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Features of the Hereditary Apparatus of Long Livers Living in Different Carpathian Ecological Regions.

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Summary. The analysis of the frequency and spectrum of chromosomal aberrations, acrocentric chromosomes' associations, micronuclei of 264 long livers and 218 persons (control group), depending on environmental conditions has been done. Analysis of the frequency of micronuclei showed the increasing of instability of all patients' hereditary apparatus, correlating with the intensity of pollution. The average group frequency of chromosomal aberrations of long livers, depended on environmental conditions was found. Therefore, for people living in positive ecological regions, the figure was (2.85 ± 0.25), in the zone of satisfactory environmental pollution - (2.98 ± 0.24) and in the regions of bad environmental conditions- (3.05 ± 0.15 %). That was respectively 1.5, 1.7 and 1.8 ($p < 0.05$) fold reduction (control group). At the same time, individual variability of chromosomal aberrations' frequency among long livers ranged from 0.2 to 5%. Studying the rate of acrocentric chromosomes' associations, the significant age and sex changes of all examined long livers were not found. The results of frequency of acrocentric chromosomes' associations correlated with indicators of the frequency of chromosomal aberrations (ranged from 0.73 to 0.9), having confirmed the negative impact of environmental conditions on the immunogenetic status and human adaptive capacity.

Keyword: Long Livers, Karyotype, Chromosomal Aberrations, Acrocentric Chromosomes' Associations.

1. Introduction

Nowadays, the problem of complex action of environmental factors on living organisms is in the focus of modern medical genetics research. Different kinds of organisms have roughly the same set of reactions to the effects of mutagens[1]. The increasing of mutagenic load to the level capable to double the frequency of mutations in a human being can lead to significant changes in the body system. This is especially true for old people, since aging enlarges the intensity of the mutation process through degradation of reparative systems, increasing sensitivity to the action of negative factors, resulting in a more rapid decreasing of vitality.

Aging inheres most of all living organisms and takes all levels of living being organization: from molecular to organismal genetics[1]. According to the modern concepts of cell theory of aging, the most important factors are the accumulation of cellular disorders, weakening mechanisms of surviving and cell and tissues' recovering. The most common among them there are spontaneous mutations in somatic cells, including structural aberrations of chromosomes. In multivariate aging the latter play significant role[2]. Studying various cytogenetic and molecular genetic disorders in most cases the frequency of their occurrence increasing with age was found. Chromosomal aberrations (CA)[3], micronuclei[4], the losing of telomeric repeats[5], mutations in

glycophorin locus[6], DNA's breakings[7], and so on. The above mentioned research confirms the important role of genetic factors in the development of such a multifactorial trait, as life expectancy. The searching of not very expensive informative tests for screening of hereditary apparatus of population is topical medical task. Among them micronucleus test (MN) takes the important place[8]. It is founded in the buccal epithelial cells of the oral mucosa (ECOM) (mucous membrane), peripheral blood lymphocytes, hepatocytes and other cells. The appearance of micronuclei shows how deep is hereditary apparatus affected, their number correlates with the frequency of chromosomal aberrations and genomic mutations[8]. The advantage of this test is that it is possible to analyze the agents in any time available for the researcher and do that repeatedly. They have an unlimited shelf life and require less material costs compared to other tests[9].

In previous studies we have found that in Ivano-Frankivsk region there are 4566 long livers at the age of 90 or above. The comparative analysis of the number of people living in different parts of the region showed their superiority in Kalush and Kolomyia region, respectively 568 and 503 long livers[10]. The least number of long livers was registered in Verkhovyna (108) and Bohorodchany (189) regions. In the area of positive environmental conditions 1,5 fold increase of long livers was revealed compared with 1.9 fold increase of long livers in the region of the satisfactory and bad environmental conditions.

The logical continuation of the research has been the study of the Carpathian long livers' chromosomal apparatus.

2. The Purpose of the Study

The research of the frequency and spectrum of chromosomal aberrations, acrocentric chromosomes' associations of the Carpathian long livers depending on environmental conditions.

3. Materials and Methods

The distribution of the Green Zone was based on the results of our previous studies of ecological passports and reports of environmental field studies[11,12]. The study group consisted of long livers, the control group was composed of the persons at the age of 30-60, not having long livers in their family tree. Among all examined people there were those living in regions of average environmental conditions (Table 1). The epithelial cells of the middle layer (ECOM) served as the material for the study of MN. To analyze the structural changes in the chromosome apparatus for MN nearly 500 cells of every person were revised. We determined the frequency of cells with micronuclei, taking into account the number and structure of MN.

Table 1: Distribution of the population of different Carpathian ecological regions

Groups (persons)	Ecological regions		
	Positive environment, n=137	Satisfactory environment, n=196	Bad environment, n=149
Study group, 264	75	107	82
Control group, 218	62	89	67

The carrying out of the cytogenetic analysis of long livers' was based on the study of the karyotype of peripheral blood lymphocytes. Collecting material was taken by sterile syringes in which 0.01 ml of heparin was added then it was placed in the bag-thermos (t= 5-7 °C) and within 1-2 hours it was delivered to an accredited genetic laboratory of SHEE "Ivano-Frankivsk National Medical University."

The cultivation of lymphocytes and doing of chromosomes' preparations was performed using reagents "PB MAX" firm "Gibco" under guidelines approved by the Ministry of Health of Ukraine[13]. Painting of metaphase plates was performed by GTG - method. The study of made

preparations was performed on optical-electronic complex "Metaskan - 2".

The metaphase plates with a good spread of chromosomes were analyzed. At least 30 metaphase plates of every person were analyzed. In addition to identifying of chromosomal aberrations the number of acrocentric chromosomes' associations were studied (AAC). The presence of AAC was evaluated according to the criteria done by D.C. Frolov and his colleagues. (1993)[14]. The specificity of the acrocentric chromosomes' location in metaphase was taken into account: no overlapping of chromosomes, acrocentrics' short arm of are oriented to each other and the distance between them without satellites (satellites) does not exceed the size of the long arm of chromosome (group G), bigger distance was taken as an association if acrocentrics were connected together by visible threads or lying on one chromosome axis. Associative index was calculated as the ratio of cells with associations to the total number of cells analyzed in terms of 100%. The average number of AAC in a cell and the average number of chromosomes in a single association was determined too.

4. Results and Discussion.

Primarily in all examined persons the micronuclei, indirectly indicating the frequency of chromosomal aberrations and being as indicator of the environmental situation were studied. It was found that the size of micronuclei in buccal epithelial cells was equal to 1,3 microns. They were located in the cytoplasm near the nucleus of primary cells having membrane and being filled with chromatin (Fig. 1). The density of the latter was lower or not higher than this basic chromatin core. Micronuclei sometimes tightly adjacent to the main core, but in this case they were surrounded by their nuclear membrane.

In all people being under study MN frequency did not depend on age (Table 2). No significant differences between the number of the MN of long livers and the control group were found. The reliable changes in the number of micronuclei depending on the region of living were proved.

The men and women living in bad ecological region, the number of MN was increased compared to the other studied groups, especially the residents of positive ecological regions. Moreover, preparations of the patients living in positive ecological regions were recorded with MN's changes. Sometimes two micronuclei per cell were met. The nuclei of epithelial cells of the majority of people (study group) were vacuolated (Fig. 2). However, being similar to micronuclei, cytoplasmic vacuoles with a dense basophilic colloid were found.

Table 2: Micronucleus rate of epithelial cells of the oral mucosa (population of different Carpathian regions) (M ± m)

Groups	Positive environment		Satisfactory environment		Bad environment	
	Women	Men	Women	Men	Women	Men
Study group	0, 63±0, 36	0, 98±0, 45°	1, 42±0, 16	1, 51±0, 15	2, 05±0, 25*	1, 68±0, 13
Control group	0, 72±0, 16	0, 81±0, 38	1, 32±0, 22	1, 37±0, 21	2, 03±0, 12*	2, 39±0, 11*

Notes: * p <0.001 – compared to positive environment
° p <0.001 – the difference between women and men

They differ from these micronuclei homogeneous by internal structure. Pyknotic cores are often recorded. Our studies confirm the literature data that the cause of micronuclei's increasing in epitheliocytes of persons, living in areas with high technological activity, is a violation of cell division and / or integrity of chromosomes[15]. Significant difference of micronuclei's number of different sexes was found in those living in positive ecological regions. The next stage of work was to study the cytogenetic characteristics of peripheral blood lymphocytes being considered as informative and reliable method for the assessment of the hereditary apparatus of man. They are karyotype changes which may indicate structural abnormalities of chromotype caused by endogenous and exogenous factors, the condition of adaptive capacity of the organism and so on.

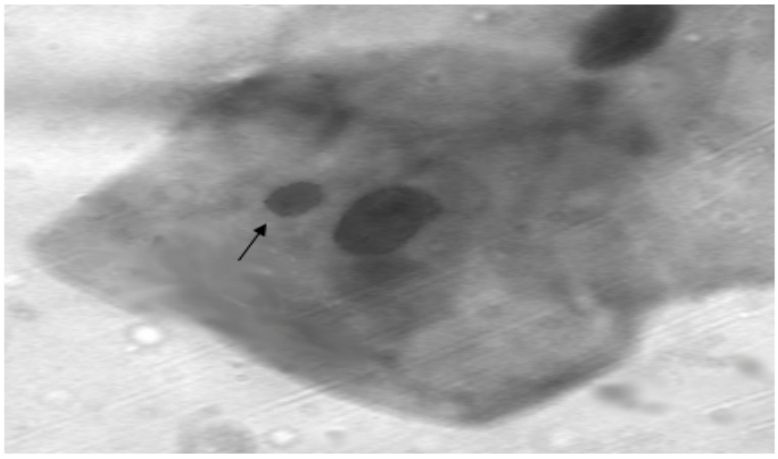

Fig 1: Microkernel (↑) in epitheliocytes of oral mucosa of long liver K., living in bad ecological region Painted by Himza. Collection: 15 x 40.

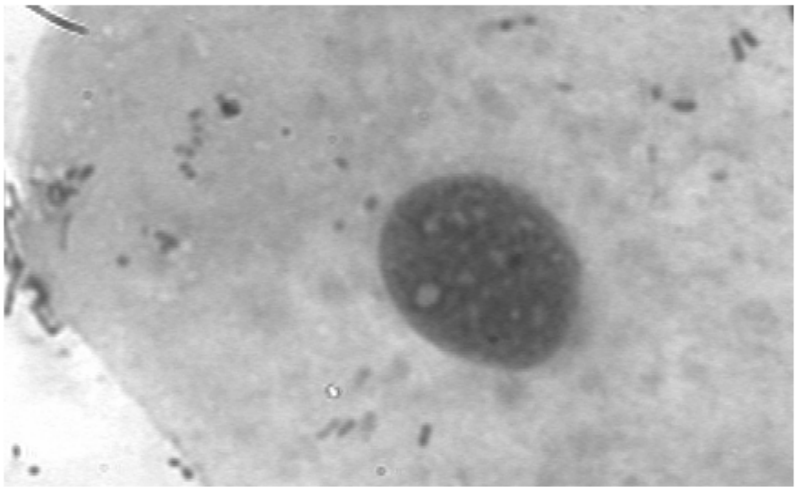

Fig 2: Epitheliocytes' vacuolised core in epitheliocytes of oral mucosa of long liver V., living in bad ecological region. Painted by Himza. Collection: 15 x 100.

Studying the metaphase plates of people (Ivano-Frankivsk region) we found that the average group frequency of CA of long livers depends on environmental conditions. Therefore, for people living in positive ecological regions, the figure was (2.85±0.25), in satisfactory ecological region (2.98±0.24) and in bad ecological region (3.05±0.15 %), compared to control group we have 1.5, 1.7 and 1.8- ($p<0.05$) fold reduction frequency of CA respectively. However, individual variability of CA frequency among long livers was determined (from 0.2 to 5%). In long livers living in bad environmental conditions, the maximum frequency of HA reached to 5%, in the control group - 7%. Individual level fluctuations of HA were caused by, we believe complex influence of radiation and chemical mutagens. Quantitative indicators' fluctuations of chromosomal apparatus were caused by both variations of reparative processes, and genetically determined mechanisms of xenobiotics' biotransformation (mutagens)[16]. These results do not object multiple cytogenetic studies having been conducted by N.P. Bochkov and his colleagues[17]. They found no changes in the total number of aberrant metaphases depending on gender and age of a person. However, in people above 80 years, the number of fragments increases and the number of chromatid exchanges decreases. This is

confirmed by academic papers by A.M. Chebotarev[18]. The investigation proves that at young age reparatory processes run more effective course.

According to one of the famous hypothesis of aging, age-related changes are mostly caused by the mutagen factors of different nature leading to numerous body damage of the genetic apparatus[1]. This is consistent with the obtained data about bigger CA incidence of the population living in bad ecological regions. HA of people (study group) had a wide range of markers of negative influence of external factors. Single fragments, chromatid bridges showed the advantages of mutagenic chemical nature background. Markers of radiation mutagenesis, dicentrics, paired fragments, breaks) were more common in people living in bad ecological region (Fig. 3). Some metaphase plates of long livers have gaps in 1 and 22 chromosomes.

Unstable chromosomal aberrations (dicentrics, rings, fragments) lead to cell death, stable (translocations, insertions) as are known accompany ontogenesis and may affect the vital functions of cells[2]. Such genetic instability in somatic cells predicts profound effect on gene expression, leading to genetic and epigenetic changes and further to degeneration and atrophy of cells, tissues. The latter is a cause of aging in general.

Studying the rate of adaptive capacity and immunogenetic status of the body – no associations of acrocentric chromosomes, significant age and sex changes in all long livers were found. Certain fluctuations in the number of AAC depending on the region of living were revealed (Table 3).

Thus, the longevity in the bad ecological regions had 2,11 average AAC fold increasing compared to positive ecological regions' data.

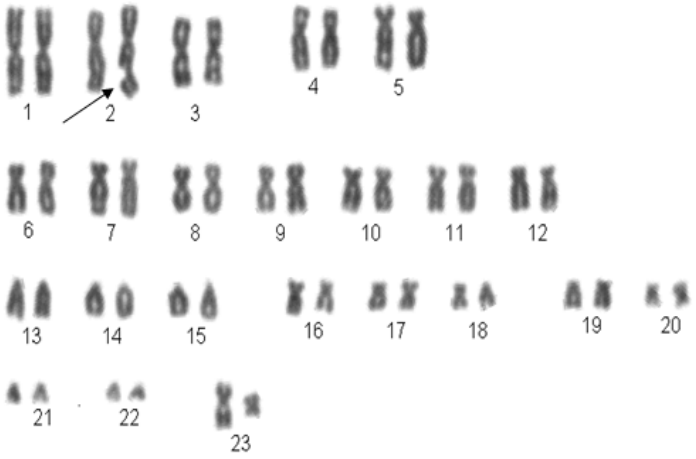

Fig3: Karyotype of long liver V., living in bad ecological region, with chromosomal aberration - a break in chromosome 2(↑) Painted by Himza. Collection: Approx. 15, vol. 100.

Table 3. The frequency of acrocentric chromosomes' association in peripheral blood lymphocytes of long livers (Ivano-Frankivsk region), M ± m

Region	Groups	Frequency of cells with AAH, %	Average number of AAH per cell, %	Number of associated chromosomes per cell, %
Positive environment	study	85, 0±0, 12*	1, 50±0, 18	1, 80±0, 31
	control	91, 0±0, 24	1, 64±0, 41	2, 1±0, 32
Satisfactory environment	study	91, 0±0, 33	1, 51±0, 19	3, 2±0, 15
	control	92, 2±0, 41	1, 67±0, 13	3, 3±0, 27
Bad environment	study	93, 0±0, 17	1, 66±0, 17*	3, 8±0, 18
	control	95, 3±0, 26	1, 97±0, 74	4, 5±0, 34

Note. * - Differences compared to control group (p <0, 05).

The frequency of cells with acrocentric chromosomes' association in long livers (positive ecological regions) was 1.07 ($p>0.05$) fold decreasing compared to control group and 1.09 fold decreasing in a group of long livers (bad ecological region).

While analyzing all possible associations between pairs of chromosomes were taken into account. The most frequent associations were found between 15, 16 and 22 chromosomes (Fig. 4).

Average number of AAC in one cell varied in long livers, depending on environmental conditions, from (1.50±0.18) to (1.66±0.17) and was lower than that in patients of control group of (1.64±0.41) to (1.97±0.74).

Thus, the biggest frequency of AAC and the number of associations in the same cell were recorded in a control group of people living in bad ecological regions.

Number of associated chromosomes in each cell was variable and was in long livers (1.80±0.31) positive ecological region and (3.2±0.15), (3.8±0.18%) satisfactory and bad ecological regions respectively. In patients of control group data were higher in 1.17, 1.03 and 1.18 times, respectively. Often AAC with two and three associated chromosomes, sometimes with three, four and five ones were met. Average values of AAH depended on environmental conditions.

The results of AAC frequency were correlated with CA frequency data (r ranged from 0.73 to 0.9), which confirmed the negative impact of environmental conditions on the immunogenetic status and human adaptive capacity.

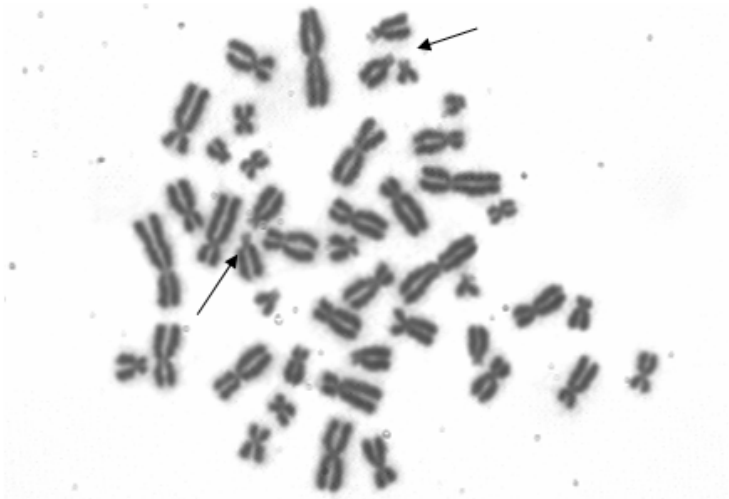

Fig 4: Association of acrocentric chromosomes in metaphase plate peripheral blood P. long liver, living in bad ecological region. Painted by Himza. Collection: Approx. 15, vol. 100.

4. Conclusions

1. The increase of micronuclei in ECOM of people living in regions with high technological capacity has been registered
2. The lower rates of CA and AAC frequency in long livers compared to people having no genetic predisposition to longevity were found.
3. The dependence of immunogenetic status of the organism (study group) on the environmental conditions has been proved. The most significant feature was the growing rate of CA frequency and AAC data in patients living in bad ecological regions.

5. Prospects for Further Research

The frequency of polymorphic variants of gene deletions of detoxication of xenobiotics (GSTM1 and GSTT1) in long livers helping in adaptation to the effects of various exogenous factors must be determined.

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