Mutation Research 814 (2017) 7–13

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Genomic health status assessed by a cytokinesis-block micronucleus cytome assay in a healthy middle-aged Korean population

Nan Young Cho [a], Kyung Woon Kim [b], Kyoung Kon Kim [a,*]

[a] Department of Family Medicine, Gachon University Gil Medical Center, 21, Namdong-daero 774 beon-gil, Namdong-gu, Incheon 21565, Republic of Korea
[b] R&D division, Bio Research Complex Co., Ltd., 9 Songdomirae-ro, Yeonsu-gu, Incheon 21988, Republic of Korea

ARTICLE INFO

Article history:
Received 16 June 2016
Received in revised form 9 November 2016
Accepted 21 November 2016
Available online 24 November 2016

Keywords:
Cytome assay
Micronucleus
Age
Sex
Body mass index
DNA damage

ABSTRACT

The aim of this study was to determine the typical incidence of micronuclei (MNi) in the peripheral blood lymphocytes of healthy middle-aged Koreans using the cytokinesis-block micronucleus cytome (CBMN-Cyt) assay. Non-smoking, low-risk alcohol-drinking healthy Korean men and women aged 30 to 59 years were recruited. Participants were divided into three groups according to age, i.e., 30 to 39, 40 to 49, and 50 to 59 years. Fifty participants were included in each age group, for a total of 300 participants. DNA damage was measured based on the number of binucleated (BN) cells with MNi, nucleoplasmic bridges (NPBs), and nuclear buds (NBUDs) using the CBMN-Cyt assay. The frequencies of BN cells with MNi in men were 14.0 ± 4.9 (mean ± SD) in 30–39 year olds, 20.0 ± 6.1 in 40–49 year olds, and 21.7 ± 7.6 in 50–59 year olds. In women, they were 19.7 ± 7.1 in 30–39 year olds, 28.7 ± 11.2 in 40–49 year olds, and 31.9 ± 12.9 in 50–59 year olds. MNi and NPBs scores were higher in females than in males. The elder groups showed higher MNi frequencies for both genders, and the NPB frequency was higher in elder groups than younger groups, but only for males. Based on a regression analysis of the CBMN-Cyt parameters, MNi frequencies showed a positive relationship with age for both genders. BMI and blood vitamin B concentration were not significantly associated with CBMN-Cyt parameters, except vitamin B6 levels, which were positively associated with MNi scores in males. These results provide the standard frequencies of MNi, NPBs, and NBUDs in peripheral blood lymphocytes in middle-aged Korean individuals with healthy lifestyles. In this group, CBMN-Cyt assay parameters varied according to gender and age; however, BMI and micronutrient levels were not significantly associated with assay parameters.

1. Introduction

Genomic damage is a fundamental cause of many chronic degenerative diseases. Accurate measurements are essential for the prevention of damage and the improvement of genomic health status. Several methods have been developed to measure DNA damage in studies of dietary antioxidants. These include the comet assay, 8-oxo-7,8-dihydro-2-deoxyguanosine (8-Oxo-dG) measurement, and micronucleus assays [1]. Among these, the measurement of micronuclei (MNi) frequency in peripheral blood lymphocytes is used to evaluate the extent of genomic damage in humans exposed to various genotoxic environments [2].

The micronucleus is a small chromosome fragment or whole chromosomes that lag behind at the anaphase stage of mitosis; it is regarded as a marker of chromosomal damage and genomic instability [2,3]. The mechanisms of micronucleus formation in mitotic cells are (1) chromosomal breakage and (2) dysfunction of the mitotic apparatus. Micronucleus formation is caused by various factors, including environmental pollutants, chronic inflammation, radiation, chemotherapy, pre-neoplastic and neoplastic conditions, genetic diseases, infections, vitamin deficiencies, etc. [3]. Therefore, MNi frequency can be understood clinically as an indicator of cumulative DNA damage resulting from exposure to environmental genotoxins as well as an unhealthy life-style, which can lead to neoplasms or inflammation-related diseases.

Abbreviations: CBMN-Cyt, cytokinesis-block micronucleus cytome; BN, binucleated; MNi, micronuclei; PHA, phytohemagglutinin; Cyt-B, cytochalasin-B; HUMN, human micronucleus; NPBs, nucleoplasmic bridges; NBUDs, nuclear buds; HBSS, Hank's balanced salt solution; DMSO, dimethyl sulfoxide; NDI, nuclear division index.

* Corresponding author.
E-mail addresses: narashya@hanmail.net (N.Y. Cho), bogunkkw1@gmail.com (K.W. Kim), zaduplum@aim.com, zaduplum@gilhospital.com (K.K. Kim).

http://dx.doi.org/10.1016/j.mrgentox.2016.11.008
1383-5718/

The cytokinesis-block micronucleus cytome (CBMN-Cyt) assay developed by Fenech et al. [2] is a method for scoring MNi in binucleated (BN) cells using phytohemagglutinin (PHA) to stimulate mitosis and cytochalasin-B (Cyt-B) to block cell division. In this method, after blocking cell division with Cyt-B, BN cells appear, some of which express MNi. An increased frequency of MNi in the CBMN-Cyt assay predicts the risk of cancer [4] and is correlated with the severity of atherosclerotic disease [5] and neurodegenerative diseases, such as Alzheimer's disease [6] and Parkinson's disease [7].

Although the increased frequency of MNi indicates cumulative DNA damage and a high risk of some diseases, the baseline frequency in a standard population should be determined in order to assess and compare the extent of genomic damage and the risk of cancer and other diseases between populations exposed to different environmental genotoxic factors. For this purpose, the international human micronucleus (HUMN) project was launched and is establishing databases on baseline MN frequencies [8]. However, there is no laboratory in Korea that collaborates with the HUMN project and the normal range of the MNi frequency is unknown. Other biomerkers of chromosomal instability in the CBMN-Cyt assay are nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) (which are indicative of dicentric chromosome formation and gene amplification respectively) however, information on base-line reference frequencies for these nuclear anomalies is even more limited than for MNi.

The aim of this study was to determine the typical incidence of BN cells with MNi, nucleoplasmic bridges (NPBs), and nuclear buds (NBUDs) in the peripheral lymphocytes of a healthy middle-aged Korean population using the CBMN-Cyt assay.

2. Material and methods

2.1. Participants

Healthy Korean men and women aged 30 to 59 years were recruited. Participants were divided into three groups according to age, i.e., 30 to 39, 40 to 49, and 50 to 59 years. Fifty participants were assembled in each age group for both males and females, for a total of 300 participants. Exclusion criteria included a history of malignant diseases, known cognitive or neurological impairments (such as Alzheimer's disease, Parkinson's disease, or stroke), current smokers and ex-smokers who quit within three years, problem drinkers, radiation exposure, exposure to toxic substances, pregnancy, intake of vitamins or mineral supplements above tolerable upper intake levels of dietary reference intakes for Koreans [9], deficiencies in folate or vitamin B12, gastrectomy, past surgical history under general anesthesia, recent medical conditions, and major medical conditions. The participants were informed of the study aim and methods during interviews. Blood sampling was performed only for participants who provided informed consent. The ethics committee of Gachon University Gil Medical Center approved this study, and the trial was performed in accordance with the Declaration of Helsinki and the guidelines of Good Clinical Practice.

2.2. Blood collection

A fresh venous blood sample after overnight fasting was collected by venipuncture in the morning, stored in a heparin anticoagulant Vacutainer blood tube, and transported to the laboratory within 6 h. Sampling, storage and transportation of the blood samples were performed in room temperature.

2.3. CBMN-Cyt assay

In general, the CMBN-Cyt assay was performed as described by Fenech [2], with some modifications. Whole blood was diluted 1:1 with Hank's balanced salt solution (HBSS, LB 003-04; Welgene, Taipei, Taiwan) and gently inverted until mixed. Diluted blood was gently overlaid onto Ficoll-Paque (17-1440-02; GE-Healthcare, Little Chalfont, UK) at a ratio of 1:3 (6 mL of Ficoll-Paque: 18 mL of diluted blood) and then centrifuged at 400 × *g* for 30 min. Buffy coat (leukocyte layer, 5–10 mL) was transferred using a plugged Pasteur pipette to a fresh tube and the leukocyte suspension was washed twice. First, it was diluted 3 × in HBSS and centrifuged at 180 × *g* for 10 min. The supernatant was discarded and the remainder was diluted 2 × and centrifuged at 100 × *g* for 10 min. The supernatant was discarded. The supernatant was removed and the remaining cell pellet was resuspended in 1 mL of culture medium (the setup method for the culture medium is described below).

2.3.1. Culture media

Two milliliters of fetal bovine serum, 200 μL of 1% L-glutamine and 200 μL of 1% sodium pyruvate were added to 18 mL of RPMI-1640 medium, after thawing fetal bovine serum (Gibco GIB-16000-044; ThermoFisher, Waltham, MA, US), 1% l-glutamine (G7513; Sigma-Aldrich, St. Louis, MO, US), and 1% sodium pyruvate (S8636; Sigma-Aldrich, St. Louis, MO, US).

2.3.2. Culture of lymphocytes

Cells were counted using the Scepter™ 2.0 Handheld Automated Cell Counter and the calculated volume of culture medium was added to obtain the cell suspension at 1×10^6 per mL in a volume of 750 μL in round-bottomed culture tubes. Ten microliters of 2.25 mg/mL PHA (Remel R 30852701; ThermoFisher, Waltham, MA, US) solution was added to culture media to stimulate mitosis in lymphocytes and cells were incubated for 44 h.

2.3.3. Addition of cytochalasin-B (Cyt-B) to culture

Five milligrams of Cyt-B (C6762; Sigma-Aldrich, St. Louis, MO, US) was dissolved in 8.33 mL of dimethyl sulfoxide (DMSO, D2650; Sigma-Aldrich, St. Louis, MO, US) to obtain a Cyt-B solution at a concentration of 600 μg/mL. Culture medium (900 μL) was added to 100 μL of the solution of Cyt-B in DMSO to obtain a 1000-μL Cyt-B solution of 60 μg/mL. After 44 h of incubation, 56 μL of medium from the top was removed and replaced with 56 μL of 60 μg/mL Cyt-B solution to obtain a final Cyt-B concentration of 4.5 μg/mL. The Cyt-B-supplemented culture medium was incubated for 28 h, and cells were harvested for slide preparation and scoring.

2.3.4. Harvesting and staining

Slides were washed with ethanol, dried, and labeled. The washed slide, a filter card, and a cytocentrifuge cup were assembled, held together with a slide holder clip, and positioned firmly in the cytocentrifuge rotor. The incubated cells in the round bottom tube were mixed gently with 60 μL of DMSO, incubated for 5–10 min, and suspended. Eighty microliters of cell suspension was loaded to the cytospin cup and spun at 600 rpm for 5 min. This step was repeated by turning the slide and card over simultaneously to prepare the second spot. The slide was placed on a rack for 10 min at room temperature to dry the cells. The slide was fixed in methanol for 10 min and dipped 10 times in orange (Diff-Quik solution 1) stain followed by 10 times in blue (Diff-Quik solution 2) stain. The stained slide was washed in tap water and washed again in distilled water. The slide was immediately placed on paper tissues and covered with paper tissues to blot away any residual moisture. The slide was dried overnight at room temperature in a fume hood.

The dried slide was placed on paper tissues in a fume hood. Two drops of mounting solution were added to the spots and a coverslip