CHROMOSOME ABERRATIONS AS BIOINDICATORS OF ENVIRONMENTAL GENOTOXICITY

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Abstract

Due to the exposure to various potentially genotoxic xenobiotics, derived from recent war activities such as NATO air strikes with antitank ammunition containing depleted uranium, we have evaluated chromosome aberrations in 84 peripheral blood samples from three local populations. One population sample included 30 individuals who lived in the Sarajevo area during and after the war (exposed to potential genotoxins), second population was presented with 26 employees of the tank repair facility in Hadžići (target of NATO air strikes), and 28 inhabitants of Posušje (not exposed to war-related activities) were treated as sample of control population. The mean of chromosome aberration frequencies for the population from Hadžići was significantly higher than the frequencies for the two other populations. Point bi-serial coefficient analysis did not reveal any relationship between the frequencies of chromosome aberrations and smoking habits or gender. Results suggest that depleted uranium could be a risk factor for human health.

KEY WORDS: environmental genotoxins, depleted uranium, human lymphocytes

Introduction

Environmental contamination presents serious threats for human health. From 1992 to 1995, the citizens of Bosnia and Herzegovina were exposed to weapons, used ammunition and waste products of ammunition decay, and they were forced to use expired pharmaceuticals and potentially contaminated food. According to The Study of the Battle and Siege of Sarajevo, the city absorbed approximately 329 shell impacts per day (1). Depleted uranium, used in NATO air strikes in 1994 and 1995, is among the potential war-related genotoxins present at certain sites in Bosnia and Herzegovina. In their 2003 report, the United Nations Environment Programme confirmed higher levels of radioactivity at localities that were targets of these strikes (2). Depleted uranium contamination and higher radioactivity were detected in samples of soil, water, air, and even lichens from three of 14 sites, including the tank repair and ammunition storage facility in Hadžići. An additional concern is that six NATO targets surrounding Sarajevo have not been examined, as their coordinates are not available (2). Although there have been several nongovernmental and SFOR (Stabilisation Force) decontamination efforts, much of the radioactive ammunition has not yet been removed and the amount of potential contaminants remains high. Depleted uranium is a waste product of the atomic energy industry (3). During decay, uranium isotopes emit α particles, which possess high energy but are poorly penetrating. Thus, uranium poses primarily an internal radiation hazard to tissue in close proximity. Uranium isotopes have relatively long half-lives of approximately 10^5-10^9 years (4), and are, therefore, a long-lasting threat. Depleted uranium has been identified as an oncogene-inducing factor both in vitro and in vivo (5). Also, depleted uranium neoplastically transforms cultured human osteoblasts (6), further suggesting that depleted uranium may be involved in cancer induction. An investigation of sixteen British Gulf War and Balkans' War veterans (two females and 14 males), who were suspected of being exposed to depleted uranium in 1990 and later on, demonstrated a statistically significant over-dispersion of dicentric and centric ring chromosomes in comparison with Bremen laboratory controls (7). An increased frequency of malignant tumors was detected in Sarajevo Canton in 1998 and a significant increase in individuals with malignant diseases, the most common being lung and breast cancers, was found after 2000 (8). Our previous research of micronuclei frequencies in Sarajevo population due to the war related environmental contamination, indicated necessity to perform additional studies (9). In the present study, we have conducted chromosome aberration analysis on peripheral blood lymphocytes from three local human populations from Bosnia and Herzegovina. One of these populations works at a location known to be contaminated with depleted uranium.

Materials and Methods

Subjects were recruited from three local populations and provided their informed consent prior to experimentation. Volunteers who were previously exposed to radiotherapy or chemotherapy, who were exposed to diagnostic X-rays, and those who were using prescription were excluded from the study. One of the study groups was composed of 30 individuals who inhibited Sarajevo during the war and the postwar period and were directly exposed to war activities. The second group included 26 workers from the tank repair facility in Hadžići, who are suspected of exposure to depleted uranium contamination as a consequence of NATO air strikes. The third group served as a reference population, and included 28 inhabitants of the west Herzegovina region (Posušje). This locality was chosen due to a lack of military activities during the war and the absence of known environmental contamination. The proportions of males and females, the average age, and the percentage of smokers in the three study groups are presented in Table 1.

Population	Number of analyzed individuals	Average age (years)	SD	Gender ratio (♂ : ♀)	Smokers
Sarajevo	30	32,37	9,90	33% : 67%	47%
Posušje	28	39,57	12,54	43% : 57%	43%
Hadžići	26	46,46	5,97	92% : 8%	46%
Total	84	39,13	11,38	55% : 45%	46%

TABLE 1. Details of study groups

Peripheral blood samples for chromosome aberration analysis were collected in LH vacutainers (BD Vacutainer Systems, Plymonth, UK) during 2002 and 2003. The blood was cultivated according to the standardized procedure described by Moorhead *et al.* (10). 400 µl of peripheral blood were added to 5 ml of 1640 RPMI medium supplemented with L-glutamine, 20% of fetal bovine serum, PHA and antibiotics (GIBCO-Invitrogen, Carlsbad, CA). The cultures were incubated in sterile, plastic, conical 15-ml tubes (NUNC, Rochester, NY) at 37°C for 48 hr. Cell division was blocked by the addition of colcemid (GIBCO-Invitrogen) 2,5 hr prior to the end of the cultivation period. For each blood sam-

ple, 100 metaphase spreads containing 46 μ 1 chromosomes were analyzed microscopically. Aberrations were scored according to International System for Human Cytogenetic Nomenclature (11). Verified aberrations were subclassified as chromosome-type (chromosome breaks, dicentric chromosomes, acentric and minute fragments) and chromatid-type aberrations (chromatid breaks). Gaps were not scored as aberrations (12, 13).

The results for each 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). Statistical analysis included analysis of variance (ANOVA), point bi-serial correlation coefficients, simple linear regression and stratified analysis conducted with *Winks 4.5 Professional* software (TexaSoft, Cedar Hill, TX).

The significance of differences between arithmetic means of the cytogenetic parameters for the study groups was determined by ANOVA followed by pair-wise comparisons with Newman-Keuls multiple comparison test. Correlations between the cytogenetical parameters and gender and 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 chromosome aberration. Stratified analysis was used to assess the possible confounding effects (14) of cigarette smoking and age on chromosome aberrations in the three study groups.

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RESULTS

In the present study, we found that approximately 60% of the chromosome aberrations in samples from the Sarajevo group were chromosome-type aberrations, while 40% were chromatid-type aberrations. The arithmetic mean of total chromosome aberrations was 1.600 per 100 metaphases, while the means for chromosome- and chromatid-type aberrations were 0,967 and 0,633 (Table 2, summarized in Table 5). For the Hadžići group, chromosome-type aberrations accounted for 95% of all aberrations and chromatid-type aberrations, 5%. Three individual samples from this population had dicentric chromosomes and one of these had two dicentric chromosomes in 100 metaphases. The arithmetic mean for chromatid-type aberrations was 0,154 and the mean of chromosome-type of aberrations was 3,115; the arithmetic mean for all aberrations was 3,269 per 100 metaphases (Table 3, summarized in Table 5). For the Posušje reference group, chromosome-type aberrations accounted for 56% of all aberrations, and chromatid-type aberrations, 44%. The arithmetic mean for chromatid aberration was 0,679 and for chromosome-type aberrations 0,857; thus, the arithmetic mean for both types of aberrations was 1,536 per 100 metaphases (Table 4, summarized in Table 5).

Sample	Aberrations/ 100 metaphases		
	(a) Chromatid	(b) Chromosome	a+b
1	0	1	1
2	1	1	2
3	2	1	3
4	1	0	1
5	0	0	0
6	0	2	2
7	2	0	2
8	1	2	3
9	0	2	2
10	1	1	2
11	0	0	0
12	0	0	0
13	0	0	0
14	0	3	3
15	2	2	4
16	1	2	3
17	0	0	0
18	2	0	2
19	1	2	3
20	1	1	2
21	1	1	2
22	0	1	1
23	1	5	6
24	0	0	0
25	1	0	1
26	0	2	2
27	1	0	1
28	0	0	0
29	0	0	0
30	0	0	0

TABLE 2. Standard chromosome aberration analysis of lymphocytes from the Sarajevo study group

One-way analysis of variance (ANOVA) followed by pair-wise comparisons revealed that the Hadžići group had a significantly higher frequency of total aberrations than the other two groups ($p = 0{,}0033$). There were no significant differences between the aberration frequencies detected for the Sarajevo and Posušje groups. The frequency of chromosome-type aberrations was significantly higher for the Hadžići group than either of the two other groups ($p < 0{,}001$), while

Sample	Aberrations/100 metaphases		
	(a) Chromatid	(b) Chromosome	a+b
1	0	6	6
2	0	7	7
3	0	2	2
4	1	1	2
5	0	4	4
6	0	4	4
7	0	3	3
8	0	6	6
9	0	10	10
10	1	1	2
11	0	0	0
12	0	9	9
13	0	0	0
14	0	0	0
15	0	4	4
16	0	0	0
17	0	0	0
18	0	0	0
19	0	5	5
20	0	3	3
21	1	1	2
22	0	2	2
23	1	2	3
24	0	8	8
25	0	0	0
26	0	3	3

TABLE 3. Standard chromosome aberration analysis of lymphocytes from the Hadžići study group

Sample	Aberrations/100 metaphases		
	(a) Chromatid	(b) Chromosome	a+b
1	0	0	0
2	0	0	0
3	0	1	1
4	0	0	0
5	0	0	0
6	0	1	1
7	0	0	0
8	1	2	3
9	0	1	1
10	0	0	0
11	0	0	0
12	1	0	1
13	2	1	3
14	1	0	1
15	1	5	6
16	0	1	1
17	0	4	4
18	2	2	4
19	0	0	0
20	1	0	1
21	1	2	3
22	1	0	1
23	1	0	1
24	1	0	1
25	0	2	2
26	3	0	3
27	3	0	3
28	0	2	2

TABLE 4. Standard chromosome aberration analysis of lymphocytes from the Posušje study group

the frequency of chromatid-type aberrations was significantly higher for the Sarajevo and Posušje groups than the group from Hadžići ($p = 0{,}0135$).

Stratified analysis revealed that cigarette smoking could be a confounder for association between depleted uranium exposure and chromatid-type aberrations as well as total aberrations, while smoking was not a confounder for chromosome-type aberrations in the three experimental groups. Stratified analysis also indicated that age could have a confounding effect on total aberrations and on chromosome-type aberrations in the individual groups as well as for the entire 84 samples.

Analysis of point bi-serial coefficients for the entire 84 samples pooled from the three study groups indicated that there was no statistically significant correlation between any of the cytogenetic endpoints and either the sex or the smoking habits of the subjects. Individuals from the Hadžići group were older than the subjects in the other two groups, however, and there was a strong positive linear association between age and the frequency of chromosome aberrations ($p < 0{,}001$).

Discussion

Industrial development is closely related with increased environmental pollution. Various mutagens present in the environment increase the genetic burden of all living populations, including humans. Environmen-

	Aberrations/100 metaphases								
	(a) Chromatid			(b) Chromosome			a+b		
	Sarajevo	Hadžići	Posušje	Sarajevo	Hadžići	Posušje	Sarajevo	Hadžići	Posušje
X_{av}	0,633	0,154	0,679	0,967	3,115	0,857	1,600	3,269	1,536
s	0,718	0,368	0,905	1,188	3,024	1,297	1,453	2,947	1,551
sX_{av}	0,131	0,072	0,171	0,217	0,593	0,245	0,265	0,578	0,293
V	113,428	238,961	133,284	122,854	97,079	151,342	90,812	90,15	100,976

TABLE 5. Arithmetic means and variability measures for chromosome aberration data from the three study groups

tal pollution is a contributing factor to the approximately 25% of inhabitants of developed countries who die from malignant disease (15). The frequency of cells with structural chromosomal aberrations in peripheral blood lymphocytes is the first biomarker for which an association has been established with cancer risk (12). In the research conducted by Liou *et al.* (16), the cytogenetic analysis of a reference group and a group of individuals who had developed cancer and who inhabited an area with a known high cancer risk, indicates that the frequency of chromosome aberration in groups at high risk for developing cancer is significantly higher in comparison with control populations. Elevated lung cancer rates have been detected in Gulf War and Balkan conflict veterans (17). We hypothesize that exposure to war-related carcinogens and mutagens, among which depleted uranium has been identified as an oncogene-inducing factor both in vitro and in vivo (5), may be involved in this increased cancer incidence.

One-way ANOVA revealed that the Hadžići group had a significantly higher frequency of total aberrations than the other two groups. As the Hadžići group was made up of individuals who work in an area known to be contaminated with depleted uranium, these data support our hypothesis that depleted uranium is a risk factor for cancer in Bosnia and Herzegovina.

Our research revealed that the blood samples of workers in the depleted-uranium-contaminated tank repair facility in Hadžići had an increased frequency of structural chromosome aberrations, including several samples with dicentric chromosomes. This observation is consistent with our previous results demonstrating a significant increase in micronucleus frequency in the group exposed to depleted uranium (18). Positive correlations have been reported previously between micronucleus frequency and the frequency of specific chromosomal aberrations, such as acentric fragments and dicentric chromosomes (19). In addition, a cytogenetic investigation on peripheral blood lymphocytes of three groups of residents from areas of south and central Serbia contaminated with depleted uranium and a group of workers occupationally exposed to X-rays detected an increased frequency of chromosome aberrations among the subjects exposed to depleted uranium. This increase, however, was bellow the frequency of chromosome aberrations in individuals occupationally exposed to ionizing radiation (20). In vitro studies with human osteoblast cells suggest that depleted uranium results in genomic instability manifested as delayed reproductive death and micronucleus formation (21).

Uranium was released into the environment of Bosnia and Herzegovina at least seven years before the sampling of peripheral blood conducted for this research. As mentioned earlier, however, uranium isotopes have a relatively long half-life and similar studies conducted years after exposure to radioactivity suggest that the genome of peripheral blood lymphocytes is a reliable biological system for cytogenetic analysis even long after exposure to genotoxins. For instance, Neronova *et al.* (22) performed cytogenetic analysis 4–13 years after the Chernobyl accident and showed an elevated frequency of acentrics, chromatid exchanges, dicentrics and rings in Chernobyl cleanup workers.

Conclusion

Results of this research suggest that exposure to depleted uranium could be a risk factor for human genome. More extensive studies, using better matched study groups, are necessary to confirm the association between exposure to depleted uranium and human health risk. Prudence dictates, however, that continuous screening should be conducted on the environment and on the health of humans in areas potentially impacted by this contamination.

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