INFLUENCE OF BASIC VARIABLES ON MICRONUCLEI FREQUENCY AND CHROMOSOMAL ABERRATIONS IN GENERAL POPULATION OF FB&H

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Abstract

The aim of this study was to determine the values of micronuclei (MN) and structural chromosomal aberrations (CA) in peripheral blood lymphocytes from 200 healthy participants of both genders from general population of FB&H, as well as to explore the influence of gender and age on MN and CA frequencies. Standard protocols for MN test, cultivation and micronuclei analysis from peripheral blood binuclear lymphocytes have been applied. MN values ranged from 0 to 8 MN per 1000 binuclear cells. The results suggest that gender and age influence MN frequency, with pronounced effect on 2 MN frequencies. Females on average have higher values of all observed variables of MN test than men. We have also found significant effect of gender – females had increased number of CAs – chromatid type; and of the age in both genders. Frequency distribution of CTAs and CSAs between male and female groups showed predominance of CTAs over CSAs, independently of gender. The results of this study will be incorporated into reference data base for comparative research in future.

Key words: *micronuclei, structural chromosomal aberrations, gender, age*

Introduction

Bio-monitoring is important part of health care for population that is either professionally or environmentally exposed to physical and chemical mutagens and/or carcinogens. It is based on measurement (or) determination of frequencies of different markers that point at the earliest, repairable bioindicators, that appear before the malignancies and/or other diseases (Kopjar et al., 2010). The main postulate for biomarker efficiency is the knowledge of its values in general, unexposed, healthy population.

Micronucleus test (MN) in peripheral blood lymphocytes is one of the most important methods in cytogenetic bio-monitoring. Besides MN-test, analyses of structural chromosomal aberrations (CA) and analysis of sister chromatid exchange (SCE) are important, and those are applied in the surveillance of professionally exposed populations.

CAs include chromosomal breaks and exchanges visible in cells arrested in metaphase-stage They are usually classified into chromosome-type aberrations (CSAs) and chromatid-type aberrations (CTAs), which can be distinguished morphologically (Collins, 2004).

MN test detects chromosomal aberrations indirectly through nucleus chromatin loses that

lead to MN formation in cell cytoplasm. MN are defined as small, round cytoplasmic bodies, that consist of DNA and are formed during cell division from either acentric chromosomal fragments or whole chromosome that are left behind during anaphase. In vitro MN test is reliable test for the detection of mutagenic factors (Krishnaja and Sharma, 2004). It is also reliable for the detection of genome instability that is related to increased risk of malignancies. Causal relation among increased number of MN and some malignancies and other diseases (diabetes and cardiovascular disease) is evident (Fenech et al., 2011; Andreassi et al., 2011). That justifies the use of MN test as suitable biomarker for long-term estimation of cytogenetic risk among human population.

In estimation of damaging and risk factors on people health, it is extremely important to have a database with biomarkers values measured or determined on large number of individuals from general (healthy) population. Regeneration of database with fresh data is recommended (every two years). According to the leading experts' recommendation, the database should include at least 20 individuals per gender per decade of age (Fenech, 1993). That objective was fulfilled by completion of this study.

The aim of this study was to determine the values of MN test and CA from peripheral blood lymphocytes in 198 healthy individuals from general population of Federation of Bosnia and Herzegovina (FB&H), with the inclusion of both genders.

Materials and methods

Study populations

The study as well as all procedures of blood sampling and handling in laboratory conditions, were performed according to ethical principles and directions for bio-monitoring of human populations. The subjects were informed about the study aim; each subject fulfilled questionnaire with the basic data needed for the study and gave his/her written consent.

The main inclusion criterion was that the subject has not been exposed to physical or chemical agents, has no acute infections or medical exposures to known agents that could interfere with cytogenetic findings. 200 examinees entered the study: 20 per gender per decade of their age. Two men had to be excluded from the study due to previous medical treatment.

Methods

Standard protocol of MN test for cultivation and micronuclei analysis from peripheral blood binuclear lymphocytes was applied. Per each sample, 1000 binuclear lymphocytes were analyzed and total number of MN, number of cells with MN and their distribution (number in cells) determined. Micronuclei were determined in accordance with recommended criteria of HUMN (Human Micro Nucleus) Project (Fenech et al., 2003). Conventional Moorhead method was used on short-term cultures for 48 hr, with all cells being in the first division (Moorhead et al., 1960). Slides from each culture were numbered and anonymously scored. At least 200 well-spread metaphases with 46 ± 1 centromeres were examined. CAs were further subclassified as CSAs (including chromosome-type breaks, ring chromosomes, marker chromosomes, and dicentrics) and chromatid-type aberrations (CTAs; including chromatid-type breaks). Gaps were not scored as aberrations.

Statistics

All variables were expressed as medians and interquartile ranges for continuous data with or without normal distribution, respectively. Nonparametric data were compared between groups using the independent samples Mann–Whitney U-test. Additionally Spearman's correlation was used as measure of association

for continues variables. P-value <0.05 was considered statistically significant. All statistical analyses were performed using the computer software Statistical Package for the Social Sciences, version 20.0 (SPSS, Chicago, IL).

Results and discussion

The study population comprised of 198 participants, 98 males and 100 females divided in 5 age groups (20-29, 30-39, 40-49, 50-59, and 60-69). In the total group MN median is determined to be (0.00-2.00), average 1.05±0.07, while individual values ranged from 0 to 8 MN per 1000 binuclear cells. Median for cells with one MN (1 MN) was 2.00, while for two MN (2 MN) was 0.00.

In 21 female and 29 male samples no MN were found. Out of total female samples (n=100), cells with 1 MN were found in 79 of them. While in the male group (n=98), cells with 1 MN were found in 69 samples (Table 1). Cells with 2 MN were found in 25/100 female samples, and in only 12/98 male participants (Table 2). Correlation between gender and MN, for cells with 1 MN showed rho=0.135 p=0.059, while the values for cells with 2 MN were rho=0.170 p=0.017. Thus, the correlation between gender and 2 MN is stronger.

Relation between age and number of cells with MN is statistically significant p<0.000. For each additional year of age the risk of having 1MN (odds ratio) increases by additional 12%, 95% CI (8-16%). For each additional year of age the risk of having 2 MN increases by 6%, 95% CI (3%-10%). Risk for 2 MN is 3 x higher in females, 95% CI (from 1 to 6 times).

As shown in Figure 1 increased frequency of CAs correlates with age in both genders, whereas in females the peak of CAs occurrence is around the age of 50, which may be linked to with the changes in hormonal status and adjustment for menopause. In males, CAs frequencies peaked at the age of 60 and all were statistically significant according to Spearman's correlation (p<0.01; rho=0.463; rho=0.470; rho=0.455; respectively). Significant positive correlation between the age and number of aberrations within all groups in healthy population is evident. (rho=0.462; p<0.01)

Mann-Whitney U test was used to determine the significance of CA frequency between male and female groups. Number of aberrations in female group is 1.0 (0.0-2.0) is higher than number of aberrations in male group 0.0 (0.0 – 2.0). This difference is statistically significant (p=0.046) (Figure 2).

Table 1. Analysis of the influence of gender status on frequencies of cells with 1 MN

			Cells with 1 MN								Total
			,00	1,00	2,00	3,00	4,00	5,00	6,00	8,00	
gender	male	Number	29	23	22	15	6	3	0	0	98
		%	29,6%	23,5%	22,4%	15,3%	6,1%	3,1%	,0%	,0%	100,0%
	female	Number	21	23	22	15	9	5	4	1	100
		%	21,0%	23,0%	22,0%	15,0%	9,0%	5,0%	4,0%	1,0%	100,0%
Total		Number	50	46	44	30	15	8	4	1	198
		%	25,3%	23,2%	22,2%	15,2%	7,6%	4,0%	2,0%	,5%	100,0%

Table 2. Analysis of the influence of gender status on frequencies of cells with 2 MN

			Cells with 2 MN				Total
			,00	1,00	2,00	3,00	
gender	male	Number	86	10	1	1	98
		%	87,8%	10,2%	1,0%	1,0%	100,0%
	female	Number	75	16	7	2	100
		%	75,0%	16,0%	7,0%	2,0%	100,0%
Total		Number	161	26	8	3	198
		%	81,3%	13,1%	4,0%	1,5%	100,0%

We have found significant effect of gender – female had increased number of CAs – chromatid type, and of the age in both genders.

Micronuclei in healthy population lymphocytes are indicators of genome damage in single cell accumulated over the years, and the mutations that appear in the first in vitro cell division (Kopjar et al., 2010). Today, cytogenetics provides numerous biomarkers for evaluating chromosomal instability and scoring of MN in lymphocytes is one that draws a lot of interest (Milošević-Djordjević et al., 2012). In this study we have analyzed the level of cytogenetic damage in healthy population from Bosnia and Herzegovina, addressing both genders and within the range of age approaching of professionally exposed and actively working population. That population could be controlled using MN-test and CA, in the future. Contrary to the published studies, in our research we did not count the total number of micronuclei (1+2 MN) they were rather been given as number of cells with 1 MN and number of cells with 2 MN. Considering that the subject group included healthy individuals with no medical record of acute infections or medical exposures or records of exposure to chemical and/or physical agents in the months preceding the study the influence of large number of environmental factors on single MN test values were minimized. Therefore gender and age were considered factors with largest influence on MN formaiton (Fenech and Bonassi, 2010; Battershill et al., 2008).

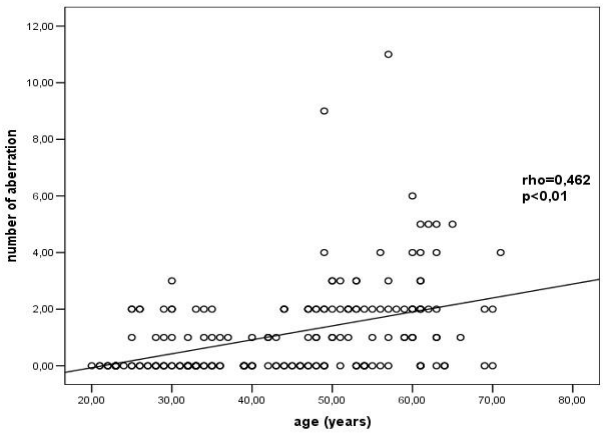

Figure 1. Relation among age and number of aberrations at all groups.

Our research, as well as others presented, show that females have higher MN count than males, on average. According to Fenech (Fenech, 1998) the frequency of MN in females is 1.2 to 1.6 times higher. Our research shows that females may have up to 3 times more cells with 2 MN than males, while this ratio is smaller when 1 MN is considered. One of the reasons

why females do have increased frequency of MN is the loss of one of X chromosomes through MN (Bolognesi et al., 2006; Kažimirova et al., 2006).

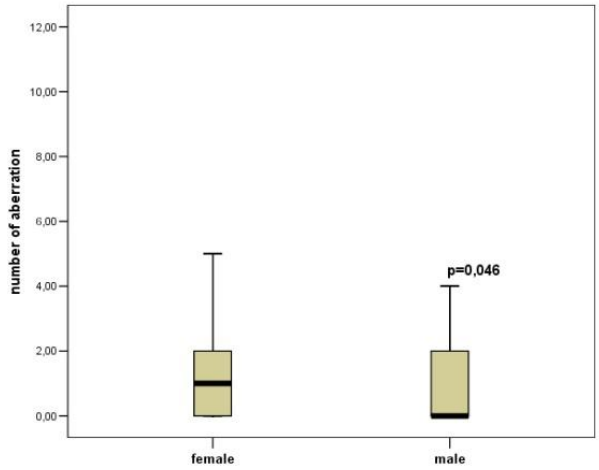

Figure 1. Relation among number of aberrations at both genders.

Positive correlation among MN frequency and age was reported in numerous studies (Veerachari et al., 2011; Nefic and Handzic, 2013; Jones et al., 2012). According to our data, age had statistically significant influence on the counts of cells with both 1 and 2 MN. The research showed that each additional year of age adds additional 6% to the risk of increase of number of cells with 2 MN, 95% CI, while that percentage is 12%, even higher when considering cells with 1 MN. Milosevic-Djordjevic et al. (2012) showed a decrease of MN frequencies in groups of older age and explained that this might be the result of declining in proliferation capacity of cells with aging. Norppa and Falck (2003) upon extensive analysis of numerous studies results, concluded that 30% to 80% of spontaneously arisen MN comprised whole chromosomes. In contrast to sex chromosomes that are more often lost through MN, autosomes appear randomly in MN and cannot be lost by ageing only (Norppa and Falck, 2003; Bukvic et al., 2001). It has also been noted that apparently healthy individuals may have large number of MN containing specific chromosome (Kopjar et al., 2010). We have found significant effect of gender – female had increased number of CAs – chromatid type, and of the age in both genders. Our results concur with other investigations done recently in other European countries, though we were not able to correlate CAs frequency and cancer risk due to inability to establish long-term follow-up. Frequency distribution of CTAs and CSAs between male and female showed predominance of CTAs over CSAs, independently of gender. An increase in chromosomal aberrations may be due to either genetic or acquired conditions conferring higher susceptibility to genetic damage. Elevated levels of chromosomal aberrations in peripheral blood lymphocytes may be seen as an indicator of an early phase of carcinogenesis, where various genetic alterations are also generated in different tissues (Mitelman et al., 2004).

Upon present experience and knowledge, MN test has great advantage over other cytogenetic tests since it allows damage estimation at functional level and integration of mitotic spindle, which other methods cannot achieve (Bolognesi et al., 2006). It should be pointed out that MN-test is useful in genome instability discovery that is in relation with increased cancer risk (Fenech and Crott, 2002). According to Bonassi research, this study provides preliminary evidence that MN frequency in peripheral blood lymphocytes is predictive of cancer risk, suggesting that increased MN formation is associated with early events in carcinogenesis (Bonassi et al., 2011).

Conclusions

The results of MN test and CA analyzed over age and gender obtained in this study are in accordance with the results determined on general healthy population in other research. These results will facilitate construction of research database for multiple genetic

categorization and for comparing and interpreting analysis in further research projects, medical diagnostics, control and bio-monitoring for professionally and environmentally exposed populations and others. That is the applicable character of this research. Also, the results are the ground for further development of laboratory database.

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