Effect of War and Postwar Genotoxins on Micronuclei Frequency in Sarajevo Study Group

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Abstract

During the 1992 – 1995 siege, as well as after the war activities, citizens of Sarajevo were most probably exposed to various potential genotoxic agents. The effects of those potential genotoxins were evaluated by micronucleus – cytokinesis blocked assay. The study included 30 individuals who resided in the area of Sarajevo during the war and the postwar period. Point bi-serial coefficient analysis did not reveal any relationship between the frequencies of binuclear cells with micronuclei as well as total number of micronuclei and smoking habits or gender. Simple linear regression revealed statistically significant positive correlation between the age and micronuclei formation. Due to the war related environmental contamination more extensive study is recommended.

KEY WORDS: war related genotoxins, binuclear lymphocytes, micronuclei

Introduction

According to The Study of the Battle and Siege of Sarajevo average daily shell impact, over the course of the siege, is estimated at approximately 329 (1). During the NATO air strikes in 1994 and 1995 localities near Sarajevo were also hit by radioactive shells containing depleted uranium. The United Nations Environment Programme, in their 2003 report, ascertained increased radioactivity in Hadžići near Sarajevo. Additional concern is the fact that six NATO targets, surrounding Sarajevo, were not examined as their coordinates are not available (2). Elimination of depleted uranium particles is a long and difficult process, thus estimations that the amount of

contaminators present is still high. Among other detrimental effects to health, depleted uranium was identified as an oncogene-inducing factor in both *in vitro* and *in vivo* experiments (3). The first reported neoplastic transformation of human osteoblasts in depleted uranium containing cell culture (4) confirms the risk of depleted uranium mediated cancer induction in vivo. In 1998, an increase in frequency of malignant tumors was detected in Sarajevo Canton. Also, a significant increase in individuals with malignant diseases was established after 2000, the most common being lung and breast cancers (5). Micronuclei are formed from fragments or whole chromosomes, which did not reach spindle poles during mitosis and remained encapsulated as separate nuclei. Micronuclei are a consequence of both clastogenic and aneugenic agents activity (6, 7). Peripheral blood lymphocytes are practical for observation of cytogentic biomarkers, such as micronuclei, even several years after the exposure. Due to the life span of human T-lymphocytes (estimated at 1,5 to 10 years) micronuclei formation in those cells provide information on genetic damage accummulated during their lifetime (8, 9). Neronova et al. (10) analyzed chromosome aberrations in peripheral blood lymphocytes of nuclear bombing survivors as well as Chernobyl workers and confirmed that lymphocytes are suitable biological models for longer periods of time. Numerous studies revealed that cytogenetic alterations could be consequence of exposure to chemicals or radiation (6). In the present study we aimed at the evaluation of genetic load, expressed as micronuclei formation in peripheral blood lymphocytes of individuals potentially exposed to various war and post-war genotoxins.

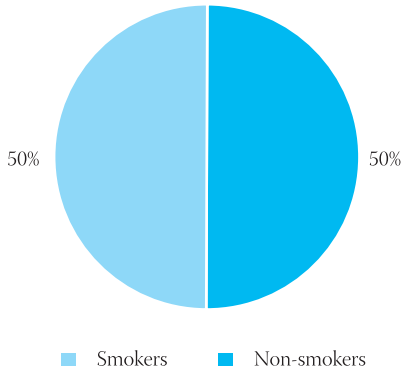

FIGURE 1. Smokers – non-smokers ratio

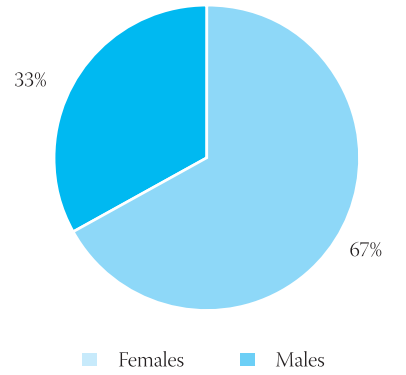

FIGURE 2. Gender ratio in the study group

MATERIAL AND METHODS

STUDIED POPULATION

This research was approved by the Scientific Council of the Institute for Genetic Engineering and Biotechnology as well as Federal Ministry of Science and Education, Federation of Bosnia and Herzegovina (grant number: 04-39-8310-1/01). Presented research was conducted on human peripheral blood of 30 individuals that were living in the area of Sarajevo during the war and postwar period. Volunteers who previously underwent radiotherapy or chemotherapy, who were exposed to diagnostic X-rays, and those using prescription, over-the-counter or recreational drugs were excluded from the study. The ratios of smokers vs. non-smokers and males vs. females, are presented in figures bellow (Figures 1, 2). Average age of individuals was 32,37 years.

LYMPHOCYTE CULTIVATION AND PREPARATION OF SLIDES

Peripheral blood was collected in heparinized tubes (BD Vacutainer Systems, Plymonth, UK) by venipuncture during 2002 and 2003. Whole-blood cultures were set up in 15-ml sterile, plastic tubes with conical bottom (NUNC, Rochester, NY) containing RPMI 1640 cultivation medium with L-glutamine, 20% of fetal bovine serum, phytohaemaglutinin and antibiotics (Gibco-Invitrogen). Micronucleus cytokinesis-block test, a reliable assay and precise method for evaluation of chromosomal damage, was performed. At the 45th hour of PHA stimulation, cytochalasin B (Sigma-Aldrich, St. Louis, MO), cytokinesis blocker, was added to the final concentration of 4,5 ug/ml. The cells were harvested by centrifugation at the 72nd hour of cultivation, resuspended in prewarmed hypotonic solution (0,075 M KCl) and, after the

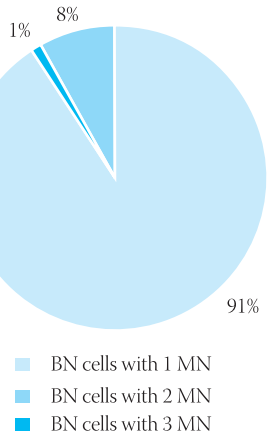

FIGURE 3. Distribution of binucleated lymphocytes with micronuclei in the Sarajevo study group

centrifugation, fixed in acetic-alcohol. The remaining lymphocytes suspended in fresh cold fixative were dropped at cold and dry microscopic slides. Air-dried slides were stained with 5% Giemsa stain (Carlo Erba, Milan, Italy) and analyzed under microscope at 400x magnification. Micronuclei (MN) occurrence in 1000 binuclear (BN) lymphocytes were registered and statistically defined. Criteria applied in identification of binuclear lymphocytes and micronuclei are defined by Fenech et al. (11).

STATISTICAL ANALYSIS

The results for the study group were expressed as arithmetic means (X_{av}) and variability measures (standard deviation - s, standard error of the mean -sX_{av}, and coefficient of variation - V). The statistical analysis included point bi-serial correlation coefficients and simple linear regression calculated using *Winks 4.5 Professional* software (TexaSoft, Cedar Hill, TX). Correlations between binuclear cells count with micronuclei and micronuclei frequencies and gender as well as cigarette smoking were tested by the point bi-serial correlation coefficient. Simple linear regression was applied in order to determine the association between age and the frequency of micronuclei and binuclear cells with micronuclei.

RESULTS

The frequencies of binuclear cells with micronuclei, total number of micronuclei in 1000 binuclear cells per sample as well as aritmetic means and variability measures are sumarized in Table 1. Individual number of binuclear lymphocytes with micronuclei varies from 3 to 31 per 1000 binuclear cells. The mean is 10,967. Absolute frequency of micronuclei in binuclear cells varies from 3 to 38, with the mean of 12. Distribution

SAMPLE	BNMN/1000 CELLS	MN/1000 CELLS
1	9	9
2	14	15
3	9	10
4	20	21
5	9	9
6	10	10
7	6	6
8	10	12
9	11	12
10	12	13
11	5	5
12	14	17
13	11	11
14	11	12
15	10	10
16	12	12
17	10	12
18	8	8
19	4	4
20	21	24
21	7	8
22	17	20
23	31	38
24	5	5
25	7	8
26	4	4
27	7	7
28	27	30
29	5	5
30	3	3
Σ	329	360
X_{av}	10,967	12
SD	6,594	7,922
sX_{av}	1,204	1,446
V	60,126	66,017

TABLE 1. Individual results and statistical parameters of micronucleus assay for the Sarajevo study group

of binuclear cells with one, two and three micronuclei in the Sarajevo study group is presented in Figure 3. The observed micronuclei frequencies were compared with the criteria for spontaneously induced micronuclei (4,4 ± 2,6 per 500 binucleated cells) given by Fenech and Morley (12). According to these criteria 5 individuals within the study population expressed an increase in frequency of binuclear cells with micronuclei. The point bi-serial correlation coefficient was applied in order to determine correlations between the binuclear cells with micronuclei and micronuclei frequencies with gender and cigarette smoking. Point bi-serial cor-

relation coefficient revealed no statistical significance neither between frequencies of binuclear cells with micronuclei and cigarette consumption nor between micronuclei frequencies and cigarette smoking. The same analysis discovered no statistically significant correlation between gender and binuclear lymphocytes with micronuclei, nor between gender and total number of micronuclei. The estimation of correlation between ages and frequencies of binuclear lymphocytes with micronuclei as well as with total number of micronuclei was achieved by analysis of simple linear regression. This analysis revealed statistically significant, positive, linear association ($r = 0{,}5716$; $p < 0{,}001$) between age and binuclear cells with micronuclei as well as between age and micronuclei frequency ($r = 0{,}5526$; $p < 0{,}001$).

Discussion

Environmental pollution, as a consequence of industrial development, affects genetic load of all living creatures. Thus, regular genotoxicological evaluations in populations exposed to increased risks are recommended and especially emphasized by the fact that 25% of inhabitants of developed countries die from malignant diseases (13).

Although citizens of Sarajevo were exposed to products of ammunition degradation, contaminated food, water and pharmaceuticals, when we compared results with our previous findings we verified that Sarajevo study group has lower frequency of micronuclei that human group exposed to depleted uranium. At the same time, Sarajevo study group shows an increase in micronuclei frequencies in comparison with the group of inhabitants that were not affected by any kind of war activities (14). Analysis of variance (ANOVA) followed by pair-wise comparisons with Newman-Keuls multiple comparison test was applied in order to evaluate significance of differences between arithmetic means of binuclear cells with micronuclei and micronuclei frequencies in Sarajevo study group and two previously described human groups (individuals living in the depleted uranium contaminated area and individuals notexposed to the war-related activities). No statistically significant difference was found between Sarajevo and group of non-exposed individuals, but the differences between these two groups and the group of individuals exposed to depleted uranium are significant both for binuclear cells with micronuclei count ($F = 4{,}09$; $p = 0{,}0201$) and for the total number of micronuclei ($F = 3{,}81$; $p = 0{,}026$).

Conclusion

The results of this research could present initial foundations for further genotoxicological investigations of Sarajevo population. Nevertheless, due to the war related environmental contamination and significant increase in malignant diseases, more extensive studies are recommended.

References

(1) Bassiouni M.C. Final report of the United Nations Commission of Experts established pursuant to Security Council resolution 780 (1992) United Nations; 1994

(2) United Nations Environment Programme (UNEP). Depleted uranium in Bosnia and Herzegovina. Geneve: United Nations Environment Programme; 2003

(3) Duraković A. Medical Effects of Internal Contamination with Uranium. Croat. Med. J. 1999; 40(1): 49-66

(4) Miller A.C., Blakely W.F., Livengood D., Whittaker T., Xu J., Ejnik J.W., Hamilton M.M., Parlette E., John T.St., Gerstenberg H.M., Hsu H. Transformation of human osteoblast cells to the tumorigenic phenotype by depleted uranium-uranyl chloride. Environ. Health Perspect. 1998; 106(8): 465–471.

(5) Obralić N., Gavrankapetanović F., Dizdarević Z., Durić O., Šišić F., Selak I., Balta S., Nakaš B. The number of malignant neoplasm in Sarajevo region during the period 1998–2002. Med. Arh. 2004; 58(5): 275–278.

(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) Fenech M. Important variables that influence base-line micronucleus frequency in cytokinesis-bloked lymphocytes – a biomarker for DNA damage in human populations. Mutat. Res. 1998; 404:155-165.

(8) Fenech M. The cytokinesis-block micronucleus technique: A detailed description of the method and its application to genotoxicity studies in human populations. Mutat. Res. 1993; 285:35-44.

(9) Bogen K.T. Reassessment of human peripheral T-lymphocyte lifespan deduced from cytogenetic and cytotoxic effects of radiation. Int. J. Radiat. Biol. 1993; 64:195-204.

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

(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) Fenech M., Morley A.A. Measurment of micronuclei in lymphocytes. Mutat. Res. 1985; 147(1-2):29-36

(13) Fučić A. Chemical carcinogens [in Croatian]. In: Boranić M. et al. Karcinogeneza – Suvremena gledišta o podrijetlu malignih tumora. Zagreb: Medicinska zaklada; 2000. p.117-141

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