COMPLEMENTARITY OF STANDARD CYTOGENETIC ASSAYS

Slavka Ibrulj[1,2], Sanin Haverić[2*], Anja Haverić[2]

[1] Faculty of Medicine, University of Sarajevo,
Čekaluša 90, 71 000 Sarajevo, Bosnia and Herzegovina

[2] Institute for Genetic Engineering and Biotechnology,
Gajev trg 4, 71 000 Sarajevo, Bosnia and Herzegovina

[*] Corresponding author

Abstract

Standard cytogenetic assays used in genotoxicology usually include chromosome aberrations analysis and micronucleus cytokinesis-block assay. Both tests originate on standard protocol for lymphocyte culture and can be used as complement or substitute to each other. Aim of this study was to evaluate complementarities between results of chromosome aberration analysis assay and results of micronucleus cytokinesis-block assay in representative sample of inhabitants from Bosnia and Herzegovina. The aim was achieved by calculating Pearson correlation coefficient and simple linear regression.

KEY WORDS: lymphocytes culture, micronuclei, chromosome aberrations

Introduction

Cytogenetic tests in human lymphocytes culture are the most frequently applied in genetic toxicology. Many of these tests are used for testing of genotoxic potential of chemical agents (1), drugs (2), as well as genotoxicological monitoring of populations environmentally (3) or professionally exposed to various mutagens (4, 5). Chromosome aberrations analysis and micronucleus cytokinesis-block assay, as standard cytogenetic tests, can be used as complement or substitute to each other. These tests are also used in cancer risk assessment studies (6). Both tests originate on standard protocol for lymphocyte culture (7). Chromosome aberrations analysis assay presents method of detection and scoring of structural and numerical chromosome aberrations in metaphase spreads. As micronuclei derive from chromosomal fragments and whole chromosomes lagging behind in anaphase, this assay can be used to show both clastogenic and aneugenic effects (6, 8). Micronucleus technique is a simple cytogenetic method proposed for measurement of chromosomal damage in mitogen-stimulated human lymphocytes (9). Aim of this study was evaluation of the correlation between results of chromosome aberration analysis assay and results of micronucleus cytokinesis-block assay in representative sample of inhabitants from Bosnia and Herzegovina.

Materials and Methods

Study was conducted over peripheral blood samples collected from three local Bosnian populations. Examined group included 84 persons (55% males and 45% females), 46% of them were smokers, average age was 39,13 years. Preparation of whole blood cultures as well as harvesting of cells was carried out according to standardized procedures. General points of applied cytogenetic tests are presented in Table 1. Microscopic observation of each sample including analysis of 100 metaphases for chromosome aberrations analysis assay and 1000 binuclear cells for micronucleus cytokinesis-block assay was performed on 1000x magnification on Olympus BX51 microscope. Two experienced scorers performed microscopic analysis. Chromosome aberrations were detected and classified according to International System for Human Cytogenetic Nomenclature (10). In total structural chromosome aberrations frequencies of metaphases with chromatid and chromosome breaks, acentric fragments and dicentric chromosomes were calculated. Gaps were not scored as chromosome aberrations. In total numerical chromosome aberrations all deviations of normal human chromosome complement (2n=46) were registered. Detailed description of the scoring criteria for the micronucleus cytokinesis–block assay used in this research defined Fenech *et al.* (11). Micronuclei frequencies were calculated in binucleated lymphocytes. In order to determine linear relationship among results of conducted cytogenetic assays, Pearson correlation coefficient and simple linear regression, conducted by *Winks 4.5 Professional software* (TexaSoft, Cedar Hill, TX) were applied. Pearson correlation coefficient and simple linear regression are measures of the linear relationship strength between two variables, in case of this research, frequencies of chromosome aberrations and micronuclei. Applied statistical methods are conducted over our previously published results (12, 13, 14).

	Chromosome aberrations analysis assay	Micronucleus cytokinesis-block assay
Culture medium	RPMI 1640 medium with L-glutamine, 20% of fetal bovine serum, PHA and antibiotics (GIBCO-Invitrogen)	
Incubation period	**48 hours** Addition of colcemid (GIBCO-Invitrogen) in 47th hour of cultivation	**72 hours** Addition of cytochalasin B (Sigma) in 45th hour of cultivation
Cell harvesting, slide preparation	0,075 M KCl hypotonic treatment, fixation with ethanol-glacial acetic acid (3:1)	Fixation with ethanol-glacial acetic acid (3:1)
Staining	5 % Giemsa stain in phosphate (Gurr) buffer	7,10±1,73
Scoring	At 100 metaphases	At 1000 binuclear cells

TABLE 1. General points of applied tests

Results and Discussion

Significant positive correlation was determined by Pearson correlation coefficient for frequencies of structural chromosome aberrations and binuclear cells with micronuclei ($p<0,001$). The same was determined for frequencies of numerical chromosome aberrations and binuclear cells with micronuclei ($p<0,001$). Comparing frequencies of total (structural + numerical) chromosome aberrations and frequencies of binuclear cells with micronuclei determined correlation coefficient was also significant ($p<0,001$). Linear relationship between the frequencies of binuclear lymphocytes with micronuclei and frequencies of lymphocytes metaphase spreads with structural and numerical chromosome aberrations are shown in Figures 1. and 2. Our results match previously published results of similar studies and reported positive correlations between micronuclei frequency and

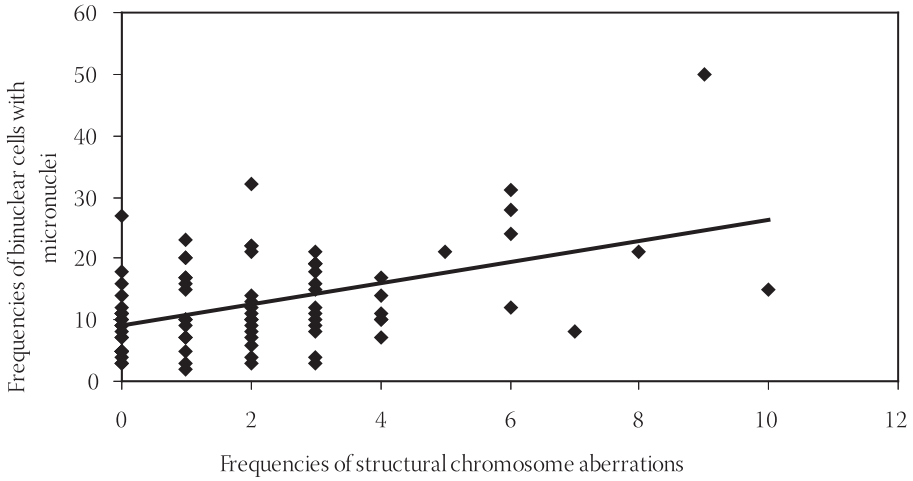

FIGURE 1. Scatterplot of simple linear regression between frequencies of binuclear cells with micronuclei and structural chromosome aberrations

the frequency of specific chromosome aberrations, such as acentric fragments and dicentric chromosomes (15). Due to its easiness and rapidness (16) as well as sensitivity (17), micronucleus cytokinesis-block assay can be used as an alternative of chromosome aberrations analysis if rapid results are needed. However, usage of both these tests simultaneously reduces likelihood of incorrect results.

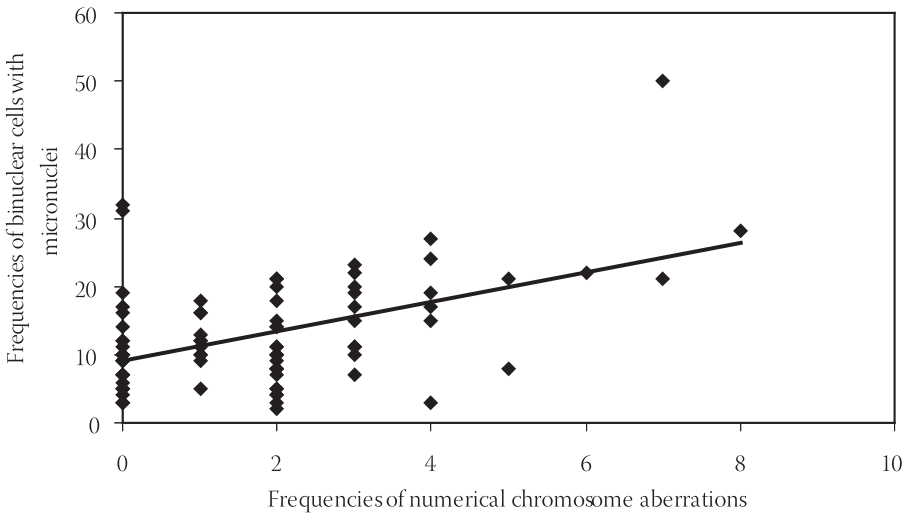

FIGURE 2. Scatterplot of simple linear regression between frequencies of binuclear cells with micronuclei and numerical chromosome aberrations

Conclusion

Applied statistical methods in study sample confirm complementarities of standard cytogenetic assays: chromosome aberrations analysis and micronucleus cytokinesis-block assay. Results of this study affirm validity and usage of both assays either independently or simultaneously in genotoxicological studies. This conclusion is additionally supported by the fact that both tests need the same infrastructure and conditions for cell culturing, harvesting, slides preparation and analysis.

References

(1) Zeiger E., Bonassi S., Chang W.P., Holland N., Kirsch–Volders M., Fenech M. Effect of smoking habit on the frequency of micronuclei in human lymphocytes: Results from the Human micronucleus project. Environ. Mol. Mutagen. 2003; 41 (3): 215.

(2) Ibrulj S., Rahmanović A., Haverić S., Haverić A., Durmić-Pašić A. Cytogenetic evaluation of paracetamol effects in human lymphocytes culture. Drug Chem. Toxicol. 2007; 30(2): 133-143.

(3) Schroder H., Heimers A., Frentzel-Beyme R., Schott A., Hoffmann W. Chromosome aberration analysis in peripheral lymphocytes of Gulf War and Balkans War veterans. Radiat. Prot. Dosimetry. 2003; 103 (3): 211–219.

(4) Neronova E., Slozina N., Nikiforov A. Chromosome alterations in cleanup workers sampled years after the Chernobyl accident. Radiat. Res. 2003; 160(1): 46–51.

(5) Lebana J.J., Patwardhan U.N, Samuel A.M. Frequency of micronuclei in peripheral blood lymphocytes from subjects occupationally exposed to low levels of ionizing radiation. Mutat. Res. 2004; 564(1): 83–89.

(6) Tucker J.D., Preston R.J. Chromosome aberrations, micronuclei, aneuploidy, sister chromatid exchanges, and cancer risk assessment. Mutat. Res. 1996; 365:147-159.

(7) Moorhead P.S., Nowell P.C., Mellman W.J., Battips D.M.A., Hungerford D.A. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp. Cell. Res. 1960; 20: 613–616.

(8) Norppa H., Falck G.C.M. What do human micronuclei contain? Mutagenesis. 2003; 18(3): 221-233.

(9) Fenech M., Morley A.A. Measurement of micronuclei in lymphocytes. Mut. Res. 1985; 147(1–2): 29–36.

(10) Mitelman F. ISCN, An International System for Human Cytogenetic Nomenclature, Cytogenetics and Cell genetics, S. Karger, Basel, 1995.

(11) Fenech M., Chang W.P., Kirsch-Volders M., Holland N., Bonassi S., Zeiger E. HUMN project: detailed description of the scoring criteria for the cytokinesis–block micronucleus assay using isolated human lymphocyte cultures. Mutat. Res. 2003; 534(1–2): 65–75.

(12) Krunić A., Ibrulj S., Haverić S. Micronuclei frequencies in peripheral blood lymphocytes of individuals exposed to depleted Uranium. Arh. Hig. Rada. Toksikol. 2005; 56: 227-232.

(13) Ibrulj S., Haverić S., Haverić A., Durmić-Pašić A., Marjanović D. Effect of war and postwar genotoxins on micronuclei frequency in Sarajevo study group. Bosn. J. Basic Med. Sci. 2006; 6(4): 54-57.

(14) Ibrulj S., Haverić S., Haverić A. Chromosome aberrations as bioindicators of environmental genotoxicity. Bosn. J. Basic Med. Sci. 2007; 7(4): 311-316.

(15) Garaj–Vrhovac V., Fucić A., Horvat D. The correlation between the frequency of micronuclei and specific chromosome aberrations in human lymphocytes exposed to microwave radiation in vitro. Mutat. Res. 1992; 281(3): 181–186.

(16) Miller B., Pötter-Locher F., Seelbach A., Stopper H., Utesch D., Madle S. Evaluation of the in vitro micronucleus test as an alternative to the in vitro chromosomal aberration assay: position of the GUM working group on the in vitro micronucleus test. Mutat. Res. 1998; 410(1): 81-116.

(17) El-Zein R.A., Schabath M.B., Etzel C.J., Lopez M.S., Franklin J.D., Spitz M.R. Cytokinesis-blocked micronucleus assay as a novel biomarker for lung cancer risk. Cancer Res. 2006; 66: 6449-6456.