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Cytokinesis-block micronucleus cytome assay parameters in peripheral blood lymphocytes of the general population: Contribution of age, sex, seasonal variations and lifestyle factors

Goran Gajski[a,1], Marko Gerić[a,1], Višnja Oreščanin[b], Vera Garaj-Vrhovac[a,*]

[a] Institute for Medical Research and Occupational Health, Mutagenesis Unit, 10000 Zagreb, Croatia
[b] ORESCANIN Ltd., 10000 Zagreb, Croatia

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ABSTRACT

The cytokinesis-block micronucleus cytome (CBMN Cyt) assay was used to evaluate the baseline frequency of cytogenetic damage in peripheral blood lymphocytes of the general population (average age, 38.28 ± 12.83 years) in relation to age, sex, body mass index, seasonal variations (season of sampling, period of sampling and different meteorological parameters) and lifestyle factors (smoking habit, alcohol consumption, exposure to medications and diagnostic radiation, physical activity, and family history of cancer). The background frequency of micronuclei (MNi) for the 200 subjects assayed was 5.06 ± 3.11 per 1000 binucleated cells, while the mean frequency of nucleoplasmic bridges (NPBs) was 1.21 ± 1.46 and of nuclear buds (NBUDs) 3.48 ± 2.14. The background frequency of apoptosis and necrosis was 1.58 ± 1.50 and 1.39 ± 1.56, respectively, while the mean nuclear division index (NDI) was 1.99 ± 0.14. The cut-off value, which corresponds to the 95th percentile of the distribution of 200 individual values, was 11 MNi, 4 NPBs and 7 NBUDs. The study also confirmed an association of the above mentioned parameters with age, sex and several lifestyle factors. Moreover, significant confounders based on our results are also sampling season, sampling period and different meteorological parameters that were dependent on the CBMN Cyt assay parameters. In line with the above mentioned, several factors should be taken into account when it comes to the monitoring of exposed populations using cytogenetic biomarkers. Moreover, the normal and cut-off values obtained in this study present background data for the general population, and can later serve as baseline values for further biomonitoring studies.

1. Introduction

Human biomonitoring is an essential tool for assessing whether and to what extent environmental substances affect the human population. It therefore provides valuable information on environmental exposure and helps to identify potential health risks. Biomonitoring can give information on total exposure of an individual at a given time, as it adds together exposure from multiple sources and routes. On the other hand, the risks these exposure scenarios may pose to human health, in which combination and at what levels, remain difficult for evaluation (Alves et al., 2014; Angerer et al., 2007, 2006; Ladeira and Viegas, 2016).

Cytogenetic methods play a crucial role in biomonitoring when it comes to the assessment of the presence and extent of chromosomal damage in human populations that have been exposed to different genotoxic agents. Spontaneous or baseline frequencies of different cytogenetic parameters can provide valuable data regarding the accumulated genetic damage appearing during the lifespan of an individual (Araldi et al., 2015; Garaj-Vrhovac and Gajski, 2010; Mateuca et al., 2006; Nefic and Handzic, 2013). One of the most extensively used cytogenetic methods is the cytokinesis-block micronucleus (CBMN) assay used for the measurement of micronuclei (MNi) in peripheral blood lymphocytes (PBL), and can be considered as a "cytome" assay covering cell proliferation, cell death and chromosomal changes. The key advantages of the CBMN cytome (CBMN Cyt) assay lie in its ability to detect both clastogenic and aneugenic events and identify cells that divided only once in a culture (Fenech, 2007, 2006; Mateuca et al., 2006, 2012; Nersesyan et al., 2016). The baseline frequency of CBMN Cyt assay parameters in a population needs to be established in order to determine acceptable, normal values for the human population.

Several parameters can be measured using the CBMN Cyt assay assuring a more detailed measurement of cytotoxic and genotoxic effects in response to different lifestyle factors. Chromosomal instability

* Corresponding author.
E-mail addresses: ggajski@imi.hr (G. Gajski), mgeric@imi.hr (M. Gerić), vgaraj@imi.hr (V. Garaj-Vrhovac).
[1] Equal contribution.

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can be measured by virtue of scoring MNi, nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs). MNi can originate during anaphase from a lagging acentric chromosome or chromatid fragments caused by misrepair of DNA breaks or unrepaired DNA breaks (Fenech et al., 2016, 2011, 2003; Samanta and Dey, 2012). NPBs originate from dicentric chromosomes, which may occur due to the misrepair of DNA breaks, telomere end fusions, and might also be observed when the defective separation of sister chromatids at anaphase occurs due to failed decatenation. NBUDs represent the process of elimination of amplified DNA, DNA repair complexes and possibly excess chromosomes from aneuploid cells (Fenech et al., 2016, 2011, 2003).

In the frame of the CBMN Cyt assay, cells can also be scored for viability status (apoptosis and necrosis). Apoptosis is considered a major mechanism of regulated cell death, employed not only upon cell damage or stress, but also during normal development and morphogenesis. Apoptosis may be triggered either by extrinsic or intrinsic stimuli in which the activation of caspases results in characteristic morphological features such as cell rounding, chromatin condensation, nucleus fragmentation and apoptotic bodies (Adams, 2003; Green, 2005; Nikoletopoulou et al., 2013; Walsh, 2014). In contrast to the apoptotic type of cell death, necrosis was thought to be a mode of unordered and passive cell death in response to acute stress typically followed by inflammatory reactions. Still, new data suggest that necrosis may also be a well-regulated process activated by rather specific stimuli (Golstein and Kroemer, 2007; Karch and Molkentin, 2015; Nikoletopoulou et al., 2013; Zong and Thompson, 2006). Moreover, the CBMN Cyt assay can be used to evaluate mitotic status which reflects the cytostatic effect and rate of mitotic division that can be calculated from nuclear division index (NDI) (Fenech, 2007; Kirsch-Volders et al., 2003).

The general population can be exposed to a variety of environmental factors that include different chemical agents, natural sources of radiation and weather conditions, all of which can influence genomic instability. Moreover, lifestyle factors such as smoking, alcohol consumption, drugs abuse, dietary habits, exercise and/or stress coupled with genetic factors such as genetic disorders, heritable diseases and/or genetic polymorphisms can also influence genome integrity (Angelini et al., 2008; Bonassi et al., 2003; Freitas et al., 2014; Huang et al., 2009; Santovito et al., 2015; Wagner et al., 2011). Therefore, in this retrospective study, we used the CBMN Cyt assay to evaluate the baseline cell and genome damage in binucleated human PBL selected from the general Croatian population. Lymphocytes are preferred for the measurement of genotoxic effects in biomonitoring studies since they are considered early warning signals for adverse health effects (Faust et al., 2004a, 2004b). The tested CBMN Cyt assay parameters have been associated with age, sex, body mass index (BMI), seasonal variations (season of sampling, period of sampling and different meteorological parameters) as well as lifestyle factors (smoking habit, alcohol consumption, exposure to medications and radiation, exercise, and family history of cancer) to investigate whether there is a correlation between cytogenetic markers in PBL of the selected subjects and the above mentioned factors. These baseline records may contribute to a better understanding of those variables that may influence the frequency of CBMN Cyt assay parameters and can later serve as baseline values for future biomonitoring studies.

2. Subjects and methods

2.1. Chemicals and media

RPMI 1640 medium and foetal bovine serum (FBS) were from Gibco, USA; antibiotics (penicillin and streptomycin) and cytochalasin B were from Sigma, USA; phytohaemagglutinin was from Remel Europe Ltd., UK; heparinised vacutainer tubes were from Becton Dickinson, USA; Giemsa dye was from Merck, Germany. All of the other reagents used were laboratory-grade chemicals from Kemika, Croatia.

Table 1
Population characteristics and lifestyle factors of the study population (mean values ± standard deviation of the mean).

	Women	Men	Total
N	144	56	200
Age (years)	38.10 ± 13.22	38.73 ± 11.85	38.28 ± 12.83
Age range (years)	19–80	20–68	19–80
BH (m)	1.68 ± 0.07	1.79 ± 0.08	1.73 ± 0.09
BM (kg)	65.53 ± 11.33	82.50 ± 11.81	72.09 ± 14.16
BMI (kg/m^2)	23.18 ± 4.06	25.60 ± 2.83	24.11 ± 3.81
Smokers	49	15	64
Alcohol drinkers	63	43	106
Sports	29	28	57
Medication exposure	62	10	72
Radiation exposure	44	12	56
Cancer history	48	17	65

N, number of subjects; BH, body height; BM, body mass; BMI, body-mass index.

2.2. Population characteristics

The present study was retrospectively conducted on a group of 200 participants (144 females and 56 males) aged 19–80 years (average age, 38.28 ± 12.83 years; median age, 33.50 years) with similar socio-economic status. Detailed population and lifestyle characteristics are presented in Table 1. All of the participants were selected from the general Croatian population. Their mean body mass was 72.09 ± 14.16 kg, mean body height 1.73 ± 0.09 m and mean BMI 24.11 ± 3.81 kg/m^2. The criterion for selection was that they were healthy at the moment of blood sampling and interviews. After giving written informed consent, the participants completed a questionnaire designed to obtain demographic data, smoking habits, alcohol consumption, health status, family history of cancer, and prior or current exposure to medication and diagnostic procedure. No private details on the subjects involved in the study have been or will be disclosed to the public. The study was part of the projects approved by the Ethics Committee of the Institute for Medical Research and Occupational Health, Zagreb, Croatia.

2.3. Blood sampling

Peripheral blood was collected by venipuncture between 1st January 2010 and 31st December 2015 into sterile heparinised tubes in the morning hours, usually between 8 and 10 a.m. After collection, all blood samples were handled in the same manner. They were randomly coded, stored at 4 °C, protected from light and processed as quickly as possible, not more than 4 h after blood sampling.

2.4. Cytokinesis-block micronucleus cytome (CBMN Cyt) assay

The CBMN Cyt assay was done in essence as described by Fenech and Morley (Fenech and Morley, 1985). In accordance with the new criteria described by Fenech (Fenech, 2007), the frequency of MNi, as well as the frequency of both NPBs and NBUDs, was scored. Whole blood (500 μL) was first added to RPMI 1640 medium supplemented with FBS, phytohaemagglutinin and antibiotics, and then incubated at 37 °C and 5% CO_2 for 72 h. Cytochalasin-B at a final concentration of 6 μg/ml was added to each sample after 44 h of incubation in order to prevent cytokinesis and the cells were harvested at 72 h. The lymphocytes were fixed in a methanol-acetic acid solution, air-dried and stained with 5% Giemsa solution.

2.5. Scoring

Microscopic analysis was done using an optical microscope with a final magnification of 400× (Olympus CX41, Japan). Every subject was analysed for total number of MNi, NPBs and NBUDs per 1000 binucleated cells (BNC), as well as for the total number of micronucleated