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Incidence of micronuclei in pregnant women and cord blood samples before and after the bombing of Serbia

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ABSTRACT

BACKGROUND: *This study provides the data regarding monitoring of population using CB-micronuclei assay in the period 1995-2001 in Serbia. The target groups consisted of 45 pregnant women of mean age 34.3(6.56) years, unaware of being exposed to chemicals drugs or other substances and undergoing cordocentesis. The incidence of micronuclei (MN) in peripheral blood lymphocytes and in fetal cord blood lymphocytes was analyzed.*

METHODS: *The study was carried out on cultures of PHA-stimulated blood lymphocytes. Three drops of blood samples ware added into 5ml RPMI-1640 (Gibco) medium supplemented with 15% of calf serum and PHA (Gibco, 2.5μg/ml). For micronuclei preparation the cytokinesis block method was used (Fenech et al., 1993).*

RESULTS: *The results of the study showed that in the year 1995, the incidence of micronuclei in pregnant women was 9.61(3.26) per 1000 binucleated (BN) cells, and 3.74(1.60) in cord blood samples per 1000 BN cells, respectively. In 2000 the incidence of micronuclei in study group was 28.26(7.87) per 1000 BN cells, and in cord blood samples 22.22(5.63) per 1000 BN cells. One year later (2001) the incidence of micronuclei in pregnant woman slightly decreased and reached the value of 26.98(4.50), while in cord blood it slightly increased up to 26.58(6.85) per 1000 BN cells.*

CONCLUSION: *The monitoring data obtained in this study have shown significantly increase of micronuclei (2- to 3-fold) in study groups in 2000 and 2001.*

KEY WORDS: *Pregnancy; Fetal Blood; Micronuclei; Lymphocytes; Radiation Effects; War; Yugoslavia*

INTRODUCTION

Micronuclei (MN) assay is one of the most sensitive markers for detecting DNA damage, and has been used in population monitoring as well as in investigation of genotoxicity of a variety of chemicals. MN testing with interphase cells is more suited as a cytogenetic marker because it is not limited to metaphases, and has the advantage of allowing rapid screening of a large numbers of cells than in studies with sister chromatid exchanges (SCE) or chromosome aberrations (CA) (1,2). Micronuclei arise from acentric chromosome fragments or whole chromosomes that are not included in the main daughter nuclei during nuclear division (3,4). Whole chromosomes damaged at the centromeric region or damaged to kinetochore or spindle proteins, may also result in unsuccessful attachment of a whole chromosome with the spindle resulting in a lagging chromosome at anaphase with subsequently increased MN (5,6). Thus, MN provide a measure of both chromosome breakage and chromosome loss and it has been shown to be at least as sensitive an indicator of chromosome damage as a classical metaphase chromosome analysis (7-9). During the bombing of Serbia (March-June, 1999), the highest contamination of the environment happened. The study provided data regarding the monitoring of the health of pregnant women in Belgrade and Pancevo during 1995-2001. The aim of this study was to evaluate potential hazardous effects on human genome.

MATERIAL AND METHODS

The study considered 45 healthy pregnant women, volunteer blood donors, with a normal genetic constitution, living in Belgrade and Pancevo (Serbia).

At the time of blood collecting for cytogenetic determination a personal history questionnaire was administered by a specially trained social assistant. Medical history included data about illnesses, prescription and non-prescription medications, type and number of X-ray diagnoses and other diagnostic procedures performed in the year previous to the time of blood sampling. Questions regarding life style included cigarette smoking, alcohol consumption and dietary habits; work exposure questions included current or past work and spare time exposure to potentially toxic substances.

Fetal cord blood samples were used for the purpose of prenatal diagnosis, according to health criteria (mothers age, familiar anamnesis, and biochemical markers in maternal blood (α-fetoprotein and β-chorionic gonadotropin - β-HCG). Around $2x10^6$ of blood leukocytes were set up in cultures containing medium RPMI-1640 supplemented with 15% of calf serum and 2.4 μg/ml of phytohemagglutinin (Gibco-BRL). For micronuclei preparation the cytokinesis block method was followed (1). Cytochalasin B at a final concentration of 4 μg/ml was added to the samples after 44 hours of culture and incubation was continued for further 24 hours. The micronuclei were prepared according to the method described by Fenech and Morley (10) with minor modification. Cells were washed with 0.9% NaCl, collected by centrifugation and treated with hypotonic solution at 37°C (8 min), consisted of

0.56% KCl + 0.9% NaCl. Cell suspension was prefixed in methanol/acetic acid 3:1, washed 3 times with fixative and dropped on to clean slide. Slides were air dried and stained in Giemsa (2%). Al least 1000 BN cells per sample were scored, registering micronuclei according to the criteria of Countryman and Heddle (11) and Fenech and Morley (10).

Statistics and index calculations. The statistical analysis of each of the parameter of interest was carried out using statistical software package Origin7.

Analysis of the incidence of micronuclei in binucleated cells and the difference in distribution cells was evaluated by Student *t* test.

RESULTS

The results of micronuclei (mean values for micronucleus yield) in 45 pregnant women of mean age 34.3(6.56) years and 45 cord blood lymphocytes are presented on Tables 1-2.

Table 1. The yield of micronuclei in peripheral lymphocytes of pregnant women

	Year 1995 Incidence of MN	Year 2000 Incidence of MN	Year 2001 Incidence of MN
	9.40	28.76	33.86
	8.26	28.06	25.88
	11.34	20.08	29.24
	10.18	19.10	20.81
	6.41	29.80	23.17
	17.30	21.30	23.88
	11.24	47.66	32.72
	7.12	40.73	24.08
	14.38	33.45	21.27
	9.20	30.00	32.20
	7.41	22.50	33.80
	9.11	21.60	24.70
	11.33	25.50	28.20
	4.20	30.60	23.40
	7.40	24.77	27.56
N	**15**	**15**	**15**
Mean	**9.62**	**28.26**	**26.98**
SD	**3.26**	**7.87**	**4.50**
Student *t* test	**11.44**	**13.91**	**23.23**
p-value	**1.73E-08**	**1.37E-09**	**1.40E-12**

Table 2. Incidence of micronuclei in cord blood lymphocytes

	Year 1995 Incidence of MN	Year 2000 Incidence of MN	Year 2001 Incidence of MN
	1.31	36.50	33.86
	3.24	22.84	25.88
	3.16	14.90	29.24
	1.34	17.18	20.81
	2.41	26.00	23.17
	4.38	16.40	23.88
	4.30	26.34	26.46
	5.27	25.57	18.36
	6.15	25.14	18.09
	3.10	25.00	32.72
	3.46	21.57	44.90
	3.21	18.40	22.40
	3.30	19.40	24.08
	4.20	22.60	29.18
	7.35	15.46	25.64
N	**15**	**15**	**15**
Mean	**3.75**	**22.22**	**26.58**
SD	**1.63**	**5.64**	**6.81**
Student *t* test	**8.91**	**15.27**	**15.09**
p-value	**3.82E-07**	**4.02E-10**	**4.71E-10**

The results of this study demonstrated that mean value of baseline MN in peripheral lymphocytes of pregnant females in the year 1995 was 9.62(3.26); in the 2000 it was 28.26(7.87), and in 2001 it was 26.98(4.50).

Mean value of baseline micronuclei in peripheral lymphocytes of pregnant women in 1995 was two-fold lower compared with years 2000 and 2001. Statistically significant difference in baseline micronuclei was observed between the period 1995-2000 (t =-8.479; p=3.21E-9) and period 1995-2001 (t=-12.108; p=1.20E-12). There is no significant difference in baseline micronuclei incidence between years 2000 and 2001(t = 0.545; p= 0.589) (Figure 1).

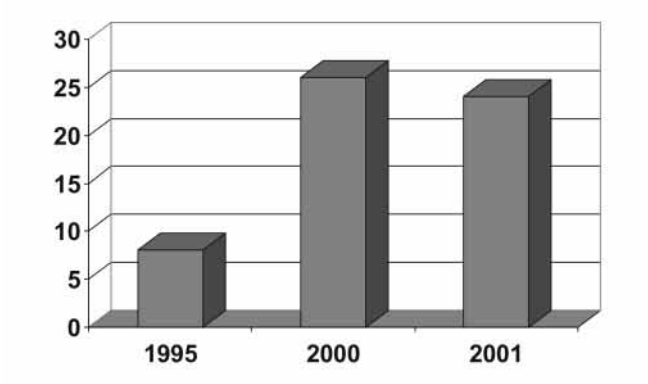

Figure 1. The yield of micronuclei in peripheral lymphocytes of pregnant women

Mean value of the incidence of micronuclei in cord blood lymphocytes per 1000 binucleated cells in 1995 was 3.74(1.6); in.2000 it was 22.22(5.63), and in 2001 it was 26.58(6.85). Significant difference was observed between the period 1995-2000, (t=-12.197; p=1.01E-12) and period 1995-2001 (t=-12.604; p=4.64E-13). There is no significant difference in baseline micronuclei incidence between years 2000 and 2001, (t =-1.89; p= 0.068) (Figure 2).

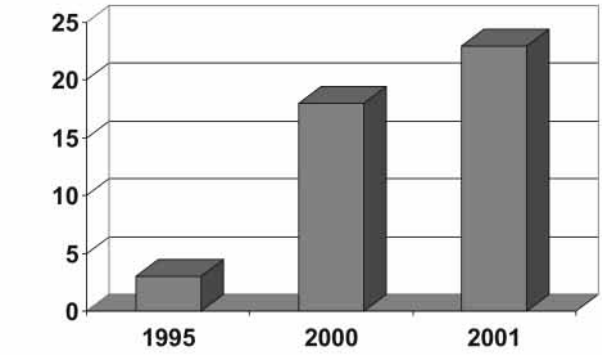

Figure 2. Incidence of micronuclei in cord blood lymphocytes

DISCUSSION

The results reported in this study have shown a significant increase of baseline micronuclei in both study groups in the year of after the bombing (2000) as compared to year 1995. Lymphocyte effects in observed period are probably caused by exposure of population to environmental toxins. Increased MN frequency found in cord blood lymphocytes might also reflect DNA damages of environmental contamination as well as the lifestyle factors like eating habits, stress conditions, smoking habits, etc., (12). Micronuclei are very sensitive biological endpoint, reflecting the effect on whole genome.

During the bombing of Serbia many different chemical compounds were generated. Environmental pollution is still taken into consideration by experts groups. Therefore, in this study we described the genetic monitoring of pregnant women before and after bombing without explaining the reasons that might have caused the observed changes. Although it is known that incidence of baseline micronuclei vary greatly between individuals, this study demonstrated that environmental genotoxic agents induce micronuclei formation in the lymphocytes (13,14). To our knowledge many literature data confirmed that genetic damage and diseases affecting parents and their offspring were associated with the risk of induced

chronic exposure to environmental agents (15,16). The problem in the evaluation of risk of induced chronic exposure to environmental agents, as the unschooled are unaware of the marked differences in the biological effects of different forms of environmental agents. The present results of MN studies with women and fetuses (cord blood samples), exposed to environmental mutagens demonstrated that CB micronucleus assay is simple and rapid method that can be detected early biological outcomes of exposure to environmental genotoxic agents.

CONCLUSION

The study provides important information that should be considered when designing the studies on environmental pollutants, such as the need to control confounding factors, to understand the impact of effect modifiers of the exposure-effect relations. The study also provides necessity for biological continuous monitoring of population employing MN test as reliable and sensitive test for DNA damages.

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