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CYTOGENETIC SURVEY OF PEOPLE FROM THE REGION OF SEMIPALATINSK NUCLEAR TEST SITE

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Abstract

The results of cytogenetic examination of people from the region of Semipalatinsk nuclear test site (the village Sarzhal). The frequency spectrum and the ratio of different types of chromosomal aberrations in the examined persons living in the most contaminated areas, confirm the deleterious mutagenic effects of ionizing radiation on chromosomes of the population in the studied group compared to controls. The high variability in the frequency of chromosomal aberrations - from 0 to 8% of the people and the lack of connection with the epidemiological data indicates heterogeneity of the population by radiosensitivity.

Key words: *chromosomal aberrations, radiosensitivity, ionizing radiation, biodosimetry, Semipalatinsk nuclear test site*

The problem of the remote consequences of action of radiation gains in recent years the special relevance connected with a number of circumstances. First of all, it is the arisen need of an assessment of a current state of genetic and somatic human health of various countries which have transferred emergencies on nuclear power plants and other sources of radiation, nuclear tests, professional contact with radiation etc. Such assessment is even more important for tracking health of future generation.

Radioactive pollution surrounding environment as a result of nuclear tests - one of large problem biosphere pollution. One of such ecologically unsuccessful regions is regions actions of the Semipalatinsk proving nuclear test (SNTS). It is obvious that the population area, adjacent to the range territory, during long time was exposed to chronically action of various doses of radiation in consequence land and underground tests of nuclear weapons. In areas much the under polygon physical measurements show meaningful excess of a radiation background and in present time (Chasnikov 1996). Thus, for 42 years of functioning SNTS the population Semipalatinsk, the Pavlodar, East Kazakhstan and Karaganda areas and some regions of Russia in various degrees was exposed to radiation influence.

The condition of radiation impurity of environment is defined by different ways and different experts. Without belittling the advantage of any approaches, it is necessary to emphasize especially big advantages of genetic methods as the general relevance of a problem is connected, first of all, with negative influence of radiation on the person. Therefore results of cytogenetic inspection have to be considered as one of the main and most objective exponents of radiation impurity of the environment. These data will be free not only from abstract judgments about possible harm proceeding from indicators of physical measurements, but also any links to incidence and mortality.

MATERIALS AND METHODS

Samples of peripheral blood for the cytogenetic analysis were taken from 110 people living in the item Sarzhal ("extraordinary" radiation risk relating region of influence SNTS the East Kazakhstan region). As control group of comparison data on cytogenetic inspection of 42 inhabitants of the environmentally friendly settlement Tausugur of Almaty area are used.

Preparation of metaphase chromosomes carried out by a standard technique (Moorhead et al. 1960). In the cytogenetic analysis of metaphase plates defined number of cages with aberrations, and also number and type of aberrations on 100 analyzed metaphases. From each individual analyzed from 200 to 400 metaphase cages. The obtained data processed statistical methods (Plohinsky 1967).

RESULTS OF RESEARCHES

There are five groups of retrospective identification of consequences of dose loads of an organism at external and internal radiation: cytogenetic, hematologic, immune bacteriological, biochemical and biophysical. At an assessment of effects of biological effect of radiation in small doses registration of cytogenetic and hematologic damages is possible: frequencies of chromosomal reorganizations, numbers of lymphocytes with microkernel, and also frequencies of cages carriers of somatic mutations in peripheral blood (Kudryashov 2004).

Cytogenetic inspection of 110 inhabitants of the item Sarzhal showed that the frequency of chromosomal aberrations at them made 3,19±0,19% in comparison with control of 0,87 ± 0,1% ($p<0,01$). The carried-out comparative analysis on types of aberrations with control group (the item Tausugur) (figure 1) showed that the frequency of aberrations of hromatidny type in the item of Sarzhal increased, by 2,3 times whereas excess of aberrations of chromosomal type is observed by 8,5 times ($p<0,01$) that points to primary influence of genotoxicant of the radiation nature. Individual fluctuations of frequencies of chromosomal aberrations made 0-8%.

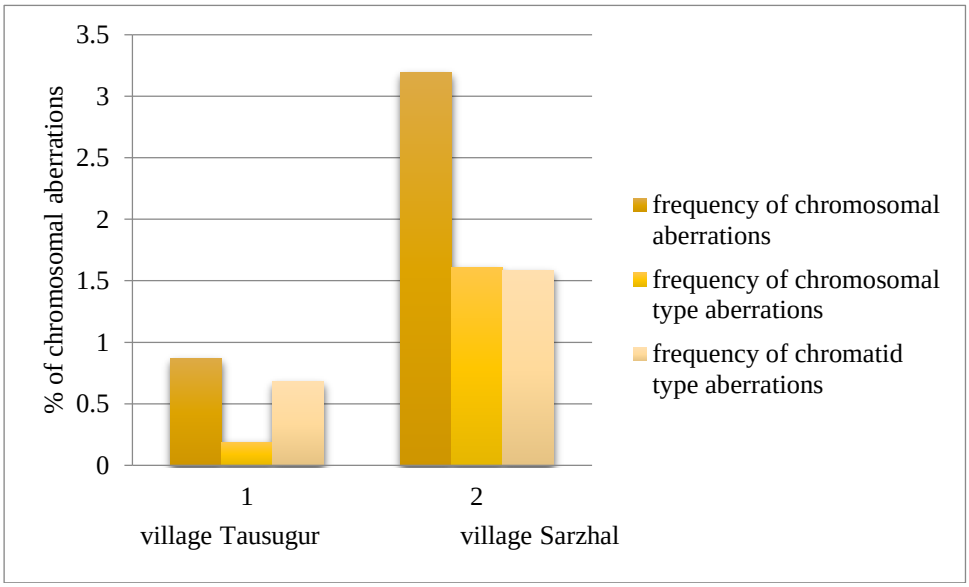
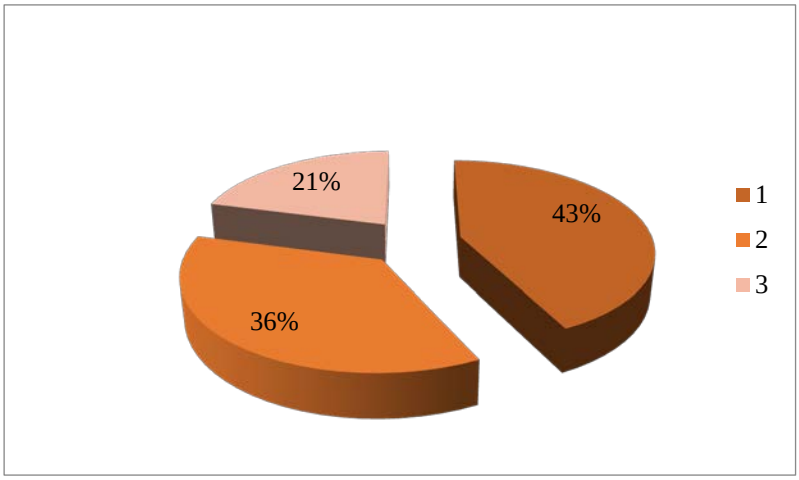


Figure 1 Distribution patterns of chromosomal aberrations in villagers Sarzhal East Kazakhstan region

Cytogenetic examination of inhabitants of the settlement Sarzhal of the East Kazakhstan region conducted in the nineties last century of Ahmatullina & Gubitskaya (2001) also revealed increase in frequency of chromosomal violations to 3,47±0,23%. Not reliability of a difference between these indicators received with a difference more than in 15 years, shows, lack of basic changes of an ecological situation in this district (Sharipov et al. 1993, Sevankaev et al. 1995, Gubitskaya et al. 1999).

The analysis of a range of cytogenetic violations of the surveyed persons showed that aberrations both chromosomal - 1,61±0,12%, and hromatidny of types - 1,58±0,12% met. Such picture of structural damages of chromosomes can be connected with action both radiation, and chemical genotoxicant. Aberrations of chromosomal type were presented by double gaps and fragments, dicentrics and the translocations, the second - single gaps, fragments and inter chromatid exchanges

At systematization of frequency of chromosomal aberrations in lymphocytes of peripheral blood all surveyed contingent was divided into three groups: the persons having chromosomal aberrations in lymphocytes of peripheral blood to 2% (1), from 2 to 4,9% (2) and from 5% and above (3). The first group includes a cohort of the people having spontaneous level, the second – raised and the third – high level of chromosomal aberrations (figure 2).



1. to aberrations 2%
2. from 2,1% to 4,9 % aberrations
3. 5% % or higher aberrations

Figure 2. Distribution of frequencies of chromosomal aberrations in villagers Sarzhal East Kazakhstan region

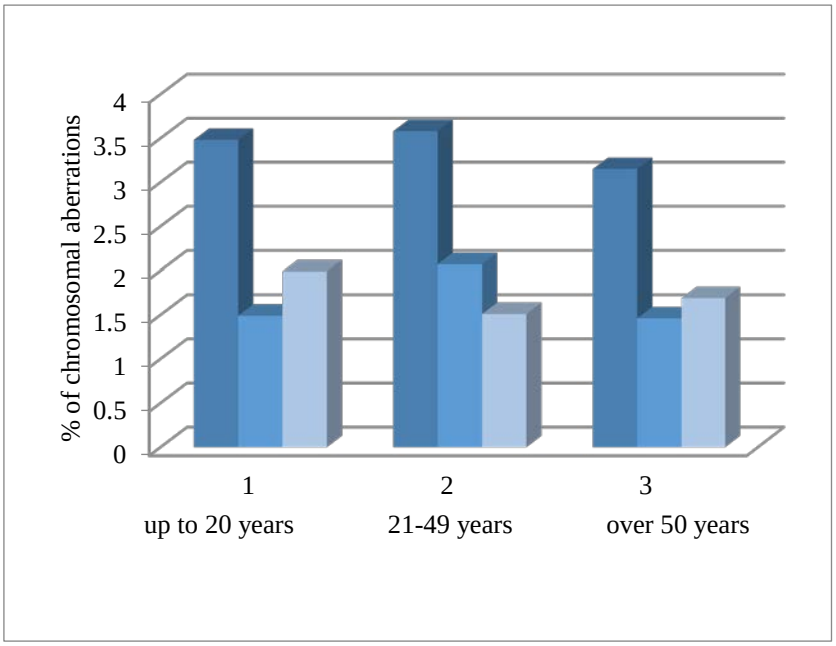


Figure 3 The distribution of the frequency of chromosomal aberrations by age groups

Apparently in figure 2, less than at a half of the studied persons (43%) the frequency of the revealed violations didn't exceed all-population spontaneous level for Kazakhstan (Gubitskaya et al. 2007), at 36% it was raised and at 1/5 (21%) – high. I.e. more than at a half surveyed (57%) the frequency of chromosomal aberrations exceeded spontaneous level by 2-5 times.

It should be noted that when carrying out cytogenetic research of the population living in the territory of polluted by radionuclide as a result of long-term land and atmospheric tests on SNTS, we consider events which occurred mainly 50-37 years ago. For expired period the situation changed: the generation of injured people, элиминировались cages with unstable aberrations of chromosomes was replaced, conditions of formation of doses of radiation that causes certain difficulties for carrying out biological indication changed. Besides the big material on age radio sensitivity of mammals and the person (Schmitz-Feuerhake et al. 1993) is now saved up.

Radio sensitivity of children, adult, elderly and old is various (Richardson & Wing 1999). The reasons of the increased radio sensitivity are various for each of age. For example, it is known that levels of bio-accumulation of the incorporated radionuclide in different bodies differ at children and adults (Bandazhevsky & Bandazhevskaia 2001). The period of semi-removal of cesium from a children's organism is 1,5 - 2 times higher, than from an organism of adults (Melnov & Lebedeva 2004). In this regard the analysis of frequency of cytogenetic violations in different age groups is carried out. The age period of 21-49 years was determined by two reasons: first, it is age of the greatest reproductive activity, secondly, the age of 49 years (for 2011), i.e. 1962 year of birth, is year of the termination of land nuclear tests (figure 3).

Apparently from the presented chart the general level of cytogenetic violations is almost identical in all age groups. However in average age group, the most active in the reproductive relation (21-49 years) observes the level of chromosomal violations raised in comparison with others. However, if to carry out the analysis on specific markers of radiation influence – to dysenteric and ring chromosomes the picture cardinally changes (figure 4). The greatest frequency of dicentrics is observed in younger age group till 20 years that confirms the thesis about bigger sensitivity of a children's organism. It is shown in many researches in the analysis of consequences of accident on the Chernobyl Nuclear Power Station (the children directly irradiated and born from irradiated parents). For example, Melnov et al. (2004) with conducted cytogenetic research of lymphocytes of peripheral blood of the children living in the territory of polluted by radionuclide of Belarus. The increase in frequency the aberrant of cages of 4,5±0,31% against 1,92±0,19% in control that can testify to increase in the general instability of a genome was noted. In parallel with it the increase in frequency of dysenteric and ring chromosomes of markers of radiation influence are noted also. Also rather high rates of frequency of chromosomal aberrations at the age of 15-20 years show instability of a genome at young people during puberty.

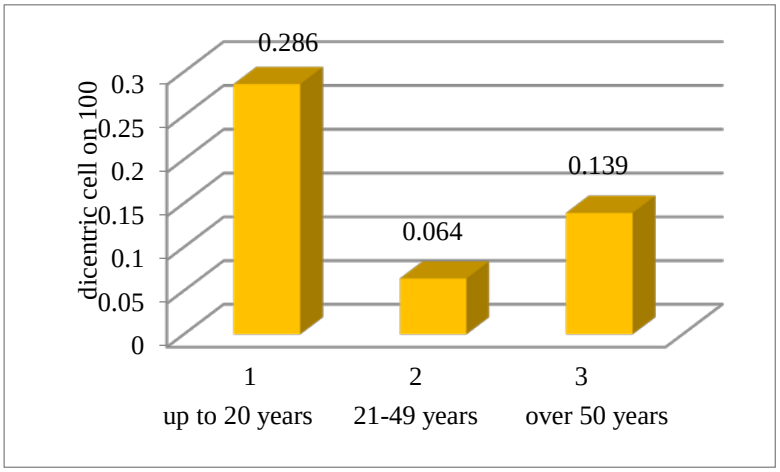


Figure 4 Dicentric frequency distribution by age groups

As at young people and children from the region SNTS, been born after closing of the range the raised level of cytogenetic violations comes to light, there is a question of the relation of the received results to a problem of transfer of genetic damages to the subsequent generations of the irradiated parents. Children in many works are considered as more sensitive system therefore the cytogenetic analysis which is carried out on their cages serves as the best indicator of action of a radiation situation on the person.

Thus, the raised level of frequency of dicentrics in younger age group show not only the current chronic influence of the raised radiation background, but also a condition of instability of the genome, transferred to them from the parents who were directly affected by nuclear tests on SNTS. For the analysis twice the bigger frequency of dicentrics in the senior age group, in comparison with average, the analysis of a ratio of frequency of dicentrics to the general frequency of aberrations of chromosomal type is carried out. This ratio reflects the real frequency of occurrence of these markers of radiation influence in each age group (figure 5). The younger age group still shows the highest rates, in the senior age group their occurrence the smallest. Decrease in frequency of chromosomal violations of this type with increase in age can be explained with a survival of the most radio steady individuals with good functioning the reparative, immunological and adaptation systems.

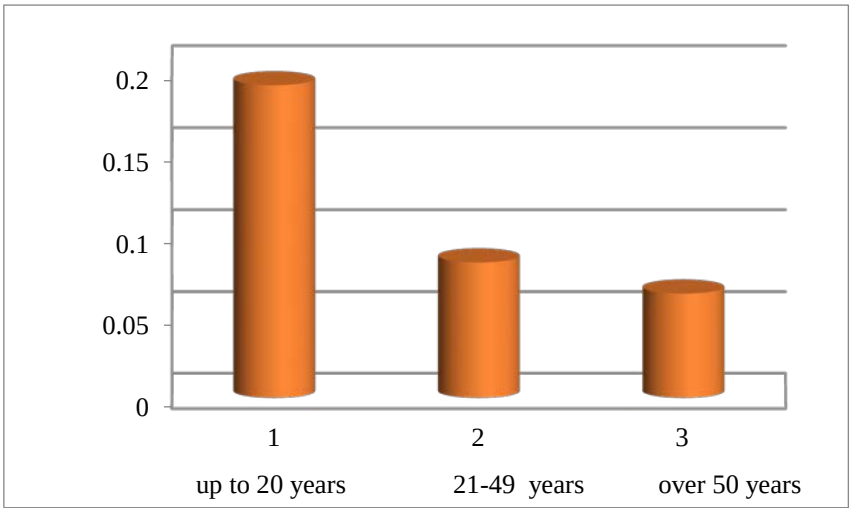
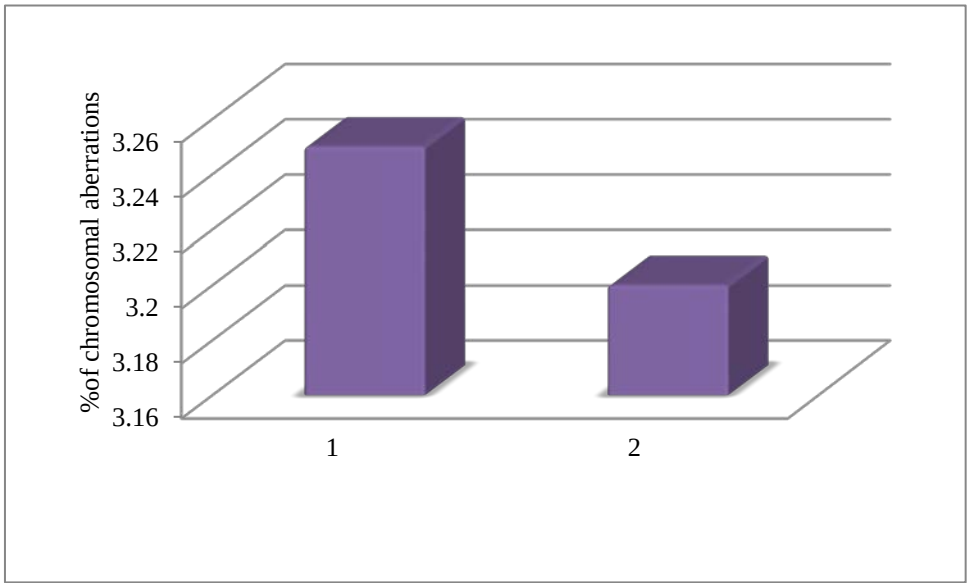


Figure 5. Dicentric ratio to the total frequency of chromosomal aberrations in different age groups

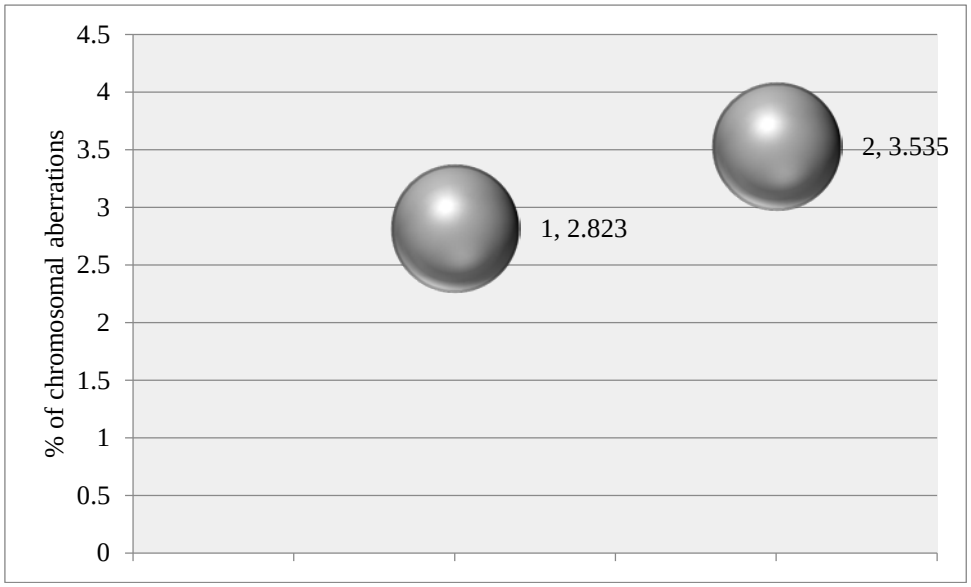
Proceeding from available data and theoretical provisions of the general biology and ecology, there is a group variability of radio sensitivity: the racial; the ethnic; the population; the sexual; the age; the physiological. Having studied age radio sensitivity, the statistical analysis of the obtained results with use of various epidemiological data on racial and a sex is carried out; at the persons having in the anamnesis abortions, dead born or children of disabled people; having blood relatives of patients or the dead from oncological diseases; with cardiovascular diseases; smokers. Having analyzed data of the conducted cytogenetic examination, in connection with questions of group radio sensitivity of various types it is shown that reliable distinctions between compared indicators aren't present. Possibly, distinctions in individual reaction to radiation are covered at genetic level and are connected with functioning and polymorphism of various genes responsible for resistance of an organism to adverse factors of environment.

As already it was told above, high variability of frequency of chromosomal aberrations at the studied group of people is observed. As they are exposed to radiation influence during different time, the analysis of dependence of this indicator from terms of accommodation of people in this settlement (till 1962 when land nuclear tests were carried out, and after it), and also from a type of work – connected is carried out or not with visit SNTS (figure 6 and 7 respectively).



1. lived in the village Sarzhal to 1962 up to the present time
2. came to the village after 1962 year Sarzhal

Figure 6. The frequency of chromosomal abnormalities depending on the length of residence in the village Sarzhal



1 - engaged in labor activity on the territory of the Semipalatinsk nuclear test site

2 - leading work on the territory of the village Sarzhal

Figure 7. Analysis of the frequency of chromosomal abnormalities in the surveyed people depending on the type of work

Apparently from the presented charts of reliable distinctions between compared indicators also it isn't revealed. I.e. the persons, being affected more long time of radiation genotoxicant, or visiting SNTS, in connection with work, don't show authentically raised level of cytogenetic violations. It also indicates the precedence of individual radiosensitivity.

The purpose of many monitoring works on the cytogenetic analysis of the people who anyway are exposed to radiation influence, the biodosimetry question is. The greatest interest among observable, damages of chromosomes is represented by dysenteric chromosomes and centric rings. On the frequency of dicentrics and centric rings establish the radiation fact, estimate level of radiation influence, and determine the size of the received dose of ionizing radiation. Essential difficulties are presented by interpretation of level of chromosomal aberrations of unstable type at the chronic and prolonged radiation. It is known that level of chromosomal aberrations observed thus is result of several independent processes: formations of chromosomal damages under the influence of radiation, elimination of cages with unstable chromosomal aberrations when passing a cellular cycle and reparation of the damages induced by radiation. However, at an assessment of averages and the collective doses received by populations, exposed to radiation (for example, the population living in territories polluted by radionuclide), it is possible to believe that influence of all listed factors is averaged therefore use of existing methods of biodosimetry in this case can be quite correct (Snigireva 2011). Now the method of the analysis of chromosomal aberrations is recommended to WHO as test system at an assessment of influence of mutagen factors of environment for the person (International Atomic Energy Agency 2001).

There are some main techniques of a retrospective assessment of doses of external radiation on the frequency of dicentrics and centric rings. For example, 1-on Lloyd's initial formula: D=y/0,022 (Lloyd et al. 1998), where D – a dose of the internal saved-up radiation (Гр), At – the frequency of dicentrics on 1 cage, 0,022 standard coefficient; 2 - to formula recommended to International Atomic Energy Agency (2001) for the fractioned radiation: $Y = \alpha+\beta D^2$, where: D – dose of the internal saved-up radiation (Гр), Y – the frequency of dicentrics on 1 cage, α – 0,03 (coefficient), β – 0,06 (coefficient); 3 - the calibration curves a dose effect received at radiation of samples of blood by gamma radiation with a power of dose of 0,1 of Gy/min. The corresponding regression dependence, has an appearance: $y = 0,1 + 1,5 D +6,3 D^2$ where y - the frequency of aberrations is at 100 cages, D - a dose of radiation (Demina et al. 2009). Using various methods of an assessment of doses results presented in table 1 were received.

Table 1 Calculation of a Dose of the Internal Saved-up Radiation for the Frequency of Dicentrics and Rings different methods at inhabitants of the item Sarzhal

Dicentric frequency on cell 100	Accumulated dose of internal irradiation (frequency of dicentrics and rings) Sv (Gy)		
	Lloyd formula D=y/0,022	$y = 0,1 + 1,5 D + 6,3 D^2$	$Y=\alpha D+\beta D^2$ (IAEA)
0,16	0,072	0,02	0,05

Also radiation doses for different age groups were calculated

Table 2 Calculation of a Dose of the Internal Saved-up Radiation for the Frequency of Dicentrics and Rings different methods at inhabitants of different age of the item Sarzhal

Age bracket	Dicentric frequency on cell 100	Accumulated dose of internal irradiation (frequency of dicentrics and rings) Sv (Gy)		
		Lloyd formula D=y/0,022	$y = 0,1 + 1,5 D + 6,3D^2$	$Y=\alpha D+\beta D^2$ (IAEA)
Up to 20 years	0,286	0,13	0,09	0,082
21-49 years	0,064	0,03	0,027	0,02
over 50 years	0,139	0,06	0,031	0,043

Using a calibration curve with average cytogenetic data at γ-облучении cultures of lymphocytes of donors in the range of doses of 0,1-1,0 of Gy at the power of doses of 0,1 of Gy/min on the general frequency of aberrations of chromosomes (Demina et al. 2009) with some share of approach it is possible to calculate the individual saved-up doses. Thus if to proceed from an individual variation of frequency of chromosomal aberrations (0-8%) the absorbed dose of radiation also varies from 0 to 0,4 Sv (Gy).

Values of doses were calculated by means of various methods. Thus we didn't consider such important factors, as the real power of a dose, nature of radiation influence (internal or external radiation), dose distribution in time, etc. as such data are absent. It is natural that there is the big share of uncertainty connected with influence on level of chromosomal aberrations of factors of not radiation nature, age, accommodation conditions. However, despite it, the received results allow to estimate retrospectively extent of radiation impact on the population of affected regions in the conditions of absence of exact data of physical dosimeter. It is known that the retrospective assessment of doses of the population is often carried out taking into account average data (a radiation condition, influence time, supervision time, etc.). As the main objective at reconstruction of doses is the assessment of the possible remote consequences of radiation, any method which will help to estimate radiation level, has the right for existence. Advantage of biological methods, in this case cytogenetic, consists that reaction of an organism to the radiation influence, mediated by specific characteristics of an organism, first of all individual radiosensitivity is estimated. The "biological" dose thus is an integrated indicator of damaging action of radiation, conditionally expressed in terms of an effective dose.

Thus, the cytogenetic analysis of inhabitants carried out by us from the item Sarzhal, revealed high frequencies radiation the induced chromosomal markers - pair fragments, dysenteric and ring chromosomes. Range and ratios of various types of the chromosomal aberrations, revealed at the persons living in the territories most polluted by radionuclide, confirm damaging mutagen action of ionizing radiation on the chromosomal device of the population in the studied group 20 years later after closing SNST. High variability of frequency of chromosomal aberrations – from 0 to 8% at people and lack of communication with epidemiological data testifies to heterogeneity of population by criterion of the radio sensitivity dependent on the genetic status of the general state of health, functioning immune and the reparative of systems of an organism.

REFERENCES

Ahmatullina, NB & Gubitskaya, EG 2001, 'Cytogenetic monitoring of the region's residents SNTS', *Proceedings of the National Academy of Sciences of the Republic of Kazakhstan*, no. 2, pp. 62-66.

Bandazhevsky, YI & Bandazhevskaia, G 2001, 'Incorporated cesium and cardiovasacular pathology', *Int. Conf. Health Effect of the Chernobyl Accident: Results of 15-years Follow-up Studies, June 4–8*, Kiev, Ukraine, pp. 11–12.

Chasnikov, IJ 1996, *Echo nuclear explosions*, Almaty.

Demina, EA, Demchenko, EN & Barylyak, IR 2009 'Character calibration curves cytogenetic dosimetry', *Herald Ukrainian partnership geneticists and breeders*, vol. 7, no. 2, pp. 184-190.

Gubitskaya, EG, Ahmatullina, NB, Vsevolodov, EB & Cherednychenko, OG 1999, 'The frequency of chromosome aberrations in residents of the Semipalatinsk region', *Genetics*, vol. 35, no. 5, pp. 838-841.

Gubitskaya, EG, Cherednychenko, OG, Baygushikova, GM & Ahmatullina, NB 2007 'Cytogenetic status of residents of Almaty region', *Bulletin of the Kazakh National University named Al-Farabi. Biology Series*, no. 2. pp. 86-90.

International Atomic Energy Agency 2001, *Cytogenetic Analysis for Radiation Dose Assessment*, Technical Reports Series no. 405, Vienna.

Kudryashov, YB 2004, *Radiation Biophysics (ionizing radiation)*, Physico-Mathematical Literature, Moskow.

Lloyd, DC, Edwards, AA & Leonard, A 1998, 'Frequences of chromosomal aberaions induced in human blood lymphocetes by low doses X-rays', *International Journal of Radiation Biology*, vol. 53, pp. 49-55.

Melnov, SB & Lebedeva TV 2004, 'Molecular genetic statuses of children and adolescents living in conditions of chronic low-dose exposure', *Radiation Biology. Radioecology*, vol. 44, no. 6, pp. 627-631.

Moorhead, PS, Nowell, PC, Mellman, WJ, Battips, DM & Hungerford DA 1960 'Chromosome preparations of leucocytes cultured from human peripheral blood', *Experimental Cell Research*, vol. 20, pp. 613-616.

Plohinsky, NA 1967, *Algoritmy biometrii*, State University, Moscow.

Richardson, D & Wing, S 1999, 'Radiation and Mortality of Workers at Oak Ridge National Laboratory: Positive Association for Doses Received at Older Ages', *Environ. Health Perspect*., vol. 107, August, p. 8.

Schmitz-Feuerhake, I, Boetticher, H & Dannheim B 1993, 'Strahlenbelastung durch Rцntgendiagnostik bei Leukдmie-fдllen in Sittensen im Landkreis Rotenburg' In: Lengfelder, E, Wendhausen, H, (Ed.), *Neue Bewertung des Strahlenrisikos: Niedrigdosis-strahlung und Gesundheit. Munchen*, MMV Medizin-Verlag, pp. 93-101.

Sevankaev, AV, Ankina, MA & Golub, EV 1995, 'The results of cytogenetic survey of residents of settlements adjacent to the Semipalatinsk nuclear test site', *Radiation Biology and Radioecology*, vol. 35, no. 5, pp. 596-606.

Sharipov, IK, Vishnevskaya, SS, & Vsevolodov, EB 1993, 'Comparative study of some ecologically unfavorable regions of Kazakhstan methods of environmental human cytogenetics', *Proceedings of the National Academy of Sciences of the Republic of Kazakhstan*, no. 3, pp. 63-66.

Snigireva, GP 2011, 'Biological dosimetry based on cytogenetic analysis', *Bulletin of the Russian Research Center of Radiology of the Ministry of Health of the Russian Federation*, no. 11, 2011, < http://vestnik.rncrr.ru/vestnik/v11/papers /snigir_v11.htm>.