
Long-term cellular effects in humans chronically exposed to ionizing radiation

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Material and methods

The study was focused on the population comprising Techa riverside residents (Southern Urals, Russia) chronically exposed to radiation (since 1949) due to the Mayak PA activities.

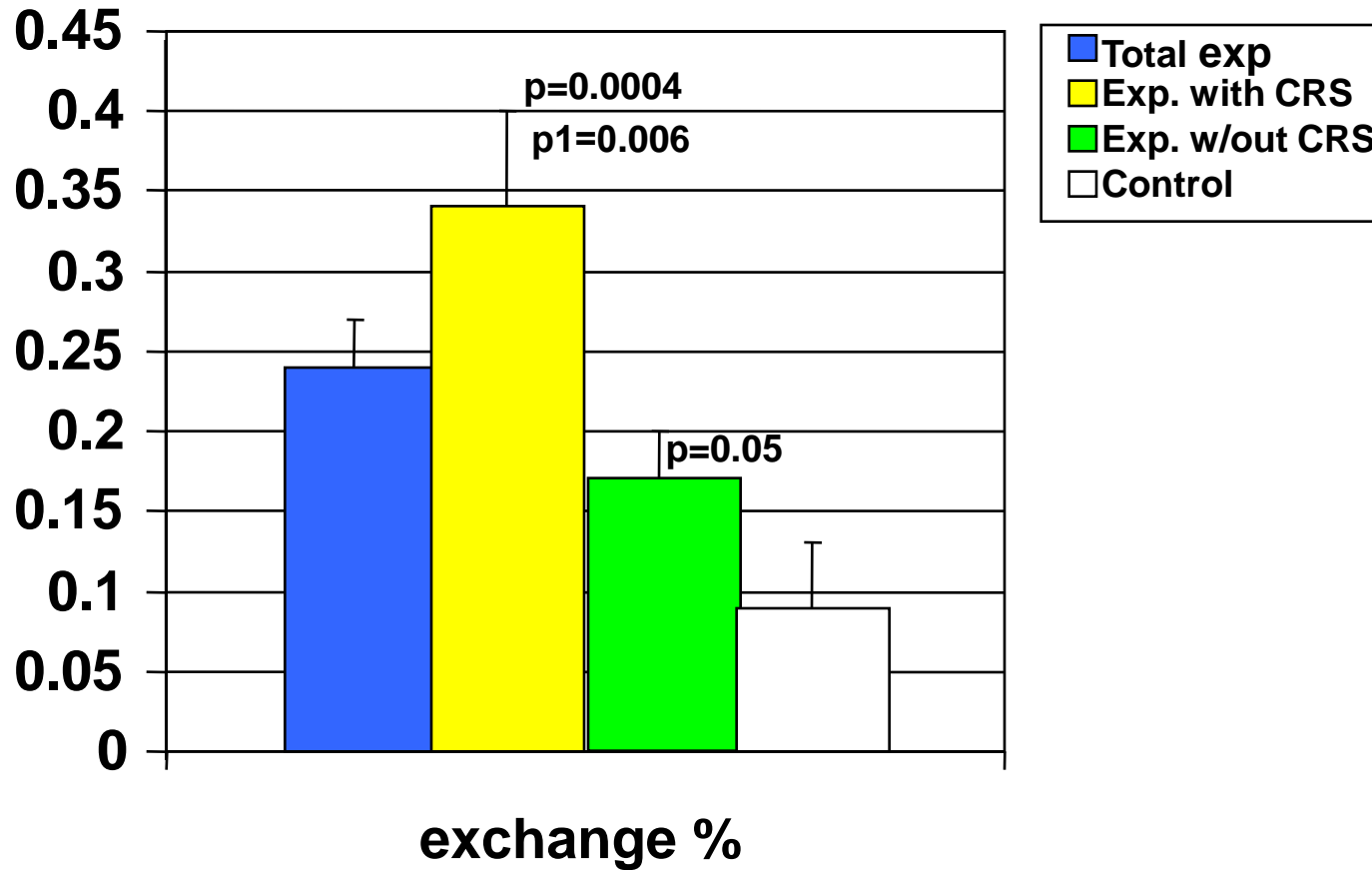
Standard research methods were used :

1. Analyses of unstable chromosome aberrations (dicentrics);
 2. Assessment of the frequency of somatic mutations in lymphocytes (CD3-CD4+ cells);
 3. Assessment of mutation frequency in the gene Tp53;
 4. Micro-nuclear test;
 5. Evaluation of oxidative processes (contents of nitrogen and its metabolites, malonic dialdehyde), status of the antioxidant system (content of Cu/Zn-superoxide dismutase);
 6. TUNEL method: assessment of the frequency of lymphocyte apoptosis;
 7. Identification of specific features of the lymphocyte cell cycle (immunofluorescent painting of the intracellular proteins Chk2 and Ki-67);
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Characterization of the study population

- The study group comprised 692 individuals chronically exposed to radiation due to production activities at the Mayak NF, (The Southern Urals, Russia) and 338 unexposed individuals.
 - Findings of total blood counts indicative of reduced number of absolute leucocytes and/or lymphocytes below the physiological norm ($4.0 \times 10^9/l$ and $1.2 \times 10^9/l$, respectively) served as eligibility criteria for inclusion in the subgroup of exposed persons with leucopenia/lymphopenia.
 - Diagnosis of chronic radiation syndrome was made on the basis of clinical examinations and laboratory investigations during the initial period of the exposure (1950-1960)
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Exchange frequency in the groups studied



p: significant differences versus control group

p1: significant differences versus persons without CRS

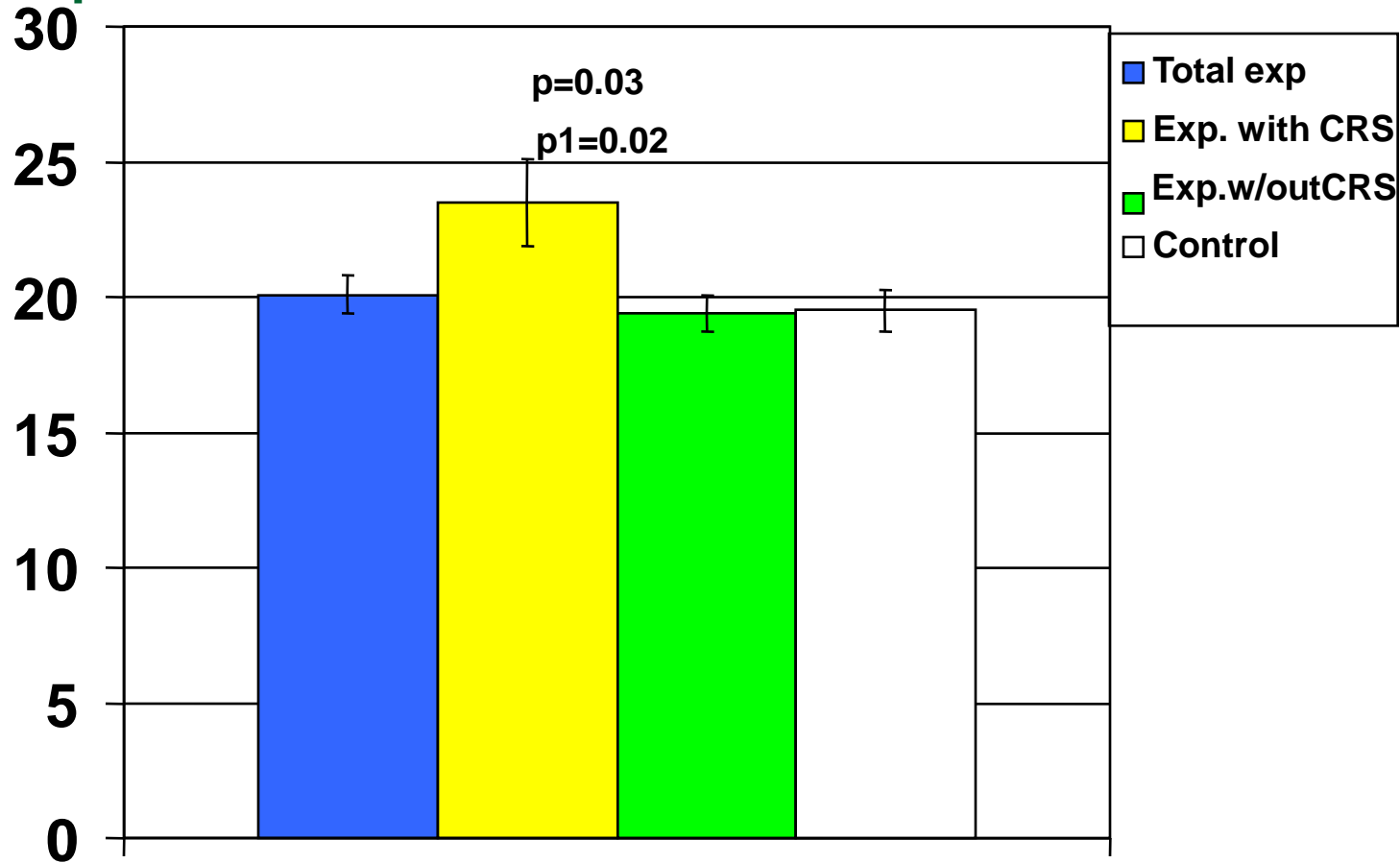
Frequency of exchange-type chromosome aberrations in subjects with cytopenic conditions

Groups	Exposed, with leucopenia	Exposed, without leucopenia	Exposed with lymphopenia	Exposed without lymphopenia	Control
Exchange-type chromosome aberrations	0.4 0.12 p=0.02 p ₁ =0.02	0.2 0.03	0.4 0.12 p=0.02 p ₁ =0.03	0.2 0.03	0.2 0.04

p: significant differences versus control,

p₁: significant differences versus the group without cytopenia

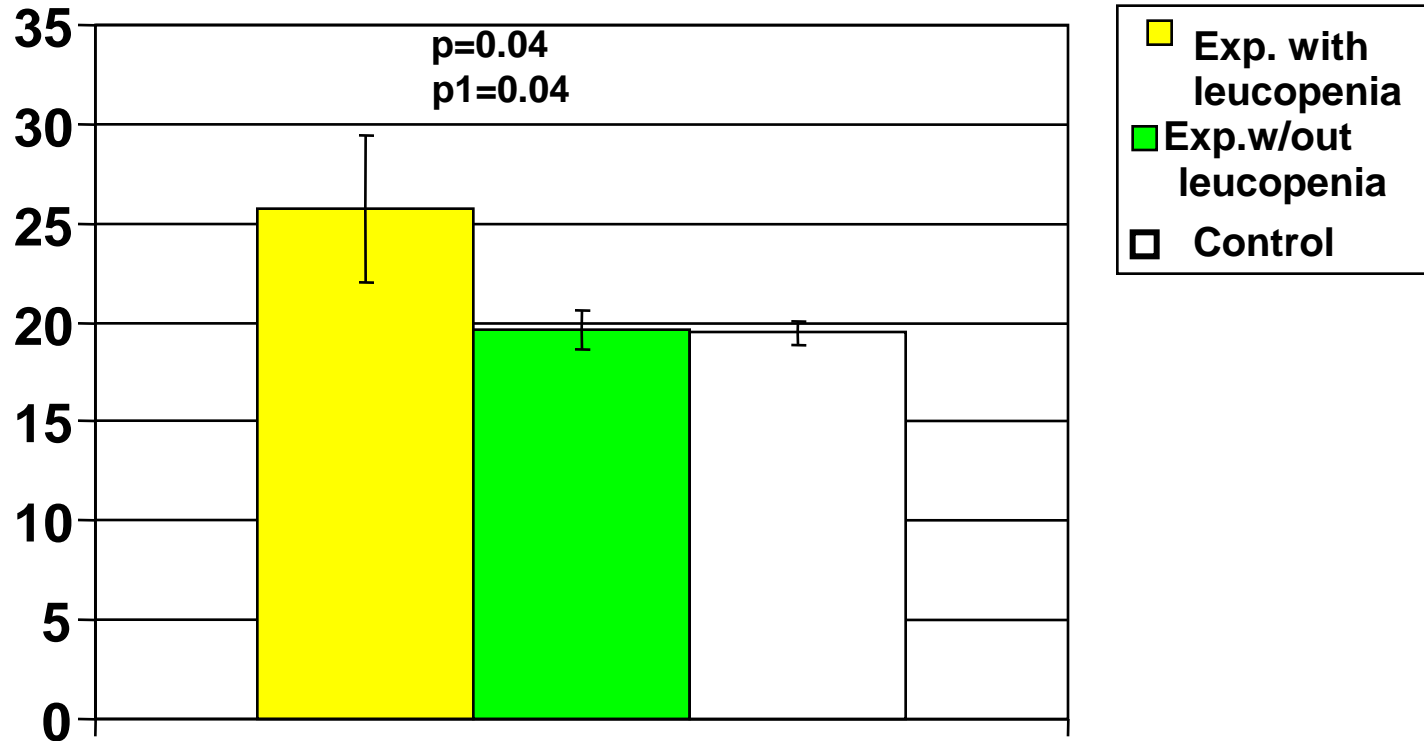
Frequency of cells with micro-nuclei among members of the groups studied.



p: significant differences versus the control group

p1: significant differences versus the CRS group

Frequency of cells with micronuclei in subjects with cytopenic conditions.



p: significant differences versus control group

p₁: significant differences versus the group free of leucopenia

Mutation frequency in loci 5,6,7,8 of the Tp53 gene for exposed individuals

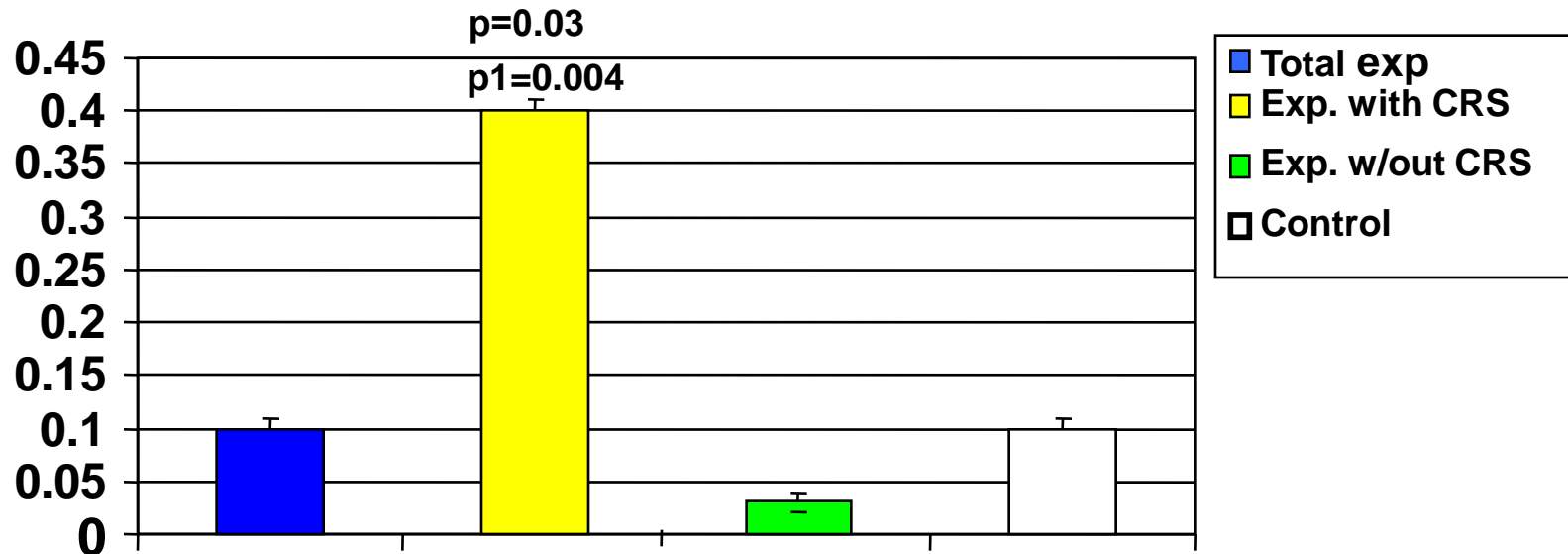
Exons	absence/ presence of mutations 0/1	Total exposed		Control	
		persons	%	persons	%
5	0	130	99.11	37	100.0
	1	1	0.99		
6	0	129	98.5	37	100.0
	1	2	1.5		
7	0	119	90.8	36	97.3
	1	12	9.2	1	2.7
8	0	126	96.2	37	100.0
	1	5	3.8		
Total for exons	0	111	84.7	36	97.3
	1	20	15.3 p= 0.04	1	2.7
p- significant difference from the control					

Mutation frequency in exons 5,6,7,8 of the Tp53 gene for subjects with leucopenia

Exons	absence/ presence of mutations 0/1	Exposed, with leucopenia		Exposed, without leucopenia		Control	
		persons	%	persons	%	persons	%
5	0	19	100.0	45	100.0	37	100.0
	1	0	0	0	0	0	0
6	0	19	100.0	44	97.8	37	100.0
	1	0	0	1	2.2	0	0
7	0	16	84.2	42	93.3	36	97.3
	1	3	15.8 p=0.05	3	6.7	1	2.7
8	0	19	100.0	44	97.8	37	100.0
	1	0	0	1	2.2	0	0
Total for exons	0	16	84.2	40	88.9	36	97.3
	1	3	15.8 p=0.05	5	11.1	1	2.7

p – significant differences from the control

Frequency of somatic mutations (CD3-CD4+ cells)



p: significant differences versus the control group
p1: – significant differences versus the CRS group

Cu/Zn SOD and MDA blood serum levels in subjects chronically exposed on the Techa River

Groups	Cu/ZnSOD concentrations, ng/ml		MDA concentration, μ mole/l	
	M m range	σ	M m range	σ
Total exposed	163.5 7.9 (18-662) (n=206)	121.0	43.4 0.8 (25-103) (n=183)	12.2
Exposed, without CRS	158.1 9.2 (18-562) (n=142)	102.5	41.5 0.83 (25.0-65.0) (n=125)	9.3
Exposed, with CRS	175.7 15.1 (18-662) (n=64)	121.0	47.3 1.5 (30-103) (n=58)	12.2
Control	146.4 12.1 (16-360) (n=49)	84.4	46.4 1.8 (25.0-71.0) (n=46)	12.1

Cu/Zn SOD and MDA blood serum levels in subjects with leucopenia

Groups	Cu/ZnSOD concentrations, ng/ml		MDA concentration, $\mu\text{mole/l}$			
	M	m range	σ	M	m range	σ
Exposed, with leucopenia	137.1	21.3 (36-334) (n=18) $p_1=0.07$	93.0	42.2	2.1 28-53 (n=14)	7.8
Exposed, without leucopenia	189.9	13.9 (18-662) (n=93) $p=0.08$	133.8	44.2	1.0 25-65 (n=84)	8.8
Control	146.4	12.1 (16-360) (n=49)	84.4	46.4	1.8 (25.0-71.0) (n=46)	12.1

p : statistical significance of differences in values versus the control

p_1 : statistical significance of differences in values versus exposed subjects without leucopenia

Levels of blood serum nitric oxide (NO) for exposed subjects

Groups	NO concentration, $\mu\text{mole/l}$	
	M m Range	σ
Exposed (n=105)	46.4 1.5 (21-108) $p=0.1$	15.8
Exposed, without CRS (n=73)	44.3 1.5 (24-77) $p=0.06$	12.5
Exposed, with CRS (n=32)	51.2 3.7 (21-108) $p_1=0.04$	20.9
Control (n=48)	54.1 6.0 (25-324)	41.3

p : statistical significance of differences in values versus the control,

p_1 : statistical significance of differences in values versus the group of exposed subjects without CRS

Levels of blood serum NO for subjects with leucopenia

Groups	NO concentrations, $\mu\text{mole/l}$	
	M m range	σ
Exposed, with leucopenia (n=11)	63.4 6.1 (39-94) p=0.06 p ₁ <0.0001	20.3
Total exposed, without leucopenia (n=49)	42.8 1.8 (24-77)	12.4
Control (n=48)	54.1 6.0 (25-324)	41.3

p: statistical significance of differences versus the total number of exposed subjects

p₁- statistical significance of differences in values versus the group of exposed subjects with leucopenia

Levels of apoptosis in lymphocytes

Group	Baseline level		5 hr. incubation		24 hr. incubation	
	M m (min-max)	σ	M m (min-max)	σ	M m (min-max)	σ
Total exposed (n=123)	0,3±0,03 (0,01-1,7)	0,37	0,9±0,18 (0,01-8,4)	1,55	1,3±0,23 (0,02-12) p=0,04	2,0
Exposed, with CRS (n=37)	0,3±0,07 (0,01-2)	0,44	1,6±0,64 (0,05-8,4) p ₁ =0,05	2,49	1,0±0,27 (0,02-6,6) p=0,07	1,3
Exposed, without CRS (n=90)	0,3±0,04 (0,01-2)	0,34	0,7±0,15 (0,01-5)	1,15	1,4±0,33 (0,04-12) p=0,04	2,3
Control (n=54)	0,2±0,05 (0,01-2,1)	0,36	0,85±0,16 (0,1-4,6)	0,97	0,4±0,13 (0,01-2,6)	0,6

p: statistical significance of differences in values versus the control

p₁: statistical significance of differences in values versus exposed subjects without leucopenia

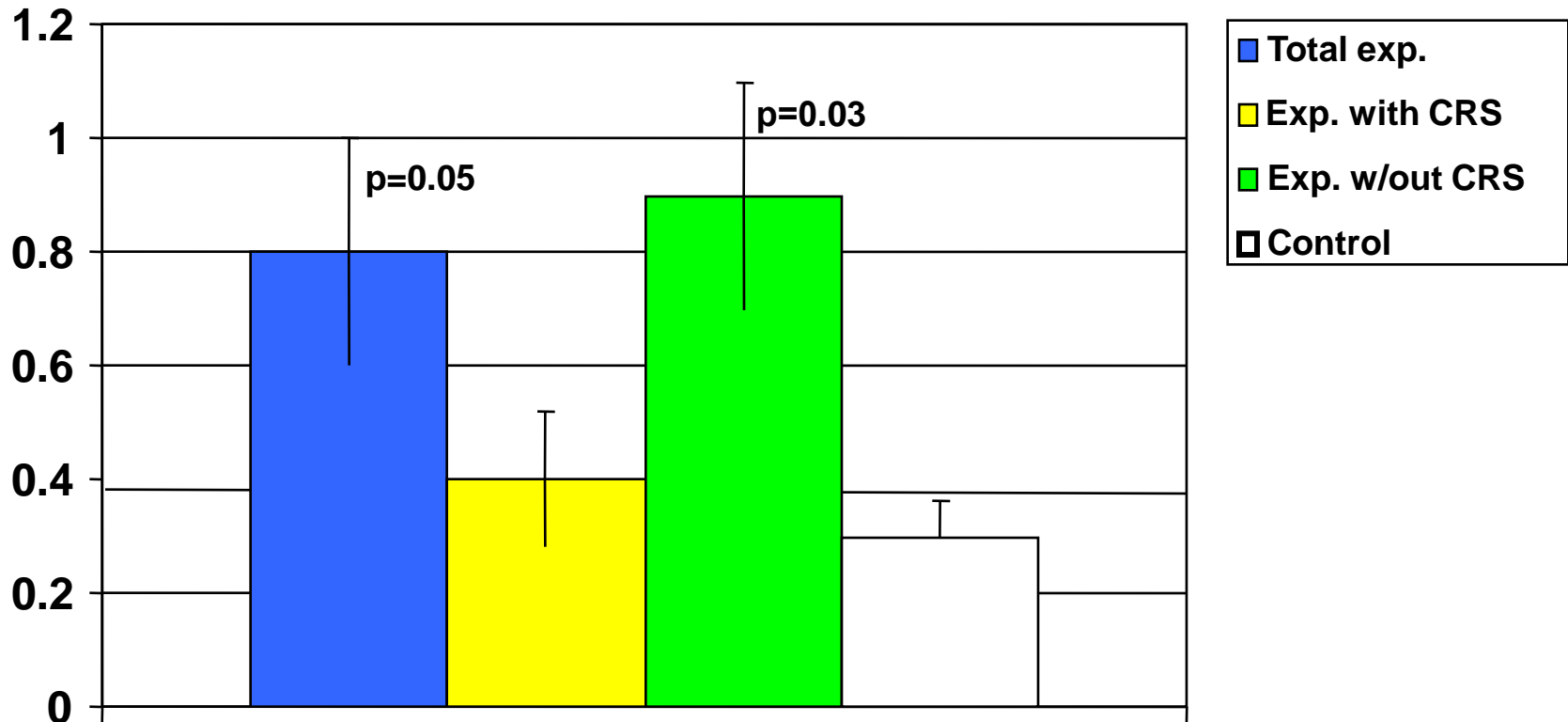
Numbers of subjects with exceeded reference values based on the frequency of apoptotic cells

Groups	subjects	
Reference value = $M+1.5 \sigma = 0.64\%$		
	N	%
Exposed, total	18	14.2 $p < 0.15$
Exposed, with CRS	8	21.6 $p < 0.05$ $p_1 < 0.05$
Exposed, without CRS	10	11.1
Controls	5	9.1

p : significant differences in values versus controls,

p_1 : significant differences in values between the exposed without CRS versus exposed with CRS

Contents of peripheral blood lymphocytes with Chk2 protein for members of the study groups.



p: differences versus the control group

p1: differences between the exp. groups without CRS and the CRS group

Contents of peripheral blood lymphocytes expressing Ki-67 protein

Groups	Ki-67 lymphocytes, %	
	M m (min-max)	σ
Exposed n=25	0.1 0.02 (0-0.39)	0.10
Exposed, with CRS n=5	0.2 0.06 (0-0.39) p=0.03	0.14
Exposed, without CRS n=20	0.1 0.02 (0-0.35)	0.07
Controls n=22	0.1 0.02 (0-0.49)	0.12

Conclusions

- Study subjects who had manifested hemopoiesis inhibition (e.g., leucopenia) and/or with diagnosed chronic radiation syndrome (CRS) during the early period of exposure (at the time of the highest dose rates) were noted to have an increased frequency of micronuclei, dicentric chromosomes, mutations in the Tp53 gene, somatic mutations (CD3-CD4+ cells). In exposed persons without cytopenia and/or CRS, the intensity of mutational processes at a late time after exposure was found to be at the background level.
- Thus, the state of the cell's functional mechanisms responsible for maintenance of the body's genetic homeostasis (antioxidant protection, cell cycle control, apoptosis) at late time after the onset of exposure can be described as an adaptively-activated one. Subjects with early effects manifested by the hemopoietic system (cytopenia, CRS) demonstrated a significant increase in the frequency of cell mutations/aberrations at later time which probably resulted from a highly probable functional disturbance of intracellular protection mechanisms.

Thus, exposure to ionizing radiation is, in all likelihood, manifested by the damage caused primarily to the category of highly radiosensitive individuals rather than by a uniform increase in the risk of induction of a genetic injury in the cells of each member of the exposed population.
