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Analysis of the Database of Frequencies of Chromosomal Aberrations in the Blood of Residents of the Industrial Region

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***Abstract* – Cytogenetic biomarkers are an important characteristic of mutagenesis and, as practice has shown, carcinogenesis, they are often used in human populations biomonitoring. The uniqueness of genetic tests in the possibility of assessment before morphological, physiological and population changes, the relevance of prevention and early diagnosis of disease, reducing the risk of genotoxic effects, the likelihood of long-term medical and biological effects. The database contains the results of cytogenetic examination of more than 800 respondents from North Ossetia.In 10 years of research analyzed more than 100,000 metaphases. The frequency of aberrant metaphases in the basic control group was found to be 0.02494, statistically significantly different from the results obtained in the group with exposure – 0.03238, p<0.001. For the entire database for the study period (from 2002 to 2011) in the study region (RSO-A) the frequency of cells with chromosomal aberrations is 0.02815. There were no statistically significant differences in cytogenetic parameters between the sexes, the study of the effect of smoking on the frequency of chromosomal aberrations demonstrated ambiguous effects. The dependence of the frequencies and types of chromosomal aberrations on age is shown. Throughout the database, the frequency of aberrant metaphases in adults and children – 3,222 and 1,815, respectively, p<0,001.**

Key words – city residents, blood, chromosomal aberrations

I. INTRODUCTION

Nowadays the pathogenic significance of the mutagenesis is obvious. Grows number of data proving the increase in DNA damage in many diseases (diabetes, asthma, hepatitis, autoimmune diseases, helminthiasis, viral and bacterial infections, malignant tumors, atherosclerosis and others), justified the relationship between the induction and accumulation of somatic mutations and the occurrence of malignant tumors, between the increase in DNA double-strand breaks in maturing lymphocytes and a violation of adaptive immunity [1, 2].

The long-term consequences of genotoxic effects are diverse and depend on the cell target (in the induction of mutations in germ cells: hereditary diseases, infertility, malignant neoplasms, premature aging, immune disorders, congenital malformations, spontaneous abortions; in the induction in somatic cells: malignant neoplasms, aging, immune disorders; in the induction in embryonic cells: congenital malformations, spontaneous abortions and diseases due to somatic mosaic; inducting in mitochondria: mitochondrial myo-, encephalo- and neuropathy, aging, diabetes). Genotoxic events are considered as prognostic biomarkers of pathogenetic processes of various genesis, biomarkers of early effects [1, 3–5].

Numerous studies of the frequency of chromosomal aberrations in blood lymphocytes of the population of territories with developed industry demonstrated an increase in genotoxic events in residents exposed to anthropogenic press [1, 7–9], showed the prospects of using the test for chromosomal aberrations in the population risk assessment of oncological pathologies [10–12].

II. PROBLEM STATEMENT

Due to the tense environmental situation in Vladikavkaz notes the increase of general morbidity [13], the number of pathologies of pregnancy [14, 15] and the gastroduodenal area [16]. According to the presented materials Rosstat RSO-A observed deterioration in public health and an increase in the number of congenital malformations [17].

A wide range of environmental factors of different nature, is a threat to the health of living and future generations. Negative biological and medical consequences of the mutation process in humans are considered as the conjugation of mutagenesis, carcinogenesis and teratogenesis. The danger of a possible increase in the frequency of mutation is associated with the additive effect of different mutagens and the delay in their action.

Under the action of genotoxicants, the rate of mutation formation significantly exceeds the spontaneous frequency, which is 1–3 % of aberrant cells for chromosomal aberrations [18]. Analysis of all possible factors over a long period of time with the collection of data in a sufficiently large sample increases the reliability of the test to assess the frequency of chromosomal aberrations in human lymphocytes. What can be achieved in the construction of a database with the introduction of the results of cytogenetic examination [19]. What is devoted to the presented long-term study of the influence of various factors on the frequency of chromosomal aberrations in lymphocytes of residents of RSO-A, as well as

comparative analysis with available databases from other regions.

III. MATERIALS AND METHODS

The database includes the results of cytogenetic examination of more than 800 individuals, the total number of analyzed cells is more than 100,000 metaphases. The database includes data from 2002 to 2011, subject to a unified methodological approach to the cultivation, fixation, preparation and analysis of chromosomal drugs [20]. Blood taken from the ulnar vein (1 ml) was cultured in a mixture of RPMI 1640 (7.5 ml) and embryonic serum (1.5 ml), 0.01 mg of phytohemagglutin was added to stimulate cell division. Hypotonized with 0.55 % KCl solution for 15-20 minutes at a temperature of 37 ° C, fixed with a cooled mixture of ethyl alcohol and glacial acetic acid (3:1), 50 µl colchicine was added 2 hours before fixation. The cell suspension applied to the cooled wet slides was dried and stained with Giemsa dye. Chromosomal aberrations were analyzed without karyotyping, gaps were not taken into account. From each Respondent investigated from 100 to 300 cells, an average of 120 metaphases.

Anamnesis was collected by oral questioning and analysis of medical records. Respondents with chronic diseases who underwent x-ray diagnostic procedures or vaccination were excluded from the study less than 3 months before the collection of material. Respondents participated in the study voluntarily, were informed about the objectives, methods and results of the study.

IV. DISCUSSION OF RESULTS

Comparison of the results of the presented work on the entire database (the total number of certain types of aberrations to the number of analyzed metaphases) with the indicators of the 30-year survey of the inhabitants of the European part of the CIS [19] and Kemerovo region [7] showed compliance and difference in some indicators. The total frequency of aberrant metaphases and acentric fragments in the database is comparable with N.P. Bochkova. Comparison of the frequency of exchange aberrations revealed differences: the number of chromosomal type exchanges in general is higher in our database than in the comparison groups. The greatest difference was found for dicentric chromosomes, the frequency of symmetric exchanges are comparable with the "Kemerovo sample", the frequency of ring chromosomes and interstitial deletions with the "European".

A. Spontaneous and induced mutagenesis

The spontaneous regional level of cytogenetic effects was assessed by the frequency of chromosomal aberrations in the culture of blood lymphocytes of North Ossetians. Table 1 presents data on the results of cytogenetic analysis in the control sample, the average frequency of aberrant metaphases in the present work exceeded the data of Bochkov N.P. et al., but was lower than the results of studies in the Kemerovo region (2.33±0.08, 2.13±0.0009 and 2.86±0.26, respectively). It follows from the table that the main statistical indicators of the basic control group correspond to the results of studies in the compared regions, with the exception of exchange aberrations. Exchanges of chromatid and chromosomal types in the present work were more common in comparison with the inhabitants of the European part of the CIS (table. 1).

Comparison of databases in the control sample with the group with impacts showed differences in the mean frequencies of cells with chromosomal aberrations (table. 2 and 3, $p<0.001$). The result of the analysis of the entire database revealed lower frequencies of all types of chromosomal aberrations in the control group.

TABLE I. BASIC STATISTICAL PARAMETERS OF CYTOGENETIC PARAMETERS FOR THE BASIC CONTROL GROUP

Individual indicators (%)	**This study**		**Bochkov N. P. et al., 2001**		**Druzhinin V. G., 2003**	
	Middle	***Standard error***	***Middle***	***Standard error***	***Middle***	***Standard error***
Aberrant metaphase	2.33	0.081080	2.13	0.000846	2.86	0.255004
Chromatid fragments	1.38	0.000694	1.41	0.000659	2.11	0.218931
Chromatid exchanges	0.15	0.000203	0.05	0.000123	0.04	0.022097
Paired fragments	0.52	0.000377	0.62	0.000516	0.89	0.134435
Exchanges of chromosomal type	0.46	0.000397	0.13	0.000184	0.08	0.012256

TABLE II. THE TOTAL FREQUENCY OF CHROMOSOMAL ABERRATIONS IN THE BLOOD OF MEN AND WOMEN OF THE BASIC CONTROL GROUP

Individual indicators	**This study**		**Bochkov N. P. et al., 2001**		**Druzhinin V. G., 2003**	
	Middle (%)	***Standard error***	***Middle (%)***	***Standard error***	***Middle (%)***	***Standard error***
Men	107		108		27	
Aberrant metaphases	2.224	0.16488	2.088	0.00126	3.593	0.52096
Chromatid fragments	1.209	0.00111	1.540	0.00114	2.667	0.48920
Chromatid exchanges	0.1955	0.00050	0.0692	0.00026	0.037	0.03704
Paired fragments	0.4955	0.00069	0.4656	0.00051	1.148	0.23084
Chromosomal exchanges	0.4862	0.00063	0.1244	0.00031	0	0
Women	284		99		83	
Aberrant metaphases	2.365	0.09374	2.049	0.00149	2.615	0.28931
Chromatid fragments	1.545	0.00145	1.397	0.00108	1.928	0.24108
Chromatid exchanges	0.1396	0.00023	0.0502	0.00019	0.036	0.02681
Paired fragments	0.5267	0.00045	0.5676	0.00082	0.807	0.16123

Chromosomal exchanges	0.4469	0.00049	0.1174	0.00026	0.0108	0.01619

B. Effect of sex

The main statistical indicators of cytogenetic examination for women and men in the cohort of basic control of this study are presented in table 2. There were no significant differences ($p>0.05$) in cytogenetic parameters among 107 men and 284 women of the basic control group.

Comparison of the databases of the control group of our work with the results of the survey of the inhabitants of the European part of the CIS [19] and Kemerovo region [7] showed comparable data, with the exception of exchange-type aberrations (table. 2).

For all analyzed cytogenetic parameters between the sexes for the basic control group. There were no statistically significant differences in the database of exposed respondents consisting of 205 men and 237 women (tab. 3, $p>0.05$).

It follows from the table that in the present study, there is no difference between affected men and women in the main cytogenetic indicators, except for chromatid exchanges. Comparison of cohort databases with the impact of the presented study with residents of the European part of the CIS [19] and Kemerovo region [7] revealed the above trends to an increased level of metabolic aberrations in respondents from North Ossetia (table. 1, 2 and 3). The analysis of male and female respondents ' databases of this work revealed a tendency to increase the frequency of chromatid exchanges in the cohort of men, most pronounced in persons with exposure. Interestingly, a study of databases of residents of the European part of the CIS [19] showed a similar trend: the frequency of chromatid exchanges in men in the group with external exposure is more than 3 times higher than for women (table. 3).

TABLE III. THE TOTAL FREQUENCY OF CHROMOSOMAL ABERRATIONS IN THE BLOOD OF AFFECTED MEN AND WOMEN

Individual indicators	**This study**		**Bochkov N. P. et al., 2001**	
	Middle (%)	***Standard error***	***Middle (%)***	***Standard error***
Men	205		467	
Aberrant metaphases	2,954	0,162473	2,939	0,001987
Chromatid fragments	1,569	0,000878	2,009	0,00188
Chromatid exchanges	0,308	0,000548	0,188	0,000389
Paired fragments	0,782	0,001032	0,849	0,000712
Chromosomal exchanges	0,445	0,000573	0,185	0,000189
Women	237		393	
Aberrant metaphases	2,844	0,007385	2,610	0,000903
Chromatid fragments	1,524	0,000768	1,764	0,000739
Chromatid exchanges	0,139	0,000263	0,051	0,0000748
Paired fragments	0,676	0,000601	0,691	0,000411
Chromosomal exchanges	0,642	0,000644	0,147	0,000145

C. Age dependence

Throughout the database, the mean age in the adult cohort was 32±0.50, in the group of children – 13.4±0.15, in the basic control group of adults – 30±0.62, children – 14±0.26. In the base cohort of exposed adults, the mean age was 35±0.76, among children-13±0.18. A group of respondents with an average age of 41±1.85 associated with harmful substances was analyzed separately.

Correlation analysis throughout the database revealed a high level of dependence of cytogenetic parameters in the blood of North Ossetians and their age ($p<0.001$), for the frequencies of cells with chromosomal aberrations, chromosomal exchanges and dicentric chromosomes Pearson correlation coefficient was equal to $r = 0.429$, $r=0.303$, $r=0.322$, respectively.

The dependence of the frequencies of cells with chromosomal aberrations and the age of the examined general basic control group was equal to $r = 0.465$ at $p<0.001$. For a cohort of the respondents connected with harmful substances the correlation dependence of age and frequencies of symmetric exchanges – $r=0,337$, $p<0,001$ is shown. For the group of patients the correlation between age and cell frequencies with chromosomal aberrations, chromatid fragments, chromosomal exchanges and dicentric chromosomes was revealed – $r=0.443$, $r=0.376$, $r=0.368$ and $r=0.395$, respectively, at $p<0.001$.

As can be seen from table 4, the division of the surveyed respondents of the general basic control group into adults and children showed differences in all cytogenetic indicators. Analysis of the database revealed an increase in the frequency of cytogenetic parameters in adult groups compared with children ($p<0.05$).

TABLE IV. THE TOTAL FREQUENCY OF CHROMOSOMAL ABERRATIONS IN THE BLOOD OF CHILDREN AND ADULTS IN THE CONTROL GROUP

indicator	**adult**	**children**	**p<**
Aberrant metaphases	0.02738	0.01193	0.001
Chromatid fragments	0.01726	0.00729	0.001
Chromatid exchanges	0.00197	0.00044	0.05
Paired fragments	0.00596	0.00312	0.01
Exchanges of chromosomal type	0.00569	0.00164	0.001

D. Smoking addiction

The study of the dependence of cytogenetic parameters and Smoking factor demonstrated statistically significant differences in the whole database (for the frequencies of cells with chromosomal aberrations, single fragments and chromosomal exchanges).

However, when you select from the database a group of healthy men, dependence on the frequencies of chromosomal aberrations and addiction to smoking not detected, $p>0.05$.

V. CONCLUSION

The results of cytogenetic examination of RSO-a residents are generally comparable with the data of databases of other long-term studies [19, 7], with some increase in the frequency of metabolic aberrations. In total, the database contains studies of frequency dicentrics of chromosomes per 100 cells 0,004569 against 0,000860 and 0,000925 respectively, with overall comparable total frequency of symmetrical exchanges, "Kemerovo" population, but not with the results of the survey of inhabitants of the European part of the CIS – 0,00043 to 0,00038 and 0,0002 respectively. The difference between the frequencies of chromosomal exchanges in the control group of Saransk from the presented data was less pronounced-0.002 and 0.005 per 100 cells, with comparable frequencies of chromatid exchanges-0.0015 and 0.0027. The increase in the

frequency of chromosomal exchanges in the Altai Mountains in the work [21] is explained by the natural radioactive background associated with increased radon emanation. The studied region belongs to the area with potentially increased radon hazard, which seems promising for further research.

The obtained results of cell frequencies with chromosomal aberrations in the general basic control group of children from North Ossetia demonstrated compliance with similar data in other regions [22–24]. The frequency of chromosomal exchanges in the group of exposed children of North Ossetia was comparable with the results of the study of the exposed group of children of the Kemerovo region – 0.26 and 0.22 respectively [25].

A number of factors could cause differences in the results of cytogenetic analysis of North Ossetians. As is known, the effects of natural (ultraviolet, radon, etc.) can be enhanced under the influence of anthropogenic substances. In particular, this seems to be related to the general trend towards an increase in the number of chromosomal exchanges over time [26].

Models of formation of exchange-type aberrations suggest their formation, mainly due to the violation of the effectiveness of the reparation system. Heavy metals can influence the repair of double fractures [27] and enhance the effects of UV radiation and other mutagens [28]. The dependence of the formation of dicentric chromosomes and telomerase activity, telomere loss.

The ability of some chemicals, in particular sulfur dioxide and cadmium, which are among the main pollutants of Vladikavkaz, to increase the number of chromosomal aberrations, dicentric and ring chromosomes is known [29].

Thus, the analysis of the database of chromosomal aberrations in the blood of North Ossetians revealed the regional level of chromosomal mutations. The study of the spectrum of chromosomal aberrations indicates the impact of a complex of mutagenic factors: of chemical and physical nature. A study of the entire database in the blood of North Ossetians showed a high level of dependence on age, but not on gender.

The analysis confirmed the feasibility of using the database for a comparative study of quantitative and qualitative cytogenetic indicators, the reliability of metaphase analysis in human lymphocytes as a reliable system for genetic monitoring, evaluation of chromosomal mutagenesis.

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