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Associations of Acrocentric Chromosomes and Unstable Chromosome Aberrations in Newborns from Different Ecological Zones

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5220 metaphase plates of 187 infants from different ecological districts of Precarpathia have been analyzed with the aim to determine the frequency and spectrum of chromosomal aberrations, associations of acrocentric chromosomes in newborns. It was determined that the total number of chromosomal aberrations in newborns from districts of ecological comfort was 2, 0 and 2, 3 times lower as compared to newborns from zones with chemical and radiation contamination. The frequency of cells with associations of acrocentric chromosomes was 7,8 % higher in children from zones of chemical contamination and 9,9 % from zones of radiation pollution as compared to the children from districts of ecological comfort. The obtained results of the frequency of associations of acrocentric chromosomes correlated with the frequency indices of chromosomal aberrations (r correlated from 0,68 to 0,84), that proved the negative influence of ecological living conditions on immunogenetic status and adaptive human capabilities.

Keyword: Chromosomal Aberrations, Associations Of Acrocentric Chromosomes, Newborns, Ecological Zones.

1. Introduction

The study of chromosomal and genomic mutations in human cytogenetics is gaining more importance due to the increased anthropogenic pollution, their occurrence is associated with the influence of environmental factors such as radiation and chemicals [1,2]. Progress made in the study of the human genome allowed to decipher the sequence of DNA nucleotides, set the number of genes and their structure. However, it remains questionable to determine the mechanisms of the implementation of genetic information, and their regulation [3]. That's why modern investigations of the state of chromatin in interphase nuclei, nucleolar apparatus and nucleus forming regions of acrocentric chromosomes are dedicated to this particular branch of functional genomics [1,4].

Traditional works on cytogenetics proved that short arms of acrocentric chromosomes are represented by heterochromatic regions [5]. They are formed by three sections: 1) short segment that adheres directly to the centromere; 2) strand – secondary constriction of the short arm; 3) small compact body, satellite that contains telomere of the short arm of acrocentric chromosome. Associations of acrocentric chromosomes AAC are related to the attraction of constrictions (regions of satellite strands forming the nucleus). Pairing of these chromosomes happens due to the homologous loci of heterochromatic zones, thus stimulating the association of acrocentrics. Considering that nucleolar organizers with ribosomal genes 28 s and 18 s RNA are localized in heterochromatic

regions the changes in AAC may correlate with the metabolic activity of the cell.

Many scientific works are devoted to the investigation of human AAC patterns [6, 7, 8-9]. The study of the number and grouping of associated chromosomes and associations of acrocentrics with other specific regions of chromosomes, showed that the changes in AAC frequency leads to the dysfunction of the immunological state of the organism and correlates with the frequency of chromosomal aberrations.

Cytogenetic control is the most informative and reliable method used to study the hereditary apparatus of the population living in ecologically unfavourable regions. Though it does not fully reflect the mutational variability, the method takes into account the level of chromosomal abnormalities in human somatic cells. Cytogenetic study of the level of chromosomal aberrations in different populations is important in terms of determining the differences in the intensity of mutagenic activity, the possibility of differentiation of chemical and radiation effects.

To assess whether the occurrence of cytogenetic abnormalities is associated with their induction or is an indicator of overall destabilization of the genetic material it is necessary to thoroughly study both the individual and intra group variability by complex of cytogenetic characteristics. Taking into account the above mentioned, the aim of the research work was to establish the frequency and spectrum of chromosomal aberrations, associations of acrocentric chromosomes in infants from different ecological regions of Precarpathia.

2. Materials and methods

Distribution of the population in Ivano-Frankivsk region into ecological zones was performed on the basis of the ecological certificate of the region and investigation data of the ecological state of Ukraine[10]. The umbilical blood of 187 newborns from different districts of Ivano-Frankivsk region was used as the test material for the investigation. (Table 1).

Table 1: Distribution of the newborns in Ivano-Frankivsk region according to the ecological living conditions

S. No	Ecological zones	Number of newborns
1	Zone of ecological comfort	80
2	Zone of preferential chemical pollution	57
3	Zone of preferential radiation contamination	50

Cytogenetic analysis of newborns was based on the study of karyotype of peripheral blood lymphocytes. The sampling was performed with the use of sterile syringes laced with 0.01 ml of heparin and placed into the cool-bag (t=5-7 °) for 1-2 h and brought to an accredited genetic laboratory of the state higher educational establishment "Ivano-Frankivsk National Medical University." Culturing of lymphocytes and slides preparation were conducted using Gibco® "PB MAX" reagents according to the methodical recommendations approved by the Ministry of Health Care of Ukraine[6]. Metaphase plates were stained using the GTG method. The derived specimens were investigated by means of optical-electronic complex "Metaskan - 2".

Metaphase plates with good spreads of chromosomes were analyzed. At least 30 metaphase plates were studied from each infant. In addition to identifying the associations of acrocentric chromosomes (AAC) the number of chromosomal aberrations (CA) was also investigated. The presence of AAC was evaluated according to the corresponding criteria[9]. Specificity of acrocentric chromosomes' arrangement in the metaphase was also taken into consideration, including the absence of chromosomal overlapping; short arms of acrocentrics were oriented at each other and the distance between them (not including satellites) didn't exceed the size of the long arm of the G-group chromosome, larger distance was taken as association if acrocentrics were connected by the visible strands or were located on the same chromosome axis. Index of association was calculated as the correlation of the number of

cells with associations to the total number of the analyzed cells, on equivalent to 100%. The average number of AAC in one cell and the average number of chromosomes in one association were also estimated. The results of the investigation were statistically processed with help of "Statistica for Microsoft V. 6.0" programme (Stat Soft Inc; 2001).

3. Results of the investigation and their discussion

The frequency of cells with associations of acrocentric chromosomes was 7,8% higher in children from zones of chemical contamination and 9,9% from zones of radiation pollution as compared to the children from districts of ecological comfort. The average number of AAC within one cell was 1, 16 times lower in newborns from zones of ecological comfort as compared to the same index in newborns from zones of preferential radiation contamination and 1, 21 times lower in comparison with the newborns from chemically contaminated zones. The number of associated chromosomes in one cell was the lowest in newborns from zones of ecological comfort, respectively this index was 1, 64 and 1, 60 times higher in infants from zones of chemical and radiation contamination.

It should be noted that the number of AAC was uneven in peripheral blood lymphocytes stimulated by phytohaemagglutinin (PHA) mitogen prior to proliferation. The average portion of associations per cell was equal to 0,89. At the same time the variability in number of associated groups of acrocentrics within the cell and the number of acrocentric chromosomes in the association was noticed. Rather important fact is that the total number of associated chromosomes in the cell characterizes their associative capability better than other indices of associations[9]. In some metaphases acrocentric chromosomes formed associations not only with each other but also with the precentromeric regions of the chromosomes 1 and 2, where the secondary constriction is located. Other investigators observed the same pattern revealing the presence of associations of short arms of satellite chromosomes with the specific regions of chromosomes 1, 2, 9, 16 [9, 11]. This feature may be associated with the DNA functions of heterochromatic regions and argue for conjugative hypothesis of AAC formation. Considerable differences between homologous and non-homologous chromosomes based on this feature cause AAC variability. Chromosomes with longer satellite strands (nucleolar constriction) join association with greater frequency than chromosomes with short satellite strands. Chromosomes with short nucleolar constrictions (short arm deletion) having no secondary constriction rarely take part in associations[6]. Hence, it follows that morphological changes of the satellite strand are interrelated with the activity of nucleolar organizer – number and activity of the ribosomal genes localized in it. Their deletion or inactivation is accompanied by the decreased ability of acrocentric chromosome to form associations, as confirmed by in situ hybridization and silver impregnation methods[12]. In addition, ectopic pairing of heterochromatic regions in metaphase, except for AAC, is manifested in closer location of homologous chromosomes, specific grouping of some non-homologous chromosomes, AAC with secondary constrictions of chromosomes 1, 2, 16, and 9 with telomeres of other chromosomes.

The next stage of the investigation involved the study of associative capability of chromosomes depending on their group specificity. Within every group the highest capability to form associations was noticed in chromosomes 21(22,11%), 13 (21,62%) and 14 (20,96%), whereas the lowest ability was observed in chromosomes 15 (18,04%) and 22 (17,27%). It should be noted that the number of associated chromosomes of D group prevailed over their number in G group. While analyzing the characteristics of forming paired associations among D group chromosomes no significant differences were found. Predominantly chromosomes 13 and 21 were forming associations within chromosomes of D-G groups. Other chromosomes formed associations with the same frequency, though chromosome 21 associated with D-group chromosomes more often than chromosome 22.

In combinations of G-group chromosomes greater tendency to associate was noticed in chromosome 21 as compared to chromosome 22. The latter may be explained by the fact that satellite associations are formed in interphase nucleus as a result of long-term conjugation of homologous loci of heterochromatic regions in satellite strands, that are transmitted through mitosis and are registered on metaphase plates[4]. Presumably heterochromatic regions of the chromosome 21 have more homologous loci with the D-group chromosomes, that was why they combined more often.

Table 2: Frequency of AAC in peripheral blood lymphocytes of newborns from Ivano-Frankivsk region, M±m

Residency area	Frequency of cells with AAC, %	Average number of AAC within one cell, %	Number of associated chromosomes within one cell, %
Zone of ecological comfort	78,2±0,22	1,32±0,16	1,74±0,22
Zone of preferential chemical pollution	86,0±0,16	1,54±0,32	2,86±0,18*
Zone of preferential radiation contamination	88,1±0,21	1,60±0,13	2,78±0,15*

Note.* The likelihood of differences in indices as compared to the zone of ecological comfort is $p < 0,05$

The next stage of the investigation was concerned with the study of the spectrum of chromosomal aberrations in order to determine the differences in intensity of mutagenic load and the possibility to differentiate between chemical and radiation exposures. The total number of CA (chromosomal aberrations) in infants from zones of ecological comfort was 2, 0 and 2, 3 times lower than in infants from zones of chemical and radiation contamination (table 3).

The specific weight of aberrations of chromosomal and chromatid types in children from districts of ecological comfort made up respectively 24,2 and 75,8%, – from districts of ecological contamination - 21,0 and 79,0% , zones of radiation pollution – 30,2 and 69,8% (see the Figure). These findings are close to the results of other studies of the relationship between aberrations of chromatid and chromosomal types as 77 and 23% [5].

Table 3: Frequency and percentage rating of CA types in peripheral blood lymphocytes of newborns in Ivano-Frankivsk region

Types of aberrations	Number of aberrations		
	Zone of ecological comfort	Zone of preferential chemical pollution	Zone of preferential radiation contamination
	Per 100 cells	Per 100 cells	Per 100 cells
Chromosomal type aberrations	0,30±0,08	0,54±0,12	1,42±0,21*
paired fragments	0,23±0,06	0,48±0,05	0,84±0,18*
abnormal monocentrics	0,04±0,21	0,04±0,002	0,23±0,13*
dicentrics	0	0	0,25±0,02
ring chromosomes	0,03±0,13	0,02±0,003	0,10±0,05*
chromatid –type aberrations	0,78±0,24	1,62±0,008	1,10±0,25
single fragments	0,710±0,003	1,40±0,15*	1,05±0,42
chromatid interchange	0,07±0,001	0,22±0,002*	0,05±0,04
Total number of aberrations	1,08	2,16	2,52

Note.* The likelihood of differences in indices as compared to the zone of ecological comfort is $p < 0,05$.

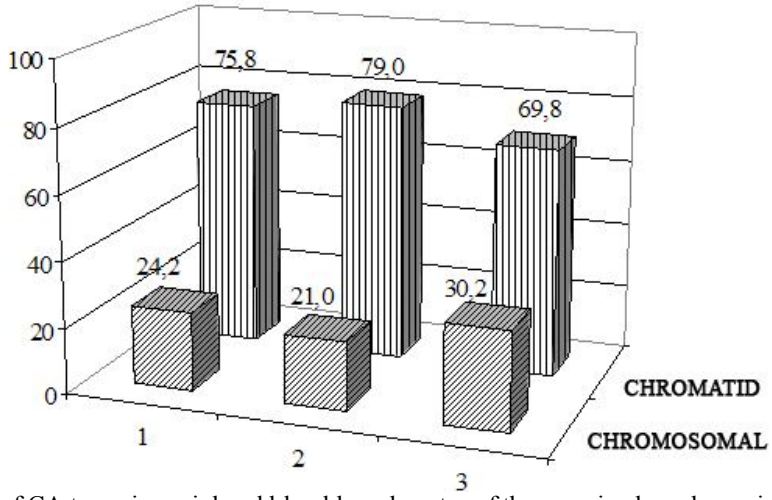

Fig 1: Percentage rating of CA types in peripheral blood lymphocytes of the examined newborns in Ivano-Frankivsk region:
1- zone of ecological comfort;
2- zone of preferential chemical pollution;
3- zone of preferential radiation contamination.

The analysis of CA types showed that aberrations of chromatid type prevailed in both residents of the contaminated zones and those from zone of ecological comfort (on the average of up to 79%) [7]. Deletions with single or paired fragments were the most common chromosomal aberrations in all the investigated infants. Considering that the frequency of unstable chromosomal aberrations of dicentrics and ring chromosomes[8] is used to evaluate the effect of small doses of ionizing radiation on the human organism, we have carried out a detailed investigation of these chromosomal abnormalities. Dicentrics were found only in newborns from mainly radiation contaminated zones. In these newborns the frequency of ring chromosomes also was 5,0 times higher than in newborns from zone of ecological comfort and 3,3 times higher than in those from chemically polluted areas. The number of chromatid-type aberrations was also significantly higher in the latter group, which argues for chemical mutagenesis. The frequency of single fragments 2,0 and 1,3 times prevailed the one in newborns from zones of ecological comfort and radiation contaminated zones. Our findings are consistent with the results obtained by other scientists regarding the absence of difference between males and females according to the overall frequency of CA and certain types of chromosomal abnormalities[5].

Consequently, presence of mutagenic effects, as stated according to the frequency and spectrum of CA in peripheral blood lymphocytes of newborns, indicated the hereditary hazards of anthropogenic contamination. The qualitative difference in CA spectrum allows considering these cytogenetic effects as indicator of environmental pollution. The obtained results of the frequency of associations of acrocentric chromosomes correlated with the frequency indices of chromosomal aberrations (r correlated from 0,68 to 0,84), that proved the negative influence of ecological living conditions on immunogenetic status and adaptive human capabilities.

4. Conclusions

1. The frequency of cells with associations of acrocentric chromosomes was 7,8% higher in children from zones of chemical contamination and 9,9% from zones of radiation pollution as compared to the children from districts of ecological comfort.
2. The number of associated chromosomes in one cell was the lowest in newborns from zones of ecological comfort, respectively this index was 1,64 and 1,60 times higher in

infants from zones of chemical and radiation contamination.

3. The specific weight of aberrations of chromosomal and chromatid types in children from districts of ecological comfort made up respectively 24,2 and 75,8%, from districts of ecological contamination - 21,0 and 79,0% , zones of radiation pollution – 30,2 and 69,8%.
4. The total number of CA in infants from zones of ecological comfort was 2,0 and 2,3 times lower than in infants from zones of chemical and radiation contamination.
5. Single dicentrics were found in newborns from mainly radiation contaminated zones; the frequency of ring chromosomes also was 5,0 times higher than in newborns from zone of ecological comfort and 3,3 times higher than in those from chemically polluted areas.
6. The number of chromatid-type aberrations was also significantly higher in the group of newborns from zones of chemical contamination, the frequency of single fragments 2,0 and 1,3 times prevailed the one in newborns from zones of ecological comfort and radiation contaminated zones.

The prospect for further research work in this field is to explore the frequency of chromosomal aberrations and associations of acrocentric chromosomes at various pathological conditions of newborns.

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