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Identification of Health Risks in Workers Staying and Working on the Terrains Contaminated with Depleted Uranium

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Health risks/Depleted uranium/Chromosome aberrations.

This study investigated health risks in workers residing and working in terrains contaminated by low ionizing radiation doses which originated from ammunition containing depleted uranium (DU). The studied population was composed of two test groups (T-I, T-II) who were occasionally exposed to DU, and two referent (R-I, R-II) groups not exposed at any time to DU. All of them were evaluated for the following: complete clinical examination and blood count, presence of immature forms and blasts, leukocyte alkaline phosphatase activity and cytogenetic tests. The probability of onset of the characteristic complete biomarkers – chromosomal aberrations, was analyzed using logarithmic function of the Poisson regression. The estimated function of the density of probabilities of Poisson distribution of the chromosomal aberrations in the test group T-II was drastically different from the corresponding distribution of the referent group R-I and to a somewhat lesser extent from the group R-II; Wilcoxon test exactly confirms the presence of a significant difference between the reference group R-II and test group T-II, $p < 0.05$. The damages to chromosomes and cells were highest in the test group T-II of workers additionally occupationally exposed to DU. The group of workers T-I, who had been exposed to DU working on contaminated terrain, have had certain risks of cell and chromosome damages, and that risk was not greater than the risk to the referent group R-II of workers occupationally exposed to ionizing radiation.

INTRODUCTION

The research studies directly related to depleted uranium (DU) and its utilisation for military purposes are relatively rare in comparison to the studies related to the natural uranium.[1)] Natural uranium is normal component of the lithosphere (in the region of Serbia, averagely ranging between 0.5–5 g/1 ton of the soil) and it is composed of 3 mutually balanced isotopes: 234, 235 and 238 (depleted).[2,3)]

Utilization of ammunition containing depleted uranium (DU) by NATO during the bombing of the South of Serbia in the middle of 1999 resulted in contamination of the terrains and continuous exposure of the living world to small ionizing radiation doses, in addition to the already existing natural (e.g., uranium) and artificial (e.g., radio cesium) radionuclides.[4–6)] Local human population living and working in the contaminated region has been also exposed to continuously increased radioactivity to DU.[7,8)]

There are two ways of DU transfer from the contaminated environment to humans. Ingestion was the predominant form of DU contamination transfer from the environment to the human bodies in the post-conflict period. DU containing in the soil finds its different ways to be included in the food chain. Through contamination of underground and ground water, radionuclide comes into the plants and animals, to be finally consumed by human population.

The mechanism of internal contamination through inhalation has been also possible. The aerosol forms were deposited in the soil, to be thereafter returned to the aerosol forms under the impacts of wind or human activities, thus coming into the human body through inhalation.[3)]

The study was aimed at identification of health risks in workers permanently or occasionally residing in the contaminated territory associated with contamination of the terrain by low ionizing radiation doses resulting from utilization of ammunition containing DU.

METHODS

Depleted uranium (^{238}U) in urine was measured applying different methods. The methods of alpha and gamma spectrometry analyzes have been used immediately after contam-

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ination, in the period from the middle of 1999 up to 2001. In this period in total of 22 urine samples three of them were contaminated with ^{238}U (8). All of them had been taken from the group T2 of patients (employees who live in the south of Serbia and who have worked in the contaminated zone). Ratio of ^{235}U and ^{238}U was less than 1, ie: 0:16, 0:21 and 0.76 respectively.

Analyses had been done by fluorimetric method did not found significantly higher concentrations of uranium in the test group in relation to the referent group. Gama spectroscopic analysis recorded the ^{137}Cs and 131J below 1 Bq-l-urine, and natural radionuclide 40K and natural uranium (Isotopes uranium in the balance). Mentioned analyses were measured according to the ICRP recommendation form 1996.[9)]

Multifactorial variance analyses showed that the investigated variables (age, sex, smoking) had no significant impact on any of the groups. Correlation with DOE was significant in health workers in the zone of ionizing radiation in the groups of R2 and T2.

Five years later, when this research had been realized, it was not expected to detected uranium in the urine, having in mind that possibly uranium had been drawn or deposited in bones. For detection of ^{238}U in the urine and blood after such a long period of time contamination of water and food in the environment would have to be very high, which was not the case here because of low level of contamination of the environment (the results of measurements are listed here after).

Detection of ^{238}U five years after contamination was possible only implementing chelating therapy as a treatment who engages ^{238}U from bone depots. This method can be used to set up the range of alpha emission of urine, which can prove the existence of depleted uranium, but it should be a live experiment, which is prohibited by the law.

According to the health consequences monitoring program on the contaminated terrains proposed by the World Health Organization in 1996,[10)] the studied population was evaluated for the following: Complete clinical examination; Complete blood count; Leukocyte formula and leukocyte/lymphocyte ratio; Microscopically observed morphological changes; presence of immature forms and blasts; Leukocyte alkaline phosphatase (L-ALP) activity; Cytogenetic tests (lymphocyte karyotype and chromosomal aberrations).

The method was limited to analysis of the peripheral blood cells and cytogenetic analysis of lymphocyte karyotype as the most sensitive parameters of low dose influence, due to short time period (5 years after initial contamination) for clinical presentation of the possible diseases. Nevertheless this was sufficiently long time of period to expect positive findings of DU blood and urine values, regardless of the previous presence of absence of contamination.

Identification of environmental radionuclide presence effects on the body was performed indirectly, using analysis of blood count parameters: total count of red blood cells, haemoglobin concentration, mean corpuscular volume (MCV), platelets, white blood cells, leukocyte formula. Particular attention had been given to lymphocyte/leukocyte ratio, leukocyte alkaline phosphatase (L-ALP) activity, chromosomal aberration and lesion frequency, probability of onset of chromosomal aberrations.[8,11–13)]

The venous blood was used for the blood cells count. An automatic counter counted erythrocytes, reticulocytes, platelets and leukocytes. Blood smears stained with May-Grunewald-Gyms were studied through the optical microscope with immersion for the differences the white cell components, presence of morphologically altered blood cells, young precursors, immature forms and blasts. The capillary blood smears were stained for alkaline phosphatase using a modified Kaplow's method.[8)] Score of enzyme activity was presented as the international units (IU).

Chromosomes were observed in peripheral blood lymphocytes. Moorhead's method and conventional cytogenetic techniques were used for preparation of lymphocytes.[11,13)] The cells in metaphase were microscopically examined in stained (2% M.G. Gyms) smears under immersion (magnification 100 × 16). The karyotypes of 200 prepared metaphase lymphocytes were analyzed, when the chromosomes were arranged in equatorial plane. The most characteristic aberrations were dicentric chromosomes. Ring chromosomes and acentric fragments were considered the equivalent to dicentric (chromosome aberrations – ca). Chromatid and chromosomal breaks and chromatid exchanges were designated as chromosomal lesions – cl. Lymphocytes having karyotype damages were marked as damaged cells – dc.[13)]

The final studies were performed during 2004; five years after the initial contamination caused by DU radioactive ammunition used in 1999, and the whole five-year period had been taken into account.

Environmental follow-up measurements were performed on several occasions (2001, 2002 and 2004) applying the well known methods, according to which, the initial contamination of the terrain was determined.[5,6)] The above evidenced chronically increased population exposure due to DU transfers into the biosphere.[8)]

Measurement of the total drinking water alpha-activity resulting from both and DU (DU) from different sources (the total of 18 points) in the contaminated region revealed the values below 1 Bq/l, i.e., averagely 10 mBq/l. The specific DU activity in water samples ranges between 0.03 and 0.21 Bq/l.

The total alpha-activity in the samples that are indicators of biosphere contamination (animals – rabbit; and plants – vegetation moss and lichen) ranges between 1340 ± 200 and 1740 ± 260 (rabbit); moss 1370 ± 210 and lichen 1860 ± 280 Becquerel at the average.

The specific activity of the DU in meat (rabbit) was 2.3 Bq/kg., while in plants it was 16–48 Bq/kg, averagely: 23 ±

5 Bq/kg.

Cesium 137 gamma activity as the indicator of radioactive contamination (even before the conflict) was lower, 0.16 ± 0.05 Bq/kg (rabbit) and 2.0 ± 0.1 (vegetation).

Statistical analysis included two groups of subjects (Table 1): referent group (without potential risk of exposure to DU) and test group (with potential risk of exposure to DU).

Referent group was designed to include two characteristic subgroups. The first referent subgroup was obtained from the population considered to be exposed one to natural phone without occupational exposure to ionizing radiation, physical or chemical mutagens. The group comprised young workers employed at the Federal Customs Administration, averagely aged 36.6 years, exclusively males with average 7.6 years of service and zero duration of exposure (group R-I).

As opposed of the above group, another referent subgroup (R-II) was introduced composed of workers employed at the Institute of Oncology in Belgrade, who had been occupationally exposed to ionizing radiation effects. They were constantly subjected to dosimetric and medical control. Dosimetric control included monitoring doses by personnel dosimeters. The absorbed external doses of ionizing radiation to the bodies were measured by personnel thermo luminescent dosimeters (TLD) for the duration of occupational exposure (DOE). The TLD measurements were expressed in mSv, as equivalent doses. Average annual doses for the observed five-year period (1999–2004) have been presented (Table 1).

Medical control included periodic check-ups based on the program proposed by the Ionizing Radiation Protection Law, incorporating ICRP recommendations, in order to get the insight into general health status, symptoms and clinical

Table 1. Statistical parameters of referent and test groups with average annual dose

Statistical parameters		**Groups**			
		Referent (R)		**Test (T)**	
		R-I	**R-II**	**T-I**	**T-II**
No (persons)		25	42	20	52
Equivalent dose (TLD)		*< 1 mSv*	*1.34 mSv*	*< 1 mSv*	*2.08 mSv*
Age	μ	36.6	42.7	53.7	43.3
(year)	σ	5.8	9.5	12.1	9.7
α_ci = 0.05	ci_lower	34.2	39.8	48	40.6
	ci_upper	39	45.7	59.3	46
Sex	M	25	12	19	31
masculinum –M, versus femininum–F	%	100	28.6	95	59.6
	F	0	30	1	21
	%	0	71.4	5	40.4
Years of services (year)	μ	7.6	15.6	28.7	14.8
α_ci = 0.05	σ	5.9	9.3	12	9.6
	ci_lower	5.2	12.7	23	12.1
	ci_upper	10.1	18.5	34.3	17.5
DOE	μ	0	13.8	0	11.1
(year)	σ	0	9.3	0	9.2
α_ci = 0.05	ci_lower	0	10.9	0	8.6
	ci_upper	0	16.7	0	13.7

TLD - term luminescence dosimeter
DOE - duration of occupationally exposure
μ – mean value – central tendency measure
σ – standard deviation
α_ci = 0.05; ci_ lower, and ci_ upper = confidence interval and deviation below and above it

signs (complete clinical evaluation).[12)]

The group was averagely aged 42.7 years, with 28.6% of males, 15.6 years of service at the average and 23.8 years of occupational exposure at the average.

Test group comprised two specific subgroups. The first test subgroup (T-I) included the workers employed at the Radio Television of Serbia from Belgrade, who had stayed, during the North Atlantic Treaty Organization (NATO) bombing of Federal Republic of Yugoslavia (FRY), on several locations including Pljačkovica hill near Vranje, the town in south Serbia, in order to repair malfunctions on the TV antenna hit by DU ammunition. The group was not occupationally exposed to ionizing radiation before that. However, due to the nature of their occupation, the test group was exposed to increased electromagnetic radiation present on TV antenna. Their average age was 53.7 years, 95% of them were males with 28.7 years of service and 0 years of occupational exposure.

The second test subgroup (T-II) included health care workers from Vranje, who were occupationally exposed to ionizing radiation since they have lived and worked in vicinity of the terrain contaminated with DU.

The workers have been constantly subjected to dosimetric and medical check-ups, similarly to R-II group.

The average age of the second test group (T-II) was 43.3 years, 59.6% of them were males with 14.8 years of service and 11.1 years of occupational exposure.

Statistical methods

The samples were compared based on the observed parameters using the estimated Poisson distribution and the significance of the difference was tested using Wilcoxon rank sum test with 95% confidence interval and significance threshold at the level of 0.05. The probability of onset of the characteristic complete biomarkers was specially analyzed using logarithmic function of the Poisson regression and it was quantified by lambda parameter (λ).

Linear regression correlation analysis and Student's t-test comparison at the probability level of 0.05 were used.

RESULTS

Measurements performed at the working sites confirmed that the exposure doses absorbed by the occupationally exposed medical professionals in Belgrade (group R-II) and Vranje (group T-II) were admissible and low. The readings were performed in the referent group II using thermo luminescent dosimeters (TLD) and the equivalent doses ranged between 1.00 mSv and 2.04 mSv (1.34 mSv/year, at the average). As for the test group II, the mean annual dose was 2.08 mSv, with minimal and maximal values being 0.9 mSv and 4.98 mSv, respectively (Table 1).

The obtained results were tabularly presented in the context of the comparative pair analysis (Table 1), both with negative clinical finding of the diseases associated with low ionizing dose radiation effects: Referent group I (R-I) and test group I (T-I); Referent group II (R-II) and test group II (T-II).

The R-I/T-I pair differs with respect to average age and average years of service, in absence of any differences related to sex and years of exposure which had been zero value in both groups. The difference in sample size may be considered negligible. The difference was found in potential exposure to ionizing radiation – it was not found in the group R-I, being probable in group T-I because of exposure to DU.

As for the pair R-II/T-II, i.e., health professionals employed in the ionizing radiation zones in two cities (Belgrade and Vranje) in Serbia, no differences were found related to age, years of service, years of exposure, occupation (approximately the same level of qualification). However, the difference was found with respect to their age, mean value (1.34 mSv/2.08 mSv) and range of annual equivalent ionizing radiation dose over the observed five-year period (1.0–2.04 mSv/0.9–4.98 mSv). The equivalent dose of ionizing radiation in T-II was by 55% higher from the dose in R-II as a consequence of the working site conditions. Workers from the test group II have had higher work load (greater number of radiological procedures per single shift). The difference between the groups was also found in potential exposure to DU, which was not found in R-II being probable in the T-II.

Multifactorial variance analyses showed that the investigated variables (age, sex, smoking) did not have significant impact on any of the groups (Table 2). There was correlation

Table 2. Frequency of chromosomal aberrations in the different groups according to age, gender and smoking

Predictors	Groups			
	Referent (R)		Test (T)	
	R1	R2	T1	T2
	Frequency of chromosome aberrations			
Age (years)				
18–39	0.07	0.10	0.00	0.20
40–70	0.01	0.07	0.21	0.28
Gender				
Female	0.00	0.08	0.01	0.28
Male	0.08	0.09	0.20	0.20
Smoking				
Smoker	0.04	0.07	0.10	0.24
Non smoker	0.04	0.10	0.11	0.24

with DOE but it was not linear and it was equal in both groups R2 and T2. Non ionizing radiation should have impacts on cytogenetic changes or DNA in a case of big doses in long time of period only on chromatid lesions, but not on specific aberrations such as dicentric specific for ionizing radiation.

The analysis included blood count elements (Tables 3 and 4). No significant difference was found between the subject pair R-I/T-I and pair R-II/T-II with respect to red blood cell line parameters (Table 3). Mean platelet values were not statistically different. All the subjects from both groups were within standard range for platelets (Table 3).

As for the test group T-II the total number of subjects found to be out of the standard range of erythrocytes was 5 (11,9%) being in the reference group (R-II) 5 or 9,5%, with their deviation ratio of 1,2. There was significantly higher number of subjects who were out of the standard interval for haemoglobin when compared to the reference group, while the number of subjects out of the standard interval for MCV was significantly lower when compared to the reference group R-II (Table 3).

As for the pair comprising reference and test group R-I/T-I pair and R-II/T-II pair (Table 4) mean values of white blood cells were within standard limits and they were not statistically different for $\alpha = 0.05$ (confidence 95%). The difference was found with respect to the criterion of deviation of the measures values from the standard interval for lymphocytes. As for the reference group I, the number of subject falling out of the standard interval was significantly lower (12%) in comparison to the test group I (45%).

Significant difference was not found with respect to other elements of the leukocyte formula. Basophiles were not evidenced. Moreover, no immature cells or blasts were observed. The L-ALP activity also was not increased. Due to specific lymphocyte sensitivity, lymphocyte-related changes were also particularly characteristic with respect to their number and correlation with white blood cells as well as with respect to chromosomal aberrations in their nuclei.

Presence of chromosomal aberrations was analyzed in the referent and test groups (Tables 5 and 6). Observation of the first pair from the groups R-I and T-I evidenced presence of chromosomal aberrations (Table 5) in the both group. Poisson distribution parameter λ differs, however the corresponding confidence intervals were crossed over for the significance threshold $\alpha = 0.05$, which indicates that the difference was not statistically significant. The mentioned was confirmed by Wilcoxon test of matching of the estimated parameters of the Poisson distribution. It indicates the presence of tendency toward chromosomal aberrations (higher risk of their onset) in the group T-I in comparison to the comparative reference group R-I. However, the difference in incidence of chromosomal aberrations was not significant, at the average (Tables 5 and 6), although the frequency of chromosomal aberrations (Table 5) was higher in the group T-I (0.21% vs. 0.08%) in comparison to the referent one.

The equivalent analysis for the test pair R-II and T-II shows similar tendencies, however they were more prominent and exceed the limits of statistic tolerance in testing of differences (do not belong to the same set - Tables 5 and 6). Estimated function of density of probabilities of Poisson distribution of the chromosomal aberrations in the test group T-II was drastically different from the corresponding distribution of the reference group R-I and to the somewhat lesser extent from the group R-II; Wilcoxon test exactly confirms presence of significant difference between the referent group R-II and test group T-II, $p < 0.05$ (Tables 5 and 6).

The difference in chromosomal aberrations was observed between the referent groups R-I and R-II which was expected since the groups were mutually different with respect to the occupational exposure to low ionizing radiation doses (Table 5). Referent group II had significantly higher incidence of the unspecific chromosomal lesions ($p = 0.0012$) in comparison to R-I group, as well as significantly higher number of the damaged lymphocytes for that reason (damaged cells – dc, $p = 0{,}0054$. Frequency of chromosomal aberrations characteristic for radiation was increased (0.17% vs. 0.08%) however the increase was non-significant ($p = 0.13$, Wilcoxon test). Poisson distribution parameter λ was higher (0.33 vs. 0.16) in the group R-II. This indicates that the probability for onset of chromosomal aberrations was higher in R-II in comparison to R-I group (Tables 5 and 6).

The results of the chromosomal aberration analysis indicate that the test group I, which has been temporarily occupationally exposed to the effects of low environmental ionizing radiation doses as well as to non-ionizing radiation, had been different from the groups R-I and T- II being at the same time the most similar with the results of the health-related risk analyzes to the referent group R-II, which was chronically occupationally exposed to ionizing radiation.

Group T-I has been significance low chromatid lesions than R-II ($p = 0.0022$, Table 6). Frequency of chromosomal aberrations characteristic for radiation was increased (0.21%) and Poisson distribution parameter λ was higher than in referent groups ($\lambda = 0{,}42$), but they were no significant.

Test group II, despite test group I, had been significantly different (confidence 95%; Table 6) from the both of referent groups (R-I and R-II) taking into consideration chromosomal aberrations in lymphocytes ($p < 0.01$) and damaged cells ($p < 0.05$).

Accordance to logarithmic function Poisson regression, complete biomarkers (chromosomal aberration – ca) showed that relative risk (RR) in group T-II was 4; in group T-I is 2 and in group R-II it was 3, while in group R-I exposed only nature radiation, relative risk had been 1.

Table 3. Statistical parameters of referent and test groups of blood count

Blood count		Groups			
		Referent (R)		Test (T)	
		R-I	R-II	T-I	T-II
No (persons)		25	42	20	47
Hemoglobin	Standardized interval	120 – 160 g/l			
(Hb)	Mean value of standardized interval	140			
α_ci = 0.05	μ	**139.1**	**133.1**	**132**	**133.2**
	σ	± 3.8	± 12.9	± 7.1	± 17.4
	ci_lower	137.5	129.1	128.6	128.1
	ci_upper	140.6	137.1	135.3	138.3
	Below limit	0	4 (9.5%)	0	10 (18.9%)
	Above limit	0	2 (4.8%)	0	2 (3.8%)
	Without limit	**0**	**6 (14.3%)**	**0**	**12 (22.7%)**
Eerythrocytes	Standardized interval	$4.0 – 5.5 \times 10^{12}$/l			
(Er)	Mean value of standardized interval	4.75			
α_ci = 0.05	μ	**4.71**	**4.418**	**4.61**	**4.64**
	σ	± 0.350	± 0.475	± 0.363	± 0.445
	ci_lower	4.56	4.27	4.44	4.51
	ci_upper	4.85	4.56	4.78	4.77
	Below limit	0	3 (7.1%)	0	2 (3.8%)
	Above limit	0	2 (4.8%)	0	3 (5.7%)
	Without limit	**0**	**5 (11.9%)**	**0**	**5 (9.5%)**
Mean corpuscular volume	Standardized interval	80.0 – 94.0			
α_ci = 0.05	Mean value of standardized interval	87.0			
	μ	**91.9**	**82.0**	**92.9**	**87.9**
	σ	± 4.0	± 23.9	± 6.3	± 7.5
	ci_lower	90.2	74.5	90.0	85.6
	ci_upper	93.5	89.4	95.8	90.3
	Below limit	0 (0%)	9 (21.4%)	0 (0%)	4 (7.6%)
	Above limit	7 (28%)	6 (14.3%)	10 (50%)	5 (9.4%)
	Without limit	**7 (28%)**	**15 (35.7%)**	**10 (50%)**	**9 (17%)**
Retikulocytes	Standardized interval	0.5 – 1.5%			
(Ret)	Mean value of standardized interval	1.0			
α_ci = 0.05	μ	**0.80**	**1.11**	**1.11**	**1.06**
	σ	± 0.316	± 0.803	± 0.174	± 0.440
	ci_lower	0.674	0.864	1.028	0.817
	ci_upper	0.934	1.365	1.192	1.305
	Below limit	0 (0%)	5 (11.9%)	0	1 (1.9%)
	Above limit	2 (8%)	11 (26.2%)	0	1 (1.9%)
	Without limit	**2 (8%)**	**16 (38.1%)**	**0**	**2 (3.8%)**
Platelets	Standardized interval	$150 – 350 \times 10^{9}$/l			
(Plt)	Mean value of standardized interval	250			
α_ci = 0.05	μ	**286**	**250**	**303**	**270**
	σ	± 22.0	± 69.1	± 50.8	± 65.3
	ci_lower	277.3	228.5	278.7	249.3
	ci_upper	295.5	271.5	326.3	291.0
	Below limit	0	2 (4.8%)	0	2 (3.8%)
	Above limit	0	2 (4.8%)	0	2 (3.8%)
	Without limit	**0**	**4 (9.6%)**	**0**	**4 (7.6%)**

α_ci = 0.05 Confidence 95%; probability p at 0.05
MCV – mean corpuscular volumeof erythrocytes
Standardized interval – adopted from the Ionizing radiation protection law and based on ICRP 2005 and WHO 1996 recommendations (7, 15)
μ– mean value – central tendency measure
σ – standard deviation
α_ci = 0.05; ci_ lower; ci_ upper = confidence interval and deviation below and above it
Below limit – below standardized interval borderline value
Above limit – above standardized interval borderline value
Without limit – below or/and above (without) standardized interval borderline value

Table 4. Statistical parameters of referent and test samples for total leukocytes, leukocyte formula and alkaline phosphatase

Leukocytes, leukocyte formula, and alkaline phosphatase		Groups			
		Referent (R)		Test (T)	
		R-I	R-II	T-I	T-II
No (persons)		25	42	20	47
Leukocyte	Standardized interval	$4.0 – 9.0 \times 10^9$/l			
Le	Mean value of standardized interval	6.5×10^9/l			
α_ci = 0.05	μ	**6.44**	**6.54**	**6.35**	**6.95**
	σ	1.41	2.18	1.69	2.27
	ci_lower	5.86	5.87	5.57	6.28
	ci_upper	7.03	7.22	7.14	7.62
	Below limit	0	2 (4.8%)	0	1 (1.9%)
	Above limit	2 (8%)	5 (11.9%)	2 (10%)	8 (15.1%)
	Without limit	**2 (8%)**	**7 (16.7%)**	**2 (10%)**	**9 (17%)**
Granulocyte	Standardized interval	0.51 – 0.61			
G	Mean value of standardized interval	0.56			
α_ci = 0.05	μ	0.598	0.577	0.630	0.597
	σ	0.060	0.113	0.152	0.087
	ci_lower	0.573	0.542	0.558	0.571
	ci_upper	0.622	0.612	0.701	0.622
	Below limit	1 (4%)	10 (23.8%)	1 (5%)	3 (5.7%)
	Above limit	0	1 (2.4%)	0%	2 (3.8%)
	Without limit	4%	11 (26.2%)	5%	5 (9.5%)
Lymphocyte	Standardized interval	0.21 – 0.35			
Ly	Mean value of standardized interval	0.280			
α_ci = 0.05	μ	0.319	0.326	0.288	0.357
	σ	0.0805	0.0815	0.0363	0.0829
	ci_lower	0.2860	0.3006	0.2705	0.3322
	ci_upper	0.3524	0.3513	0.3045	0.3809
	Below limit	2 (8%)	6 (14.3%)	8 (40%)	4 (7.6%)
	Above limit	1 (4%)	5 (11.9%)	1 (5%)	11 (20.8%)
	Without limit	3 (12%)	11 (26.2%)	9 (45%)	15 (28.4%)
Monocyte	Standardized interval	0.04 – 0.08			
Mo	Mean value of standardized interval	0.060			
α_ci = 0.05	μ	0.0500	0.0657	0.0425	0.0430
	σ	0.0144	0.1008	0.0234	0.0203
	ci_lower	0.0440	0.0343	0.0316	0.0370
	ci_upper	0.0560	0.0971	0.0534	0.0489
	Below limit	3 (12%)	12 (28.6%)	6 (30%)	12 (22.6%)
	Above limit	1 (4%)	3 (7.1%)	0	1 (1.9%)
	Without limit	4 (16%)	15 (35.7%)	6 (30%)	13 (24.5%)
Leukocyte alkaline phosphatase	Standardized interval	20.0 – 80.0 IU			
L-ALP	Mean value of standardized interval	50.0			
α_ci = 0.05	μ	**64.9**	**57.1**	**74.9**	**67.1**
	σ	8.8	15.3	9.8	16.3
	ci_lower	60.3	51.8	70.3	61.8
	ci_upper	69.5	62.3	79.5	72.3
	Below limit	0	0	0	0
	Above limit	0	0	7 (35%)	4 (7.6%)
	Without limit	0	0	**7 (35%)**	**4 (7.6%)**

α_ci = 0.05 – Confidence 95%; probability (p) at 0.05
Standardized interval – adopted from the Ionizing radiation protection law and based on ICRP 2005 and WHO 1996 recommendations (7, 15)
μ – mean value – central tendency measure
σ – standard deviation
α_ci = 0.05; ci_ lower, and ci_ upper; = confidence interval and deviation below and above it
Below limit – below standardized interval borderline value
Above limit - above standardized interval borderline value
Without limit – below or/and above (without) standardized interval borderline value

Table 5. Poison distribution of chromosomal aberrations, chromosomal lesions and damaged cells in the referent and test groups

Chromosomal aberration, lesion, and damaged cells			Groups			
			Referent (R)		Test (T)	
			R-I	R-II	T-I	T-II
No (persons)			25	42	19	41
No (cells)			5000	8050	3800	7920
Chromosomal aberrations - ca	No persons with ca		4	7	6	20
	%		16.0	16.7	31.6	48.8
	Total ca		4	14	8	32
	% - frequenci ca		**0.08**	**0.17**	**0.21**	**0.48**
	Poison distribution	λ	**0.1600**	**0.3333**	**0.4211**	**0.9268**
	α_ci = 0.05	ci_ lower	0.0269	0.1483	0.1353	0.5853
		ci_ upper	0.5038	0.6390	0.9778	1.3892
Chromatid lesions - cl	No persons with cl		1	16	0	17
	%		4.0	38.1	0.0	41.5
	Total cl		1	29	0	24
	%		0.02	0.36	0.00	0.30
	Poison distribution	λ	0.0400	0.6905	0	0.5854
	α_ci = 0.05	ci_ lower	0.0002	0.4049	NaN	0.3233
		ci_ upper	0.2972	1.0947	0.2789	0.9694
Damaged cells - dc	No persons with dc		5	18	6	26
	%		20.0	42.9	31.6	63.4
	Total dc		5	27	8	47
	%		0.10	0.34	0.21	0.60
	Poisson distribution	λ	0.2000	0.6429	0.4211	1.1463
	α_ci = 0.05	ci_lower	0.0431	0.3688	0.1353	0.7614
		ci_upper	0.5660	1.0356	0.9778	1.6516

λ – lambda parameter of density of probabilities of Poisson distribution
α_ci = 0.05 Confidence 95%; probability (p) at 0.05
α_ci = 0.05; ci_ lower, and ci_ upper; = confidence interval and deviation below and above it

Table 6. Significance of difference in chromosomal aberrations and lesions between the referent and test groups

Referent groups	Chromosomal aberration, lesion, and damaged cells	Test group 1		Test group 2	
		Confidence 95% alfa = 0.05	**p**	Confidence 95% alfa = 0.05	**P**
Referent group 1	Chromosomal aberration	No	0.2103	**Significance** < *0.01*	p = 0.0041
	Chromatid lesion	No	0.4089	*Significance*	9.86e-004
	Damaged cells	No	0.3531	*Significance*	1.29e-004
Referent group 2	Chromosomal aberration	No	0.3449	**Significance** < *0.01*	p = 0.0045
	Chromatid lesion	*Significance*	0.0022	No	0.7582
	Damaged cells	No	0.3513	*Significance* < *0.05*	p = 0.0203

α_ci = 0.05 - Confidence 95%; probability (p) at 0.05

DISCUSSION

Identification of field contamination resulting from application of radioactive ammunition risks on the health on individuals living and working on the contaminated territory was significant for studying of the admissible levels of absorbed radiation of different population groups, particularly individuals occupationally exposed to the ionizing radiation as well as their patients and general population.

The obtained results indicate the presence of the objective risk if exposure of workers living and working on the known contaminated terrain on the south of Serbia as a result of DU originating from the military conflicts that took place on the terrain in 1999.

DU ammunition has become a source of environmental contamination. On one hand, it was superimposed to the natural phone, while on the other, it penetrates to the soil and ground water to come thereby into the biosphere.[4–6] Thus, DU becomes a residual radiological risk for human life in the course of their life on the affected terrain.[14] As for the non-occupationally exposed population, each dose above 1 mSv/1year was conspired to be a significant dose. Low environmental doses were cumulated during the stay in such regions and within five-year period, if they were above 1 mSv per year, they may reach 6 mSv, which represents the upper limit for the occupationally exposed individuals in the category B zone.[11] For this reason, the individuals working and living on the contaminated territories were subjected to health monitoring.[3,6,9] Lack of specificity of the blood count elements as health indicators for assessment of ionizing radiation risk points out to need of analysis of chromosomal aberration presence.[13–15]

As for the workers living in a wider region with verified contaminated zones who were occupationally exposed to low doses of ionizing radiation, statistical differences were identified upon their comparison with the referent group of the occupationally equivalent subjects, particularly with respect to onset of the chromosomal aberrations. For that reason, the number of damages lymphocytes was higher, which was essential for their organism defence role.

No statistically significant difference was found with respect to unspecific, single-stranded chromatid changes (lesions), which do not form chromosomal figures (dicentrics). Most probably, they were also the result of other causes, in addition to radiation (chemical metal toxicity, habits, smoking etc.) and they may influence karyotype instability.[13]

Additionally, it has also been evidenced that onset of these lesions on DNA was accompanied by the same number of the stable aberrations (deletion, inversion and translocation), which indirectly suggests the probability of onset of mutations (due to stable aberrations fixed in the cell division).

Test group II (workers in the radiation zone in the contaminated region in the southern Serbia) had been composed of two groups of subjects. The first one was composed of individuals at risk of uniform low-dose irradiation on their working sites, while the second one comprised the individuals at risk of contamination with DU. For this reason, the radiation risk was highest in this group and it has been quantified by the highest probability of onset of chromosomal aberrations and the highest frequency of chromosomal aberrations and damaged lymphocytes. Damaged lymphocytes lost function because since 50% of them tend to disappear after the initial divisions, and before division ten, all the remaining damages ones also disappear.[8,11,13,15]

Only referent group I was free of any of the 2 above mentioned risks – radiation at the working site or radiation at the contaminated terrain. Therefore, it was clear why the referent groups were also mutually different, since the referent group II had been composed with the individuals chronically exposed to low ionizing radiation doses at their work sites. Nevertheless, the difference was found between the reference groups I and II with respect to chromosomal lesions, i.e., higher risk expressed as probability of onset of lesions was found in the referent group II composed of the occupationally exposed health care professionals from Belgrade. The group was also proved to have higher frequency of unspecific lesions in comparison to the test group I, in which occupational exposure was not continuous but only occasional during repair works on the TV antenna on the contaminated terrain. This group was not exposed to increased radiation and did not have relative risk due to exposure to DU. It was not significantly different from other referent groups with respect to other parameters, however if was different from the test group II composed of subjects continuously staying in the vicinity of the contaminated region.

Accordingly, statistically significant differences related in comparison with the referent group had not been observed, although changes values of certain parameters were evidenced in workers who had lived out of the contaminated regions but who were occupationally exposed for some time due to their stay in the contaminated zone performing their professional tasks.

Therefore, life in the contaminated region was associated with higher radiological risk, particularly for those who have worked in the ionizing radiation zone and probability of potential occupational diseases may be higher and thus workers rights related to occupational diseases, treatment and assessment of their working abilities should be considered. Additionally, the criteria for recognition of radiation-induced occupational diseases must be also reconsidered in the new circumstances associated with increased risk of environmental contamination as well.

The above fact points out need for further investigations of the association between occupational exposure to the ionizing radiation effects and effects of the underlying envi-

ronmental contaminants, particularly within the context of the increased relative risk that way quantitatively verified in this study. Accordingly, the aspect of legal regulations in the fields of occupational protection should be also reconsidered, since the situation is new and uncovered by the current regulations. This is particularly important for the health risks of medical professionals in the ionizing radiation zone in southern Serbia, having in mind the fact that their work load was higher due to higher morbidity rate among the population in the underdeveloped region.[9,16)]

CONCLUSIONS

For all the above, the latent period before onset of the disease was prolonged, and thus the consequences of the DU effects were yet to be expected. The former points out to the need of reassessment of the admissible level of radiation in case of occupational exposure. Decrease of occupational exposure (admissible dose level) may suppress the influence of the environmental doses, since natural phon was increase by superimposed contamination with DU.

Increased health risk of the workers exposed to ionizing radiation caused by profession and additionally by contamination from the contaminated environment by DU has become because of cumulative radiobiology effects of small doses over the continual exposition and it depends to the time of period of exposure duration.

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