CHROMOSOMAL ABERRATIONS AND MICRONUCLEI FREQENCIES IN BULGARIAN CONTROL POPULATION

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Abstract

The aim of this investigation is to represent the frequency of spontaneous chromosomal damages in peripheral blood lymphocytes of Bulgarian control population.
Material and methods. The investigated group includes persons belonging to both sexes and different ages. Each of them is interviewed of their social and health status. Sixteen persons are examined using the chromosomal aberrations analysis and fourtyfife with micronucleus test.
The frequency of chromosomal aberrations varied between 0 - 2.4% and the mean value is 1.00%. The frequency of cells with micronuclei varied between 4.5 - 24.5‰ and the mean value 12,9‰.
Further work on the investigation of spontaneous frequency of chromosomal damages is in progress.

Key words: chromosomal aberration, micronucleus test, spontaneous frequency

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Introduction

Chromosomal Aberrations and micronuclei formation are widely used cytogenetic bioindicators for monitoring exposure to ionizing radiation, chemicals and biological agents of the environment. They are also proved as biodosimeters of radiation exposure in case of radiation accidents (5). The correct estimation of monitoring data and a correct design of cytogenetic investigation require a good information about the spontaneous frequencies of the cytogenetic biomarkers recorded and the factors which influence them. A large amount of data was accumulated in respect to the chromosomal aberrations used for the purposes of radiation dose estimation. The information is limited in respect to spontaneous levels of micronuclei in cytochalazin bloked binuclear cells.

The influence of some factors of culturing and slides preparation on cytogenetic alterations analysed was reported (2, 6). In the present report we present the protocol used for chromosomal aberration test and micronucleus test.

The aim of the present investigation is to accumulate data on the frequency of chromosomal aberrations and micronuclei in control Bulgarian population.

Material and Methods

In this investigation two cytogenetic methods are applied - chromosomal aberration analysis (16 donors) and micronucleus test (45 donors).
Human whole blood was obtained by venepuncture in tubes containing litium heparin. The blood samples were proceed as follow:

Chromosomal aberration assay
In brief:
1. 1 ml blood sample was incubated at 37 oC in RPMI medium supplemented with 10% bovine serum, and antibiotics. Phytohaemagglutinin (PHA) was added to the cultures to stimulate lymphocytes to divide.
2. After a culture time of 48 hours, the cell cultures were arrested at the metapahase stage with 1 ml 0,01 mM Colchicine solution was added for 2 hours at 37 oC to .
3. The cells were then fixed and preparations of metaphases were made by standard procedure (2).
4. Giemsa staining 10% for 15 min.

Micronucleus assay.
The cytochalasine B method in lymphocytes has been introduced by Fenech and Moorley(1985**)** and described in detail (3). There are experimental conditions, i.e. timing, CB concentration that may require adjustment from laboratory to laboratory (4).
In our study we use the following protocol :
1. The blood samples were incubated in the presence of PHA for 44 hours at 37 oC
2. Cytochalasine B was added at 44 h at concentration 6mkg/ml.
3. After a culture time 72 h the cells were collected and fixed by standard procedure.
4. Giemsa staining 5% for 10 min.
5. Scoring is done after coding the slides. At least 2000 binucleated lymphocytes are scored.

Results and discussion

At present several biological indicators of genotoxic occupational and professional exposure are available. Chromosomal aberrations are quantitative, but also qualitative indicator of genotoxic exposure. Using chromosomal aberration assay , it is possible to identify different types of chromosomal damage. Dicentric chromosomes are recognized as a biodosimeter of radiation exposure and are widely used for biomonitoring of radiation exposure and dose estimation in case of radiation accidents (5). Micronucleus test is a fast and simple assay system broadly applied for in vitro and in vivo genotoxicity testing (3, 4). Micronuclei are produced during mitosis via various mechanisms (acentric fragments, multicentric chromosomes, damaged kinetochores, spindle defects) and can be detected in the cytoplasm besides the cell nucleus as a small nucleus like particle(3, 4).
The frequency of chromosomal aberrations in control Bulgarian population is given on table 1. The estimated frequency chromosomal aberrations in 16 investigated subjects varied between 0 - 2.4% and the mean value is 1.00%.

TABLE1.
CHROMOSOMAL ABERRATIONS FREQUENCY IN BULGARIAN CONTROL POPULATION

№	№	cels	chromossomal	chromossomal	chromatide	chromatide	№
investigated	scored	with	fragments	changes	fragments	changes	CA%
persones	cells	CA%					
16	6300	1.00%	0.70%	0.016%	0.20%	0.05%	1.00%

The results of MNT are shown on table 2.
The frequency of cells with micronuclei varied between 4.5 - 24.5%o and the mean value is 12,9%o.

TABLE2.
MICRONUCLEUS FREQUENCY IN BULGARIAN CONTROL POPULATION

№	№	Cells	Total
Cases	Scored	with MN	№
	cells	%	MN%
45	9950	12.9	13.99

There are number of possible confounding factors in the cytogenetic assays to be considered, like age, sex, smoking, alcochol consumption, recent diagnostic X-rays. These confouders must be considered in the cytogenetic monitoring studies.
Several autors have investigated the spontaneous frequency (6, 7, 8).
From the results presented it appears that the frequency of chromosome aberrations control Bulgarian population is
1,0%, estimated on 16 examined subjects and the spontaneous frequency of micronuclei is 12,9%o estimated on 45 examined subjects .

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