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Research Article

Frequency and Spectrum of Chromosomal Aberrations, Acrocentric Chromosome Associations Among Long Livers with Arterial Hypertension and Osteoarthritis Residing in the Carpathian Region

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Abstract
The frequency and spectrum of chromosomal aberrations, acrocentric chromosome associations among 264 long livers with arterial hypertension and osteoarthritis residing in the Carpathian region were analyzed. The obtained results were compared between patients with arterial hypertension and osteoarthritis, patients with arterial hypertension only, patients with osteoarthritis only and healthy individuals. The indices of the average frequency of chromosomal aberrations in all long livers was as follows: (2.82±0.27) in long livers with arterial hypertension and osteoarthritis and (2.17±0.47) in healthy individuals. In long livers with arterial hypertension and those with osteoarthritis, the frequency of chromosomal aberrations was 1.38 times higher compared to the control group (healthy long livers). The frequency of chromosomal abnormalities in long livers with arterial hypertension and those with osteoarthritis was (2.93±0.09) and (2.64±0.37), respectively.
At the same time, there was observed the individual variability in chromosomal aberration frequency from 0.2 to 5%. In the spectrum of chromosomal aberrations, unstable chromosomal aberrations (dicentrics, rings, fragments) predominated in all long livers. When studying the index of acrocentric chromosome associations there was revealed that the difference in the indices between studied groups was identical to that when studying the frequency of chromosomal aberrations. In long livers with arterial hypertension and osteoarthritis, the index of the average number of acrocentric chromosome associations per cell was 1.07 times higher than that in long livers with arterial hypertension only, 1.32 times higher compared to that in long livers with osteoarthritis only and 1.75 times higher compared to healthy individuals ($p<0.05$).

Keywords
arterial hypertension; osteoarthritis; long livers; karyotype; chromosomal aberrations; acrocentric chromosome associations

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Problem statement and analysis of the recent research

The ageing is typical for almost all living organisms; it occurs at all levels of organization of living things: from the molecular genetic level to the organismal one [1]. According to modern concepts of cellular theory of ageing, the important factors are the accumulation of cellular disorders as well as weakened mechanisms of both cell and tissue survival and recovery. Spontaneous mutations in somatic cells, structural chromosomal aberrations (CA) in particular, are the most common cellular disorders. They play a significant role in a multifactorial process of ageing [2]. The study of various cytogenetic as well as molecular genetic disorders (CA [3], micronucleus formation [4], loss of telomeric repeat sequence [5], mutations at the glycophorin locus [6], DNA breakage, etc.) revealed that in most cases, their incidence increased with age. CA are one of the signs of karyotype destabilization as well as somatic mutagenesis activation. This indicator which combines a group of phenomena being different in the mechanisms of development has been traditionally used to assess genotoxic effects. A number of scientists have studied the state of the chromosomal apparatus in patients with arterial hypertension (AH) and those with a comorbidity [8]; however, there are currently no studies on the peculiarities of the chromosomal apparatus state in long livers with AH and osteoarthritis (OA) as well as healthy individuals residing in the Carpathian region.

Karyotype changes may indicate structural abnormalities in a chromotype caused by both endogenous and exogenous factors, the state of the adaptive capacity etc. From this perspective, we have assessed the frequency and spectrum of CA as well as acrocentric chromosome associations (ACA) in peripheral blood lymphocytes of long livers residing in the Carpathian region.

The objective of the research was to studythe frequency and spectrum of CA and ACA as well as to compare the obtained results between long livers with AH and OA, patients

with AH only, patients with OA only and healthy individuals residing in the Carpathian region.

1. Materials and methods

To study the features of the karyotype, all studied long livers were divided into 4 groups considering their health status: Group I included 83 long livers with stage II AH and stage II OA; Group II comprised 76 long livers with AH; Group III included 49 long livers with OA; Group IV included 56 apparently healthy long livers. Cytogenetic analysis of long livers was based on studying the karyotype of peripheral blood lymphocytes. Blood sampling was performed using sterile syringes; then, 0.01 ml of heparin solution was added; the material was thereafter placed in a cool bag (t=5-7 °C) and delivered to the accredited genetic laboratory of the Ivano-Frankivsk National Medical University within 1-2 hours. Lymphocytes were cultivated and chromosome slides were prepared according to the methodological recommendations approved by the Ministry of Health of Ukraine [9]. The preparations were studied using electrooptical complex MetaScan 2.

Metaphase plates with well-spread chromosomes were analyzed. At least 30 metaphase plates were analyzed for each individual. In addition to the identification of CA, the number of ACA was studied. The presence of ACA was assessed according to the criteria developed by O.K. Frolov et al. [10]. Specific location of the acrocentric chromosomes in metaphase was considered: no chromosome overlapping; the short arms of the acrocentric chromosomes with the orientation towards each other, the distance between them without including the satellites did not exceed the size of the long arm of G-group chromosome; a larger distance was considered as an association if the acrocentric chromosomes were bound to each other with visible filaments or were localized at one chromosomal axis.

The index of association was calculated as the ratio of the number of cells with associations to the total number of analyzed cells expressed in percentage terms. The average number of ACA per cell as well as the average number of chromosomes per association was determined.

2. Results and Discussion

Cytogenetic analysis was primarily conducted among long livers with AH and co-existent OA. The obtained results were compared with those obtained in long livers without the aforementioned pathology. The average indices of CA frequency were as follows: (3.01±0.27) in long livers with AH and OA and (2.17±0.53) in healthy individuals. In long livers with AH or OA, the differences in CA frequency as compared to the control group were even less significant - (2.56±0.33) and (2.45±0.27), respectively. The comparison of the obtained indices indicated the tendency toward the increase in CA frequency depending on long livers' health status. In individuals with AH and OA, the frequency of CA was 1.7 times higher compared to that in long livers with AH only and 1.22 times higher than that in long livers with OA only, respectively.

There was observed the individual variability in CA frequency from 0.2 to 5%.

The analysis of CA frequency in males and females of all groups revealed no statistically significant sexual dimorphism (Table 1). However, there was observed a tendency toward higher incidence of disturbances in the chromosomal apparatus in male long livers.

When studying CA, all chromosome-type as well as chromatid-type aberrations which may be recognized in group karyotyping on uniformly stained metaphase chromosomes were considered (Table 2).

Unstable CA (dicentrics, rings, fragments) lead to cell death while stable CA (translocations, insertions) are known to accompany the ontogenesis; they may affect vital cellular functions as well. Genetic instability of somatic cells affects gene expression leading to both genetic and epigenetic changes which result in the degeneration and atrophy of cells and tissues. The latter is the cause of the aging of an organism as a whole [11].

The obtained results were compatible with numerous cytogenetic studies carried out by N.P. Bochkov et al. [12, 13]. They revealed the absence of changes in the total number of aberrant metaphases depending of the person's age and gender. However, after 80 years of age, the number of fragments increases, while the number of chromatid interchanges decreases. It was confirmed by A.M. Chebotarev [4]; according to him, it is due to more effective course of reparation processes in young people.

The study of the adaptive capacity index as well as the immunogenetic status of the organism – ACA revealed no significant sexual changes among all studied long livers [14]. There were detected some variations in the number of ACA depending on health status. The average frequency of cells with ACA in long livers with AH and OA predominated over that in individuals without the aforementioned pathology (Table 3).

All possible variants of associations between chromosomes were considered. In long livers of Group I, the index of the average number of ACA per cell was 1.07 times higher than that in long livers of Group II, 1.32 times higher compared to that in long livers of Group III and 1.75 times higher compared to Group IV ($p<0.05$). Similar tendency persisted when analyzing the number of associated chromosomes per cell.

There was observed an unequal number of associated chromosomes per cell: in healthy long livers, it was (1.80±0.31) (Fig. 1a) and in patients with AH and OA - (3.2±0.15) and (3.8±0.18 %), respectively. The comparison of this parameter in long livers with a comorbidity revealed its predominance over that in healthy long livers by 1.18 times indicating decreased immunocytogenetic status of the body. ACA with two and three associated chromosomes were most commonly seen; ACA with three, four, five associated chromosomes were rare

(Fig. 1b).

The frequency of associations involving two and three acrocentric chromosomes in long livers with AH and OA was (80.93±1.01) and (19.67±0.31) %. Associations involving four and five acrocentric chromosomes were rarely seen - (3.68±0.21) and (1.02±0.09) %, respectively. In two long livers with AH and OA, ACA involving six chromosomes were observed (0.03±0.01) %. There were observed no significant differences in these indices between long livers of Group II, Group III and Group IV. Therefore, long livers with AH and OA developed a higher number of cytogenetic abnormalities (CA, ACA) in peripheral blood lymphocytes as compared to healthy individuals.

Table 1. Frequency of chromosomal aberrations among long livers of Ivano-Frankivsk region depending on gender, M±m

Groups	Males, n=113	Females, n=151
Group I, n=83	3.12±0.26	2.91±0.37
Group II, n=76	3.04±0.12	2.82±0.06
Group III, n=49	2.81±0.22	2.47±0.52
Group IV, n=56	2.31±0.31	2.03±0.62

Note.

* - the probability of a difference between the given indices and those of healthy individuals ($p<0.05$).

3. Conclusions

We have determined the following feature of the increase in CA frequency among long livers of the Carpathian region: healthy individuals<patients with OA<patients with AH<patients with AH and OA.In the spectrum of CA, unstable CA predominated in all long livers.When studying the index of ACA, there was revealed that the difference in the indices between studied groups was identical to that when studying the frequency of CA. In long livers with AH and OA, the index of the average number of ACA per cell was 1.07 times higher than that in long livers with AH only, 1.32 times higher compared to that in long livers with OA only and 1.75 times higher compared to healthy individuals ($p<0.05$).

4. Prospects for further research

The determination of the frequency of polymorphic variants of the GSTT1 and GSTM1 gene deletions among long livers which contribute to the adaptation to the effects of various exogenous factors is promising.

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Table 2. Structure of chromosomal aberrations in long livers of Ivano-Frankivsk region

Indices	Frequency of aberrations per 100 cells			
	Group I, n=83	Group II, n=76	Group III, n=49	Group IV, n=56
CA	3.01±0.27	2.56±0.33	2.45±0.27	2.17±0.53
Chromosome-type aberrations	1.87±0.12	1.53±0.22	1.43±0.31	1.12±0.13
Paired fragments	1.17±0.04	1.14±0.08	1.04±0.05	1.06±0.13
Chromosome breaks	0.13±0.05	0.12±0.07	0.12±0.03	0.11±0.02
Chromosome gaps	0.19±0.06	0.18±0.03	0.17±0.02	0.15±0.07
Dicentrics	0.06±0.02	0.05±0.04	0.05±0.03	0.04±0.02
Chromatid-type aberrations	0.96±0.32	0.84±0.21	0.74±0.14	0.68±0.12

Note.

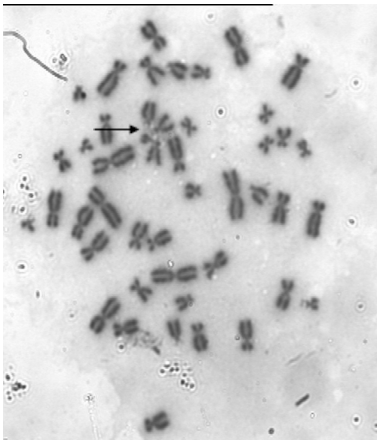
* - the probability of a difference between the given indices and those of healthy individuals ($p<0.05$).

Table 3. Frequency of acrocentric chromosome associations in peripheral blood lymphocytes of long livers residing in Ivano-Frankivsk region, M±m

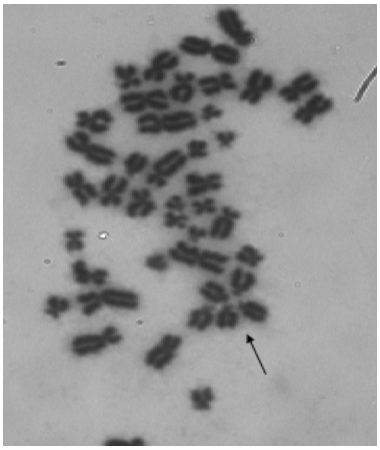
Group	Frequency of cells with ACA, %	Average number of ACA per cell, %	Number of associated chromosomes per cell, %
Group I, n=83	93.68±0.27	3.24±0.41*	4.1±0.22*
Group II, n=76	90.21±0.63	3.01±0.22*	3.72±0.11*
Group III, n=49	88.73±0.31	2.45±0.33	3.21±0.42
Group IV, n=56	82.83±0.61	1.85±0.54	2.5±0.14

Note.

* - the probability of a difference between the given indices and those of healthy individuals ($p<0.05$).



(a) 1a



(b) 1b

Figure 1. Acrocentric chromosome associations (↑) in metaphase plate from peripheral blood of a healthy long liver K (a) and a patient with arterial hypertension and osteoarthritis (b). Giemsa staining. Mag.: oc.15, ob. 100.

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