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A retrospective analysis of spontaneous chromosomal aberrations in human lymphocyte cultures of individuals from Bosnia and Herzegovina

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Abstract

Spontaneous chromosomal aberrations are structural or numerical changes of chromosomes that occur naturally, without exposure to external genotoxic factors. They are not inherited, occur randomly in the karyotype, and do not have direct clinical significance. However, they can affect genomic instability and disease predisposition. They can result from DNA replication or repair processes errors, and typically are observed in cells that are actively dividing. Spontaneous chromosomal aberrations may arise due to the natural chromosomal instability and can be elevated in individuals exposed to mutagens. We analyzed frequencies of spontaneous chromosomal aberrations in 137 individuals subjected to karyotype analysis at the Laboratory for Cytogenetics and Genotoxicology, University of Sarajevo – Institute for Genetic Engineering and Biotechnology, during 2008-2023. Whole blood samples were cultivated for 72 hours with the thymidine added in the 48th hour. Metaphases were arrested by colcemid 60 minutes before harvesting. GTG banding was performed and slides were analyzed under 1000x magnification in accordance with An International System for Human Cytogenetic Nomenclature and E.C.A. Cytogenetic Guidelines and Quality Assurance. Constitutionally aberrant karyotypes were found in 2.92% of analysed individuals as well as altered karyotypes considered as normal chromosomal variants. In the total of 3092 analyzed metaphases, 20 spontaneous chromosomal aberrations were found in 13 individuals. This study contributes to the limited knowledge of the cytogenetic status of the Bosnian and Herzegovinian population. Further monitoring of spontaneous chromosomal aberrations incidences is recommended.

Introduction

Chromosomal aberrations are the changes in karyotype that can affect the number of chromosomes (increased - hyperdiploidy; decreased - hypodiploidy) or their structure if chromosome regions are exchanged, inverted, deleted, or duplicated. They can be produced by errors in normal cellular processes including DNA replication, DNA repair, transcription, and cell division (Preston, 2014). Chromosomal aberrations in an organism may be constitutional or acquired. Constitutional chromosomal aberrations affect all or a majority of cells in an organism since they arise during gametogenesis or early embryogenesis, while acquired chromosomal aberrations, which are involved in the pathogenesis of neoplasms, typically develop during the life from a single cell (McFadden and Friedman, 1997; Queremel and Tadi, 2023).

Spontaneous chromosomal aberrations in human lymphocyte cultures refer to changes in the structure or number of chromosomes that occur naturally without exposure to external factors, such as radiation or chemicals (Russell, 2002). They occur spontaneously but do not have an impact on the individual's clinical symptoms. These aberrations are not inherited and occur randomly in the karyotype. They are typically observed in cells that are actively dividing, such as lymphocytes during cultivation. Spontaneous chromosomal aberrations may arise due to the natural chromosomal instability and can be elevated in groups environmentally or occupationally exposed to mutagens. The most common types of spontaneous chromosomal aberrations include aneuploidy, deletions, duplications, inversions, and translocations. Different laboratory setting conditions, donor characteristics, but also media used for lymphocyte cultivation can impact the frequencies of spontaneous chromosomal aberrations (Jha, 1992). Regarding spontaneous aneuploidies, Richard et al. (1993) found that chromosome losses are more frequent than chromosome gains and that chromosome losses and gains are significantly higher in 72-h compared to 48-h cultures. Although spontaneous chromosomal aberrations do not have direct clinical significance, they can provide valuable insights into genomic instability potential and disease predisposition. Farkas et al. (2016) confirmed an association between numerical chromosomal aberrations and cancer risk in a large healthy cohort of 2145 healthy individuals.

It has been shown that karyotype abnormalities in peripheral blood lymphocytes are responsible for azoospermia, oligospermia, amenorrhea and abnormal gonad development in adults (Zhang et al., 2018).

The aim of this research was to reveal the incidence of spontaneous chromosomal aberrations and to find the rates of their different types in cultivated lymphocytes of individuals subjected to the karyotype analysis in our laboratory during the 15-year period.

Material and methods

Frequencies of spontaneous chromosomal aberrations were analyzed in the group of 137 individuals (71 males and 58 females) subjected to the karyotype analysis in the Laboratory for Cytogenetics and Genotoxicology of the University of Sarajevo – Institute for Genetic Engineering and Biotechnology during the 15 year period (2008-2023). No selection criteria were applied and all samples received in the stated period are included in the study, regardless of the personal data and lifestyle factors, as those were not collected.

Prior karyotyping procedure, all individuals were informed to provide blood samples at the medical facility and to deliver venous blood samples collected into lithium-heparinized tubes to the University of Sarajevo – Institute for Genetic Engineering and Biotechnology right after the sampling. Whole blood cultures were initiated in 4h in complete medium: 400 µl of heparinized

blood in 5 ml of PB-MAX™ Karyotyping Medium (GIBCO-Invitrogen, Carlsbad, CA, USA) for 72 hours with the thymidine added at 48th hour to synchronize cell culture. Metaphases were arrested by colcemid (0.18 µg/ml) 60 minutes before harvesting.

Following hypotonic treatment with 0.075M potassium chloride (25 minutes at 37˚C), cells were fixed three times in a fresh ice-cold absolute ethanol/glacial acetic acid fixative (3:1, v/v), and cell suspension was dropped on coded slides.

Slides were banded with conventional GTG banding procedure (resolution of 400-550 bands per haploid chromosome set), and analyzed under 1000x magnification on Olympus BX51 microscope (Olympus, Tokyo, Japan), in accordance with An International System for Human Cytogenetic Nomenclature (Shaffer and Tommerup, 2005) and E.C.A. Cytogenetic Guidelines and Quality Assurance (Hastings, 2012). Each detected spontaneous chromosomal aberration was confirmed by additional metaphase analysis to eliminate mosaicism. This retrospective study was conducted in accordance with the Declaration of Helsinki and ensuring privacy of the data. The study was approved by the Ethics Committee of the Institute for Genetic Engineering and Biotechnology of the University of Sarajevo (No. 587/23).

Results and Discussion

Among 137 analyzed individuals 129 (94.17%) had normal karyotypes, four individuals (2.92%) had altered karyotypes considered as normal chromosomal variants, and four individuals (2.92%) had constitutionally aberrant karyotypes (Table 1). In a total of 3092 analyzed metaphases over 137 individuals included in the study, 20 spontaneous chromosomal aberrations in lymphocyte cultures of 13 individuals were found. Among 20 detected spontaneous chromosomal aberrations 12 were aneuploidies and 8 were structural chromosomal aberrations (Table 2).

The combined incidence of all detected spontaneous chromosomal aberrations was $64.68x10^{-4}$ per metaphase (20 of 3092), with $25.87x10^{-4}$ structural aberrations and $38.81x10^{-4}$ aneuploidy incidence. Among the structural chromosomal aberrations, translocations involving chromosomes 7 and 14 were the most frequent, which has also been previously reported (Dewald et al., 1986; Hecht et al., 1987; Haverić et al., 2022a).

Tawn and Whitehouse (2001) also reported the highest frequency of translocations in G-banded chromosomes in the control group. In the prenatal diagnosis study conducted over 3800 patients in the republic of North Macedonia, the frequency of

Table 1. Constitutional karyotypes distribution in analyzed individuals (N=137)

		Karyotype	Absolute frequency	Relative frequency (%)
Normal	**male**	46,XY	71	51.83
	female	46,XX	58	42.34
Normal chromosomal variations		46,XX,21ps+	2	1.46
		46,XX,1qh+	1	0.73
		46,XX,inv(9)(p11q13)	1	0.73
Aberrant		47,XX,+21	3	2.19
		47,XXY	1	0.73

Table 2. Frequencies of detected spontaneous chromosomal aberrations

Chromosome aberrations	Karyotype	Absolute frequency	Relative frequency in total of numerical/structural aberrations (%)	Incidence in total of analyzed cells (N=3092)
Numerical	47,XX,+10	1	8.33	$3.23x10^{-4}$
	47,XX,+21	4	33.33	$12.94x10^{-4}$
	47,XY,+X	3	25.00	$9.70x10^{-4}$
	46,X,-Y,+X	1	8.33	$3.23x10^{-4}$
	47,XX,+X	1	8.33	$3.23x10^{-4}$
	45,X,-X	2	16.67	$6.47x10^{-4}$
Structural	46,XX,t(7;14)(p13;q11)	1	12.50	$3.23x10^{-4}$
	46,XY,t(7;14)(p21;q21)	2	25.00	$6.47x10^{-4}$
	46,XY,t(1;3)(q25;p22)	1	12.50	$3.23x10^{-4}$
	46,XX,t(7;14)(q11.22;q11.2)	1	12.50	$3.23x10^{-4}$
	46,XY,t(7;19)(q33;q13.4)	1	12.50	$3.23x10^{-4}$
	46,XX,t(7;14)(p21;q23)	1	12.50	$3.23x10^{-4}$
	46,XY,der(21)	1	12.50	$3.23x10^{-4}$

42% translocations was reported including 0.29% of balanced and 0.13% of unbalanced translocations (Vasilevska et al., 2013). Translocations, as highly represented aberrations in tumors, present the most important biomarker of cumulative exposure (Mateuca et al., 2006). Among the aneuploidies the most frequent were the gain of chromosomes 21, X, and 10, and the loss of X chromosome. Structural chromosomal aberrations were more frequent among the male individuals, while numerical aberrations were more frequent among the females. In a previous study that aimed to analyze the effects of sex and aging on spontaneous chromosomal damage among healthy individuals from Bosnia and Herzegovina, the frequency of structural chromosomal aberrations was higher in females but numerical chromosomal aberrations were more frequent in males (Nefić and Mušanović, 2014). Also, an increased frequency of chromatid-type chromosomal aberrations was evidenced in females (Aganović-Mušinović et al., 2014). In the study by Mackic-Djurovic et al. (2018), chromosomal aberrations were found in 16 out of the 100 Bosnian women with confirmed reproductive problems (sterility and habitual abortions). The most frequent was 46,XX, inv9(p11;q13) karyotype with the frequency of 4%. However, there is a lack of data about spontaneous chromosomal aberration frequencies in lymphocyte cultures, and their causal connection with reproductive problems and miscarriages.

Commonly, chromosome aberration frequencies are reported in giemsa stained chromosomes. Median frequency of structural chromosomal aberrations of 2% has been reported for the control group in the population of B&H (Haveric et al., 2022b). Previously, an increased CA frequency among females in B&H was reported (Nefić and Mušanović, 2014; Mačkić-Đurović et al., 2017), and the values were also higher compared to the background level of CA in the population of Croatia (Rozgaj and Kasuba 2000; Kasuba et al.,

2008). Generally, the level of chromosome aberrations may differ between the countries and this could be attributed to lifestyle differences, geographical location, weather conditions, and pollution levels across Europe that could affect the frequencies of such parameters (Sierra-Torres et al., 2004; Sabirov et al., 2020; Musilova et al., 2023). On the contrary, certain lifestyle factors have beneficial effects in maintaining genomic integrity (Fenech et al., 2023; Santovito et al., 2023). Accordingly, limitations of this study include lack of anthropometric or lifestyle factors data (e.g., smoking status, cancer history of cancer, alcohol consumption, etc) for included individuals.

Conclusion

This retrospective study contributes to the limited overall knowledge of the cytogenetic status of the Bosnian and Herzegovinian population. Since chromosomal aberration analysis in cultured human lymphocytes remains a fundamental method in genetics and genomics, with practical applications in environmental monitoring and medicine, further monitoring of spontaneous chromosomal aberration incidences in human lymphocyte cultures is recommended.

Conflict of interest

The authors declare that they don't have any conflict of interest.

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