments, dicentric, rings, chromosomal exchanges.

Total analyzed 11000 metaphase nuclei (the rate of 200 metaphases / 1 pers.). For each metaphase nucleus made on 3 shots, according to the used filters. The presence of chromosomal aberrations was established by image processing metaphases. Table 4 summarizes the cytogenetic analysis FISH-stained metaphase spreads.

It should be noted that the frequency of aberrations detected in this case was higher than the frequencies identified in routine analysis. This is due, firstly, with a large number of metaphases studied and, secondly, the spread spectrum revealed aberrations in FISH-analysis.

Table 4.

Results of the analysis of chromosomal aberrations in the offspring of residents living in the area covered by the former Semipalatinsk nuclear test site.

Breaks		Fragments		Dicentric (%)	Chromosome change (%)	Deletion (%)	Duplication (%)	Translocations (%)
Chromatid (%)	Chromosome (%)	Single (%)	Double (%)					
Sarzhal								
0,375 ± 0,097	0,4 ± 0,1	0,425 ± 0,103	0,475 ± 0,109	0,05 ± 0, 035	0,2 ± 0,071	0,075 ± 0,043	0,025 ± 0,025	0,25 ± 0,025
Total chromosome aberrations - 2,15 ± 0,229								
Chromatid type- 0,850± 0,145				Chromosome type - 1,300 ± 0,179				
Dolon								
0,171 ± 0,049	0,286 ± 0,064	0,214 ± 0,055	0,271 ± 0, 062	0,071 ± 0,032	0,029 ± 0,020	0,114 ± 0,040	0,100 ± 0,038	0,100 ± 0,038
Total chromosome aberrations - 1,414 ± 0,141								
Chromatid type - 0,400± 0,075				Chromosome type - 1,014± 0,120				
Total two settlements								
0,245± 0,047	0,327± 0,054	0,291 ± 0, 051	0,345 ± 0,056	0,064 ± 0,024	0,091 ± 0,029	0,100 ± 0,030	0,073 ± 0,026	0,073 ± 0,026
Total chromosome aberrations - 1,682 ± 0,123								
Chromatid type - 0,564± 0,071				Chromosome type - 1,118± 0,100				

Group average frequency of chromosomal aberrations in the villagers Sarzhal above (2,150 ± 0,229), than in the village of Dolon (1,414 ± 0,141). This is true for both routine and FISH-analysis. In both groups there is excess frequency of chromosomal aberrations (1,118±0,100) over chromatid (0,564±0,071), which corresponds to modern ideas about the influence of the radiation factor. As a comparison of the results, we use the well-known data on the number of spontaneous mutations revealed by FISH-analysis.

However, for this cohort of residents of East Kazakhstan region (EKR), the frequency of chromosomal aberrations were lower than those of other Kazakh researchers who conducted the study population cytogenetic EKR (previous showed higher frequencies of chromosomal aberrations: at the level of 3% [9]. The explanation for this is that in our study were examined indicators descendants (2-3 generation) residents living in the contaminated areas. However, because of the small amount of data in the literature regarding the cytogenetic changes in the offspring of exposed individuals, determined their frequency of aberrations of stable and unstable type of routine and FISH methods exceed levels spontaneous mutation, which does not exclude the effect of the radiation factor.

In the calculations, the cumulative dose of radiation, we used published data on the specific dose dependence of the frequency of stable chromosome aberrations obtained after irradiation of blood samples by gamma irradiation at a dose rate of 0.1 Gy / min and subsequent FISH-analysis.

It should be noted that in the Snegireva G.P. [10] obtained similar dose dependence as taking into account the frequency of dicentric / centric rings, as well as taking into account the translocations identified by FISH method. This is undoubtedly an important result confirms the correctness of the formula proposed (Lucas et al., 1993) for converting the frequency of translocations involving chromosomes stained the entire genome. To determine the dose of irradiation, we used the following regression relationship:

$y = 0,24 + 0,70\ D + 0,14\ D^2$, where

y - frequency translocations involving chromosomes 1, 4, and 12 100 cells

D - dose. The significance of the model:

$p = 6,3 \times 10^{-4}$.

Use of this dependence to calculate the cumulative dose of radiation frequency of translocations allowed us on the basis of the results FISH-analysis to evaluate the retrospective dose cohorts surveyed residents of East Kazakhstan region: the v.Sarzhal - 0.6 cSv; v.Dolon - 2.5 cSv. The entire cohort of residents EKR - 1.82 cSv.

It is noted that dosage values were calculated using a calibration curve for gamma [10] obtained by a specific dose. In this case, were not taken into account important factors such as the real dose rate, the nature of radiation exposure (whether internal external radiation), the dose distribution in time and relative biological effectiveness of radiation, since such data are not available. Naturally, there is also a great deal of uncertainty associated with the impact on the level of non-radiation factors translocations nature, such as age, living conditions, factors of production. However, despite this, the results make it possible to retrospectively assess the degree of radiation effects on the population of the regions affected by radiation in the absence of precise data of physical dosimetry.

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