

*Short reports***CHROMOSOMAL ABERRATIONS AND CONGENITAL MALFORMATION IN THE INHABITANTS OF INDUSTRIAL CITIES OF WESTERN SIBERIA**

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Background

The importance of the test for chromosomal aberrations for forecasting diseases of a human being has not been studied completely yet. Five epidemiologic studies, from Northern Europe, Italy, the Czech Republic, Taiwan and Central Europe have reported an association between high frequency of chromosomal aberrations and increased cancer risk [1-5]. Chromosomal aberrations which children have with various types of congenital malformation are actively investigated [6]. However interrelation between accumulation of chromosomal aberrations in the healthy donors and frequency of congenital anomaly occurrence in a population are still not studied completely. Similar researches get a special topicality in the populations living in the conditions of constant genetically-toxic pressure of the environmental factors.

Novokuznetsk and Kemerovo (Western Siberia, Russia) are of great interest for epidemiological and genetic researches. The environment of Novokuznetsk is influenced first of all by the metallurgical enterprises. Kemerovo is the center of chemical and coal-mining industries of Kuzbass. The total emissions of polluting substances in these cities are the highest in Kuzbass. According to this, the aim of the research is to study the frequency of chromosomal infringements in the populations and frequency of congenital developmental anomalies of children in these cities.

Materials and methods

Work is carried out on samples of blood of voluntary unrelated donors, the inhabitants of Kemerovo and Novokuznetsk. Venous blood was taken from each subject using heparinized tubes. Duplicate lymphocyte cultures were set up adding 0,5 ml of whole blood to 5 ml of RPMI medium, supplemented with 20% inactivated fetal calf serum, antibiotics and glutamine. Lymphocytes were stimulated by 1% phytohaemagglutinin. For the evaluation of chromosomal aberrations, the culture were incubated for 72 h at 37°C. A final concentration of 0,4 µg/ml of colchicine was added 2 h before fixation. Slide preparations and staining of chromosomes were carried out by standard cytogenetic methods [7]. Four categories of chromo-

somal aberrations were evaluated, i.e., chromatid and chromosome breaks, and chromatid and chromosome exchanges [8, 9]. All cells carrying breaks or exchanges were counted as aberrant cells (AB.C.). Gaps were not scored as aberrations. 100-500 well-spread metaphases has been scored.

Revealing children with congenital anomaly was carried out within the period from 1994 to 2005. The material for this research was taken from the data of the medical documentation of maternity hospitals, laboratory-diagnostic service of the zonal prenatal center, polyclinics, hospitals and prozection. All form of congenital anomaly of newborns, deadborn and foetuses were taken into account and placed in common computer database according to classification of WHO. The data on frequency of the congenital anomaly in Kemerovo were used for the comparative analysis [10]. Nonparametric methods were chosen for the data did not follow a normal distribution. The Mann-Whitney rank sum U-test for comparison of two samples were used.

Results and Discussion

The results concerning the frequency of chromosomal aberrations in group from industrial city western Siberian are presented in Table 1.

For comparison our last given the data about a background level of chromosomal aberrations in group of inhabitants of ecologically clean villages in the Kemerovo region [11].

From data Table 1 it follows, that the basic cytogenetic parameter – AB.C. is significantly increased in groups of Kemerovo and Novokuznetsk in comparison with basic control group ($p < 0,05$). Value of separate categories of aberrations: chromatid and chromosome breaks of inhabitants of industrial city also were significantly higher in comparison with basic control group. High values of cytogenetic damages specify, that a human population in industrial city is constantly influenced by genetically-toxic factors.

It is revealing, that the average value of metaphases with aberrations in group of the inhabitants of Kemerovo ($3,27 \pm 0,14$ %) coincides practically with the number of metaphases with aberrations, registered in the inhabitants of Novokuznetsk ($3,29 \pm 0,28$ %). So close there are also frequencies of separate types of aberrations in compared groups. The analysis of possible influence of such factors as a gender, age and addiction to smoking, made in the group of inhabitants of industrial cities revealed, that none of the listed factors had caused significant modification of frequencies of the studied parameters. This fact, and also the selection of the staff of the studied groups, except people with chronic pathologies, vaccination, allow to conclude, that the main cause of the increase of the frequency of chromosomal aberrations in the inhabitants of Kemerovo and Novokuznetsk is the influence of factors of the city environment.

Table 1. Chromosomal aberrations in groups of Kemerovo, Novokuznezk and basic control group

Group	No. of cells scored	A.B.C.(%).	breaks		exchanges	
			chromatid	chromosome	chromatid	chromosome
Kemerovo	27700	3,27 ± 0,14 ^{a*}	2,31 ± 0,12*	1,01 ± 0,08*	0,03 ±	0,07 ±
Novokuznezk	10100	3,29 ± 0,28*	2,21 ± 0,26*	1,09 ± 0,13*	0,01	0,02
Basic control group	11200	2,86 ± 0,26	2,08 ± 0,22	0,89 ± 0,14	0,02 ±	0,05 ±
					0,01	0,02
					0,04 ±	0,08 ±
					0,02	0,03

a - Mean ± SE

*-p<0,05

Congenital anomaly

The average frequency of congenital anomaly in Novokuznetsk was 23,1 ‰, and in Kemerovo - 17,6 ‰. The received results have appeared quite comparable to data of other cities of Russia and Europe [12, 13]. The analysis of dynamics of frequency congenital anomaly has shown significant fluctuations of average annual values. The maximal values have been marked in Novokuznetsk in 1996 (42,10 per 1000 newborns)

and in 2000 (33,10 ‰), to Kemerovo in 2000 (21,2 per 1000 newborns). The frequency of congenital anomaly among the inhabitants of rural areas of Kemerovo region is considerably lower and it is 2,21‰ in Yashkino area and 12,28‰ in Tashtagol area (Tolochko T.A. - the private messages, non published data).

Table 2. Frequency congenital malformation among newborns and fetuses of Novokuznetsk and Kemerovo (‰)

Anomaly Subgroup	Novokuznezk	Kemerovo	EUROCAT (1994-2004)
Anencephalus	0,44	0,37	0,03 – 0,64
Spina bifida	0,41	0,69	0,28 – 1,72
Encephalocele	0	0,36	0,02 – 0,29
Hydrocephaly	1,19	1,20	0,06 – 1,75
Microtia/Anotia	0	0,25	0,005 – 0,08
Cleft palate	0,55	0,52	0,29 – 1,37
Cleft lip with or without palate	0,03	0,30	0,46 – 1,68
Transposition of great vessels	0,08	-	0,12 – 0,52
Hypoplastic left heart	0,11	-	0,07 – 0,51
Oesophageal atresia	0,19	0,29	0,065 – 0,39
Ano-rectal atresia	0,19	0,18	0,13 – 0,78
Hypospadias	0,53	2,84	0,18 – 2,61
Congenital constriction bands/ amniotic band	0,14	1,32	0,002 – 0,15
Polydactyly	0,06	1,06	0,19 – 1,16
Diaphragmatic hernia	0,22	1,06	0,07 – 0,54
Bilateral renal agenesis including Potter Syndrome	0,39	0,29	0,05 – 0,57
Omphalocele	0,22	0,07	0,08 – 0,62
Gastroschisis	0,33	0,15	0,045 – 0,62
Down syndrom	0,44	0,69	0,80 – 3,60
Multiple congenital anomaly	1,41	1,64	0,90 – 2,40

In general structure of congenital anomaly in Novokuznetsk the defects of the development of the nervous system - 22,4 ‰; multiple congenital anomaly - 19,33 ‰, urinary anomaly - 16,94 ‰; musculo-

skeletal anomaly - 15,28 ‰ were predominated. In Kemerovo congenital heart disease (35 ‰) prevailed. Hypospadias took the second place (16 ‰), the third - multiple congenital anomaly (9 ‰). In Novokuznetsk

boys had congenital anomaly (61,58 %) more often, than girls (38,42 %). And predominance of male sex formed due to such forms, as hypospadias (boys have - 13,6 %, girls – don't have) and hydrocephaly (boys have - 16 %, girls have 7,7 %). In Kemerovo boys had congenital anomaly more often. The boys` average annual frequency of congenital anomaly made up - 23,89 cases per 1000 newborns, and at girls` average annual frequency of congenital anomaly - 14,58 cases per 1000 newborns. It was registered, that in Kemerovo boys had congenital defects incompatible to life (multiple congenital anomaly, hydrocephaly, spina bifida) more frequently. The comparison of frequency of occurrence of separate forms of congenital anomaly in Novokuznetsk, Kemerovo and other cities of Russia and Europe has shown the absence of significant differences for the majority of the given types of defects (table 2.) Plural developmental anomalies

(frequency - 1,41 %) and hydrocephaly (1,19 %) were registered more often in the structure of this congenital anomaly. In the formation of the congenital anomaly a special role is played by the accumulation of mutations in the sexual cells, leading to the appearance of fetuses with abnormal genotypes. The analysis of genotypes of abortuses in Novokuznetsk wich had been done from 2001-2005 showed that abnormal genotypes were met in 22 % of cases (62 from 282 analyses). The spectrum of the registered abnormal genotypes is presented in Figure 1. Among all the abnormal genotypes additional autosome: Down syndrome (+21), syndrome Patau (+13), Edwards's syndrome (+18) were met more often (46,7 %). Also the frequency of polyploidy (69, XXV; 69, XXX; 92, XXYY) was high (29,7 %). The prevalence of the other kinds of abnormal genotypes was not high.

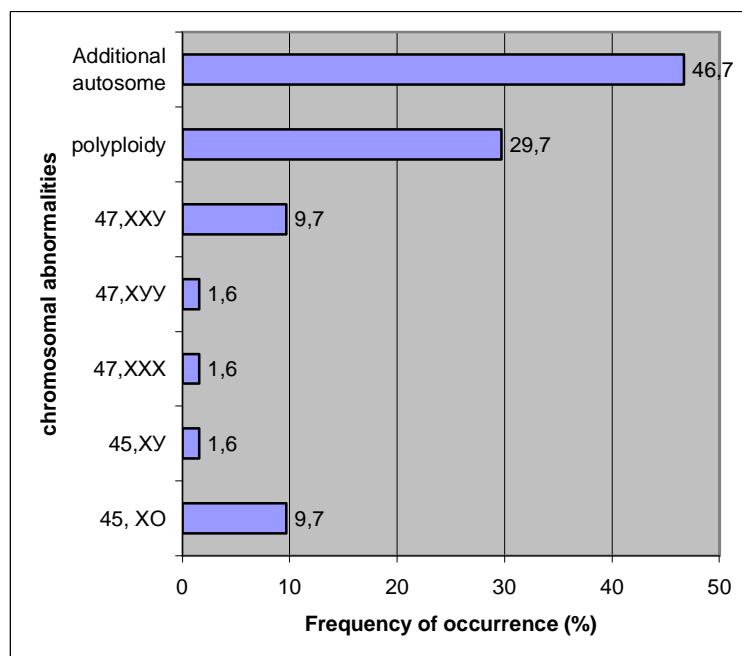


Figure 1. Anomal genotypes in abortus of Novokuznetsk

As a result of the work wich has been done it is possible to draw up the following conclusions:

1. The frequency of chromosomal infringements in the inhabitants of the industrial cities is significantly higher, than the control donors have living in a country side.

2. Among the genetic damages causing the congenital anomalies and spontaneous abortions, additional autosome and polyploidy are taking the leading part.

3. The Epidemiological analysis has shown the increase of frequency of congenital developmental anomalies of children and fetuses in population of Kemerovo and Novokuznetsk in comparison with the inhabitants of rural areas of Kuzbass.

The received results testify to a coordination of ecologically caused processes of teratogenesis and mutagenesis in the inhabitants of industrial cities. In our opinion, it gives the basis to consider a high level of chromosomal aberrations as one of factors of population risk of congenital anomaly formation.

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SEARCH FOR ANTIOXIDANT THERAPY MEANS BY KINETIC METHODS

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At the present time the hypothesis about biomembranes' permeability disturbance due to the lipid peroxidation intensity change has been adopted as the molecular mechanism of many pathologies' development. A special attention is paid to the mechanism of free radical aging, adaptation to the silent epidemic, labour activity regime and also cancerous diseases development [2].

For prevention and treatment of various physiological states and pathologies an antioxidant therapy is widely used [1, 4]. It is evident that in the

progress of the therapy is possible on the basis of effective antioxidant testing methods development. All the known antioxidants are divided on their physico-chemical properties into hydrocarbon soluble ones and their water soluble derivatives. Kinetic approaches are developed for water insoluble antioxidants testing. For this purpose the chain interruption velocity constants in the inhibitor (K_i) or the induction time of ethyl benzene, cumene, styrene, decalin oxidation in the presence of phenols and aromatic amines [3, 5] are used. For lipid oxidation processes inhibition we offered a methyl linoleate model. For this purpose the methyl linoleate oxidation kinetics in the solution of benzene chloride at $60^\circ \pm 0,2^\circ \text{C}$ at the presence of the initiator depending on the concentration of 2,6-ditertbutyl-4-methyl phenol (ionol), 2, 5, 7, 8-tetra methyl-2-(4, 8, 12-trimethyl tridecyl)-6-oxichromane (α -tocopherol). The participation of ionol is shown in the chain interruption reaction only. The chain interruption velocity constant in ionol equal to $2,6 \pm 0,4 \cdot 10^4 \text{ l} \cdot \text{mol}^{-1} \cdot \text{c}^{-1}$ is defined by kinetic research methods. Similar calculations of K_i for α -tocopherol showed their changes depending on concentration and complicated mechanism of its action. For such inhibitors' efficiency estimation the method of mathematical treatment of kinetic curves (KC) by means of their approximation by functions and the following differentiation. As a result of this approach five kinetic parameters, which allow the inhibition efficiency to be evaluated on the initial velocity and inhibition time. On the maximum velocity, acceleration and acceleration time finish the mechanism of the inhibitor's action is evaluated.

However, the biomembranes' lipids conditioning the level of free-radical oxidation, the biomembranes' permeability change and the development of pathologies represent water-emulsion systems including amino acids, proteins and enzymes. That is why bio-adequate models are needed to search the means of antioxidant therapy. To select such a model the micelle formation of ethyl oleate has been investigated. For this purpose the micelle formation in two- and three-component systems has been studied first of all: ethyl oleate – water; ethyl oleate – water – emulsifier. On the minimal value of the critical concentration of micelle formation the composition of water-lipid substrate including ethyl oleate and water in the ratio 1:3 (in volume) and cetyl trimethyl ammonium bromide as emulsifier in the concentration of $(1-3) \cdot 10^{-3} \text{ mol/l}$ was defined.

Further, the kinetics of the water-lipid substrate oxidation at the presence of salts CuCl_2 , FeCl_2 , FeCl_3 , CoCl_2 , NiCl_2 depending on the concentration was studied. It was shown that the most active catalyst is cupric chloride, and the activity of other salts falls off in the series $\text{Cu}^{2+} > \text{Fe}^{2+} > \text{Fe}^{3+} > \text{Co}^{2+} > \text{Ni}^{2+}$. In further investigations cuprum cations in the concentration of $(1-3) \cdot 10^{-3} \text{ mol/l}$ were chosen as a catalyst.

For the estimation of antioxidant testing possibility in the water-lipid substrate the influence of ionol