RESIDENTS OF INDUSTRIAL REGIONS: LONG TERM DYNAMICS OF SPONTANEOUS MUTAGENESIS

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Abstract: The studies shows results of the 10-years research related to the frequency of chromosome aberrations in peripheral blood lymphocytes of North Ossetia inhabitants. The study of cytogenetic indexes in the blood of healthy individuals showed an increase in the average frequency of chromosome aberrations as time passed. The long-term cytogenetic study of North Ossetia residents' blood showed the growth of medium frequency of aberrant metaphases from 2.07 ± 0.37 to 3.04 ± 0.16 in the group of adults and from 0.5 ± 0.25 to 1.9 ± 0.2 in the groups of children. The most significant increase among all studied cytogenetic indexes in groups of North Ossetia adults and children was in 2009 due to accidental release at one of the metallurgical enterprises in the capital city. All studied groups were characterized by prevailed acentric fragments among chromosome disorders, with an increase of all types of aberrations as time passed. There was a growth in the proportion of chromosome aberrations (adults had an increase of double fragments from 0.20 ± 0.11 to 0.60 ± 0.15 and chromosome exchanges from 0.27 ± 0.15 to 0.64 ± 0.13, children: from 0.13 ± 0.13 to 0.26 ± 0.12 and from 0 to 0.26 ± 0.20 respectively). Sex and smoking factors had no significant effect on the studied samples during the observation period. The 10-year (2002-2011) dynamics analysis of cytogenetic disorders has shown an intensification of mutagenic effects on residents of the republic, which were the most severe after accidental releases in 2009. The results of cytogenetic studies suggest that a mutagenic impact of chemical and radioactive nature takes place.

Keywords: chromosome aberrations, mutagenesis, anthropogenic factors, environment, heavy metals

INTRODUCTION

There is a considerable amount of facts that clearly show the negative impact of ecopollutants on genome and the need to study population genetic processes in the areas of industrial pollution [1–3]. A variety of anthropogenic factors modifying the structure of the genetic material and understanding of etiopathogenetic role of genome genotoxic lesions has gone beyond the boundaries of the hereditary and oncology pathologies [4, 5]. Genotoxic lesions are considered to be the most common factor in the development of CVD (cardiovascular diseases), neurodegenerative diseases and aging on the whole [6]. The article describes reproductive disorders under the influence of chemical mutagens and reprotoxicants. The use of complex cytogenetic markers and hygienic criteria in assessing chromosomal abnormalities in the presence of risk factors contributes to examining the cause-effect system analysis "environment – health" [7].

This examination is particularly relevant for some regions including the Republic of North Ossetia – Alania (North Ossetia), and in particular, the industrial center of the republic Vladikavkaz, where people have been living under high anthropogenic load for decades. Long-term activity of metallurgical enterprises has led to extremely high levels of soil contamination of residential areas, including playgrounds. The polluted soil of residential areas dozens of times exceed permissible levels of lead, cadmium, copper and zinc. If we focus on the German standards, almost the entire territory of the Industrial District exceeds the norm of 400 mg/kg. Contaminated soil of other urban area is classified as high risk category. The level of ecopollutants in some distant suburban areas remains dangerous. The long-term storage of chemical and radioactive waste at the factory premises in the center of Vladikavkaz is the most alarming fact. Orographic and aero climatic conditions degrade pollutants scattering process in the atmosphere and contribute to high levels of air pollution [8-10]. The situation is made worse by accidental releases of metallurgical enterprises, when a significant part of the population is suffering under their influence as it was in 2009.

The purpose of this study is to determine the dynamics of the frequency of cells with chromosomal aberrations in the culture of leucocytes of North Ossetia residents for the 10 year period.

OBJECTS AND METHODS OF STUDY

Spontaneous mutagenesis was assessed in the blood of healthy residents of North Ossetia from 2002 up to 2011. Blood was taken for cytogenetic examination from: 285 adults (the median age is 30.00 ± 0.62) and 106 children (the median age is

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13.90 ± 0.27), who had no viral diseases for over 3 months, had no X-ray examination within 6 months and were not exposed professionally to harmful production. 60 men and 225 women were examined among adults, 47 boys and 59 girls – among children. Metaphase chromosomes of lymphocytes in peripheral blood cultured under conditions *in vitro* were analyzed, according to standard procedures [11]. From 100 to 300 metaphases for each donor were analyzed, taking into account the percentage of cells with chromosomal aberrations, the number of single fragments, chromatid exchanges, double fragments and chromosomal exchanges. Dicentric chromosome, centric and acentric ring chromosomes, symmetrical exchanges (abnormal chromosomes) were considered to be chromosomal exchanges. Achromatic gaps in the number of aberrations were not included and were taken into account separately [11]. 48900 metaphase plates were analyzed in total.

Statistical processing of factual material was carried out with the help of programs "Microsoft Office Excel 2007" and "Statistica 10". A comparison was carried out using the non-parametric method of Mann-Whitney U-criterion.

RESULTS AND DISCUSSION

Tables 1 and 3 present the average frequency of aberrant metaphases in culture of examined residents' lymphocytes. Statistically significant differences are found when comparing the frequency of cells with chromosomal aberrations for different years of observation. Analysis of the dynamics of cytogenetic effects in the blood of examined adults and children showed an increase in the frequency of metaphases with aberrations as time passed. The minimum frequency of aberrant metaphases indicators in the blood of adult population occurred in 2005, 2006 and 2007, the maximum was in 2009, when the figure exceeded the upper limit of accepted population standards for healthy individuals of the Russian population [12]. Cytogenetic analysis of the children's population showed a similar trend: the minimum frequency was up to 2009, the increase in the frequency of aberrant cells was observed in 2010 and 2011. While analyzing frequencies of individual types of aberrations it was found out that frequency of chromatid and chromosome aberrations in groups of surveyed residents also changed periodically with the same position of highs and lows. However, the most pronounced increase over time was marked for chromosomal aberrations: double fragments and chromosomal exchanges (Tables 2 and 4).

The survey results showed no pronounced gender effect on the studied cytogenetic characteristics of North Ossetia residents. There was a tendency to an increase in the average frequency of chromosome aberrations in groups of men, but the differences were significant only if all adults for the whole 10-year period of the study ($p = 0.013$) were included in the analysis. Assessing the impact of the sexual factor in the formation and / or accumulation of various types of chromosomal aberrations in all examined groups also showed no significant differences. In the group of adults there was a tendency to an increase in a mid-frequency of chromosomal aberrations in the blood of men compared to women ($p = 0.0012$). In the group of children there were no significant differences on the frequency of chromosomal aberrations and the formation of various types of aberrations due to gender effect.

Smoking analysis as a modifying factor showed no pronounced effect on cytogenetic indicators of surveyed residents during the observation period.

A comparison of the frequency of chromosomal aberrations in the blood of the surveyed residents of North Ossetia and literature data shows that the average frequency of aberrant metaphases in our region is higher than similar data of other authors [13–15]. According to [12], the average value of the frequency of aberrant metaphases in the group of basic control from the number of inhabitants of the European part of Russia and CIS countries is 2.13 ± 0.09%, which is lower than in our region (2.75 ± 0.09). The findings of the work are comparable with the results of research in industrial regions [2]. Comparison of cytogenetic research results presented in this paper with the research cited above has shown that the greatest difference is achieved through exchange aberrations.

Certain differences in the overall frequency and type of chromosome aberrations given in the present study and the results of other authors may be associated with the presence and specificity of climatic conditions of the region under study. Perhaps the observed cytogenetic effects may also be influenced by general planetary environmental changes, since this study was carried out at a later date than the period of studies in the cited works. In [16] it is shown that the control group and the group connected with the production and environmental harm have the most significant increase in chromosomal exchanges over time. The changing magnetic field of the Earth is able to lead to a significant variation in the average level of spontaneous chromosome aberrations.

The results obtained in the present study confirm the fact that the spontaneous level of chromosomal aberrations in human lymphocytes is not rigidly fixed and it depends on external factors. Analysis of the results of long-term study of spontaneous mutagenesis in North Ossetia has showed that cytogenetic parameters of blood is not constant in time and they changed upward during the survey period from 2002 to 2011. Thus, the results of long-term monitoring of cytogenetic effects in a group of adults and children have shown steady reproducing clastogen influence with a clear tendency to increase. Cytogenetic biomarkers had the maximum values in 2009 and remained high in 2010 and 2011.

The lack of significant effect of smoking on blood cytogenetic indicators in surveyed people may be due to the formation of the studied group, which consisted of a large number of children and women, who could not confess in it even if they had this bad habit. In addition smoking may be a weak factor modification under the conditions of anthropogenic load.

Table 1. The dynamics of the frequency of chromosomal aberrations in the blood cells of the adult population in North Ossetia

Year	Number of examined	Number of metaphase	Average age (X± S_X)	Metaphases with aberrations (M±m), %			Gap (%)	The frequency of cells containing more than 1 CA (%)
			total	total	male	female	total	total
2002–2003	15	1500	24.00 ± 1.77	2.07 ± 0.37	2.75 ± 0.82	1.82 ± 0.40	0.40 ± 0.21	0
2004	26	2950	24.00 ± 1.00	2.18 ± 0.27	3.41 ± 0.61	1.63 ± 0.28	0	0
2005	9	900	25.00 ± 2.10	1.67 ± 0.43	0	1.67 ± 0.43	0	0
2006	22	2200	31.00 ± 3.07	1.59 ± 0.27	1.50 ± 0.61	1.61 ± 0.30	0.14 ± 0.10	0
2007	8	800	30.00 ± 4.39	1.75 ± 0.46	0	1.75 ± 0.46	1.30 ± 0.82	0
2008	8	800	35.00 ± 5.64	2.50 ± 0.55	2.50 ± 0.78	2.50 ± 0.78	0.13 ± 0.13	0
2009	51	5100	28.00 ± 0.89	3.26 ± 0.25	3.50 ± 0.92	3.23 ± 0.26	3.12 ± 0.39	0.14 ± 0.06
2010	58	8650	33.00 ± 1.64	3.06 ± 0.19	3.40 ± 0.36	2.95 ± 0.22	1.15 ± 0.26	0.17 ± 0.09
2011	88	12230	31.00 ± 1.02	3.04 ± 0.16	3.42 ± 0.32	2.92 ± 0.18	0.84 ± 0.22	0.16 ± 0.07
2002–2011	285	35130	30.00 ± 0.62	2.75 ± 0.09	3.19 ± 0.19*	2.63 ± 0.10	1.09 ± 0.13	0.12 ± 0.03

Note. A comparison according to Mann-Whitney U-criterion with women * p = 0.013.

Table 2. The dynamics of types of chromosomal aberrations in the blood cells of the adult population in North Ossetia

Year	frequency of aberrations (%)					
	Single fragments	Chromatid exchanges	Double fragments	Chromosomal exchanges		
	total	total	total	total	men	women
2002–2003	1.60 ± 0.21	0	0.20 ± 0.11	0.27 ± 0.15	0.25 ± 0.25	0.27 ± 0.20
2004	1.22 ± 0.22	0.14 ± 0.07	0.54 ± 0.16	0.31 ± 0.14	0.67 ± 0.31	0.15 ± 0.12
2005	1.00 ± 0.47	0.22 ± 0.15	0.22 ± 0.15	0.22 ± 0.24	0	0.22 ± 0.24
2006	0.82 ± 0.20	0	0.64 ± 0.19	0.14 ± 0.10	0	0.17 ± 0.10
2007	0.75 ± 0.31	0.25 ± 0.25	0.63 ± 0.42	0.13 ± 0.13	0	0.13 ± 0.13
2008	1.63 ± 0.57	0	0.50 ± 0.19	0.38 ± 0.18	0.50 ± 0.29	0.25 ± 0.25
2009	1.53 ± 0.15	0.26 ± 0.08	0.80 ± 0.13	0.86 ± 0.17	1.25 ± 0.48	0.83 ± 0.18
2010	1.67 ± 0.24	0.09 ± 0.05	0.74 ± 0.17	0.84 ± 0.20	1.14 ± 0.35	0.72 ± 0.24
2011	1.85 ± 0.24	0.36 ± 0.10	0.60 ± 0.15	0.64 ± 0.13	0.66 ± 0.19	0.63 ± 0.16
2002–2011	1.58 ± 0.11	0.21 ± 0.04	0.63 ± 0.07	0.62 ± 0.07	0.78 ± 0.14*	0.57 ± 0.08

Note. A comparison according to Mann-Whitney U-criterion with women * p = 0.012.

Table 3. Dynamics of frequency of chromosomal aberrations in the blood cells of children from North Ossetia

Year	Number of examined	Average age (X ± S_X)	Number of analyzed metaphase	Metaphases with aberrations (M ± m), %			Gaps (%)	The frequency of cells containing more than 1 CA (%)
				Total	Male	Female	Total	Total
2005	8	12.0 ± 1.06	800	0.50 ± 0.25	0.50 ± 0.35	0.50 ± 0.35	2.13 ± 0.40	0
2006	10	13.3 ± 1.12	1000	1.0 ± 0.32	0.67 ± 0.33	1.50 ± 0.61	0.4 ± 0.27	0
2007	18	12.8 ± 0.55	1800	1.33 ± 0.27	1.0 ± 0.30	1.86 ± 0.51	0.67 ± 0.31	0
2008	38	14.4 ± 0.36	3800	0.63 ± 0.13	0.71 ± 0.20	0.57 ± 0.16	0.95 ± 0.24	0
2010	8	14.6 ± 1.02	1330	2.66 ± 0.44	3.65 ± 0.77	2.33 ± 0.56	1.50 ± 0.66	0.30 ± 0.38
2011	24	14.5 ± 0.60	4690	1.85 ± 0.20	1.54 ± 0.36	1.97 ± 0.24	0.51 ± 0.27	0.06 ± 0.09

Table 4. Dynamics of the types of chromosomal aberrations in the blood cells of children from North Ossetia

Year	Frequency of aberrations (%)			
	Single fragments	Chromatid exchanges	Double fragments	Chromosomal exchanges
2005	0.25 ± 0.25	0.13 ± 0.13	0.13 ± 0.13	0
2006	0.5 ± 0.27	0	0.4 ± 0.27	0.1 ± 0.11
2007	0.56 ± 0.17	0	0.39 ± 0.12	0.39 ± 0.18
2008	0.34 ± 0.10	0	0.24 ± 0.09	0.05 ± 0.04
2010	1.88 ± 0.90	0.30 ± 0.27	0.68 ± 0.67	0.68 ± 0.67
2011	1.22 ± 0.36	0.06 ± 0.07	0.26 ± 0.12	0.26 ± 0.20

CONCLUSION

1. Cytogenetic studies in the blood of North Ossetia residents show the intensification of mutagenesis in this population, and suggest the increase of anthropogenic pressure factors on the genome of the examined population for the period of study.
2. The obtained results on the dynamics of cytogenetic indexes in blood of healthy and sick residents of North Ossetia with a peak in 2009 show significant effects of anthropogenic factors. These data are consistent with the findings of some researchers who consider that the main source of increased accumulation of ecopollutants in this region is the metallurgical industry [8–10] and underline the need to strengthen disease prevention measures.

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