COMPARATIVE CYTOGENETIC ANALYSIS OF CHROMOSOMAL ABERRATIONS AND PREMATURE CENTROMERE DIVISION IN PERSONS EXPOSED TO RADIONUCLIDES

Dubravka Jovičić[1], Boban Rakić[2], Tanja Vukov[3], Jelena Pajić[2], Snežana Milačić[2], Radomir Kovačević[2], Milena Stevanović[4], Danijela Drakulić[4], Nenad Bukvić[5]

- [1] Faculty of Applied Ecology "FUTURA", University – Singidunum, Belgrade, Serbia dubravka.jovicic@futura.edu.rs
- [2] Serbian Institute for Occupational Health "Dr Dragomir Karajović", Belgrade, Serbia
- [3] Institute for Biological Research "Siniša Stanković", Belgrade, Serbia
- [4] Institute for Molecular Genetics and Genetic Engineering, Belgrade, Serbia
- [5] DIMIMP – Medical Genetic Section, University of Bari, Italy

INTRODUCTION

Many researchers have found strong positive correlation between increase in incidence of chromosome aberrations and the impact of small radiation doses among persons occupationaly exposed to ionizing radiations. Beside conventional method for analysis of chromosomal aberrations (CA) we paid special attention on detection of premature centromere division (PCD), Fig. 2. This phenomenon, found in lymphocytes of peripheral blood, is characterized by divided chromatides of chromosomes already in metaphase of cell cycle. The premature division of constitutive heterochromatin in centromeric region (PCD) can cause appearance of aneuploidy. In different types of neoplasia it represents the manifestation of disorders of spatial and temporal regulation mechanisms of mitoses that leads to genomic instability. Fluorescent *in situ* hybridization (FISH) was used to confirm presence of PCD at certain chromosome in metaphases and interphase nuclei as well as to detect at which stage of the cell cycle centromere regions split, Fig. 3,4.

Figure 1. The metaphase chromosomes

Figure 2. Premature centromere division (PCD)

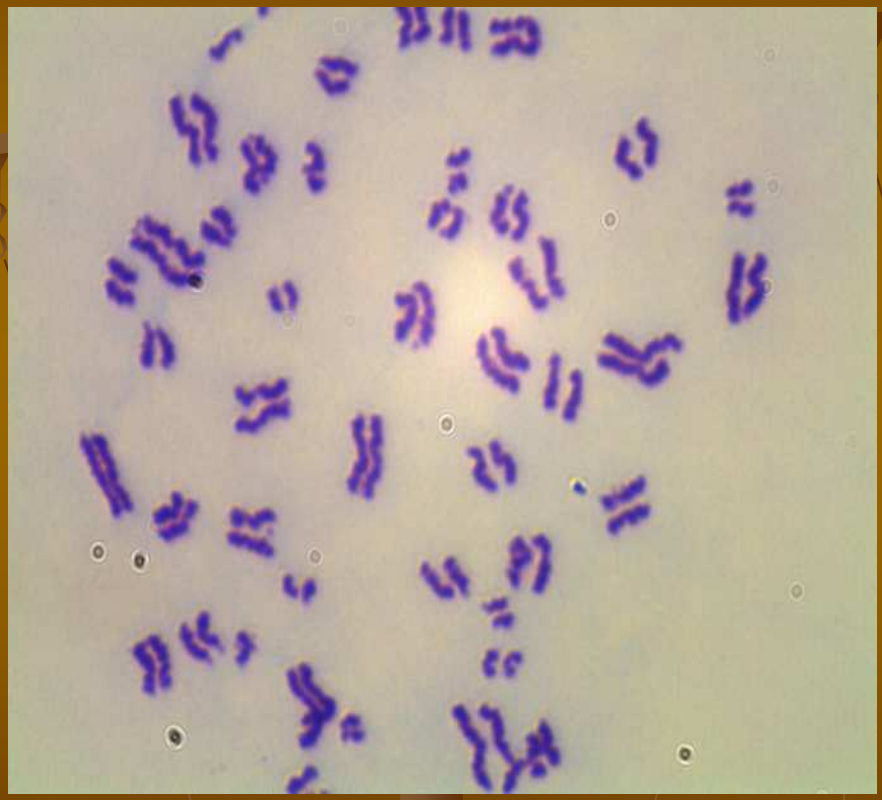


Figure 3. The presence of fluorescent signals in centromeric region of the metaphase chromozome 18 indicates exsistance of PCD

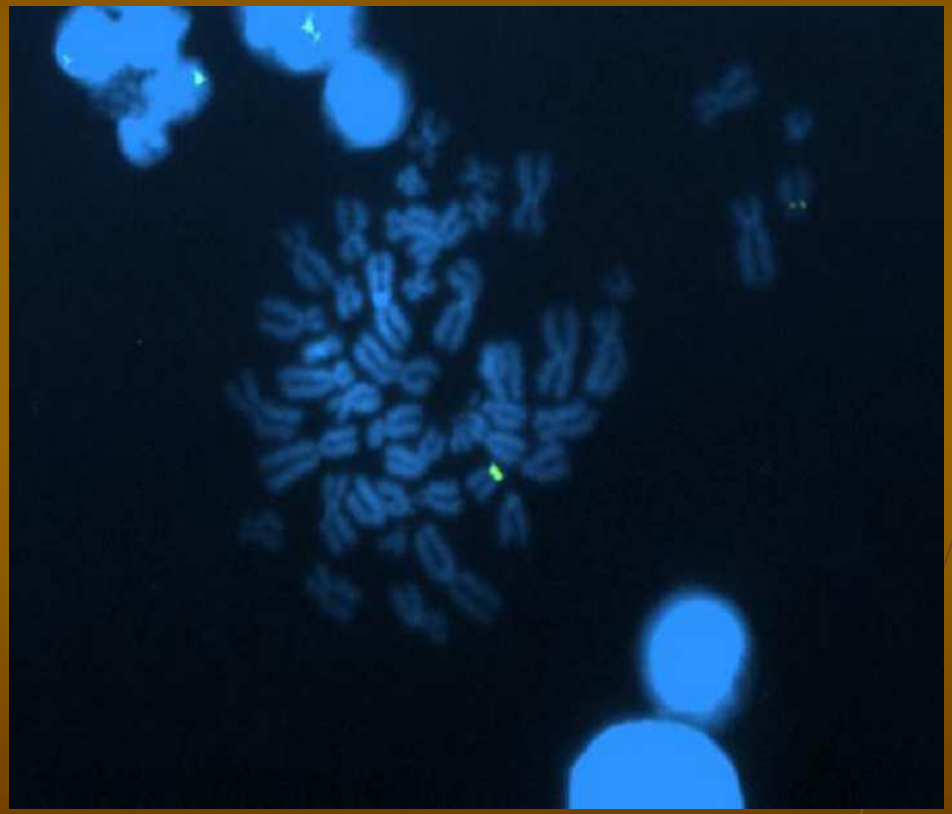
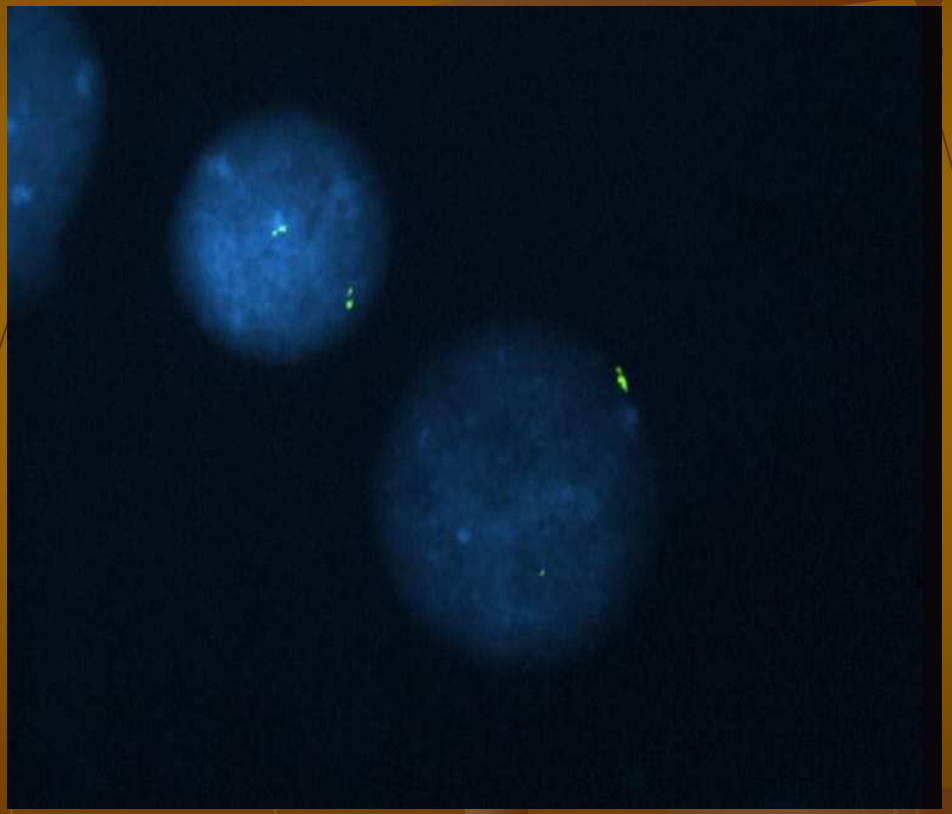
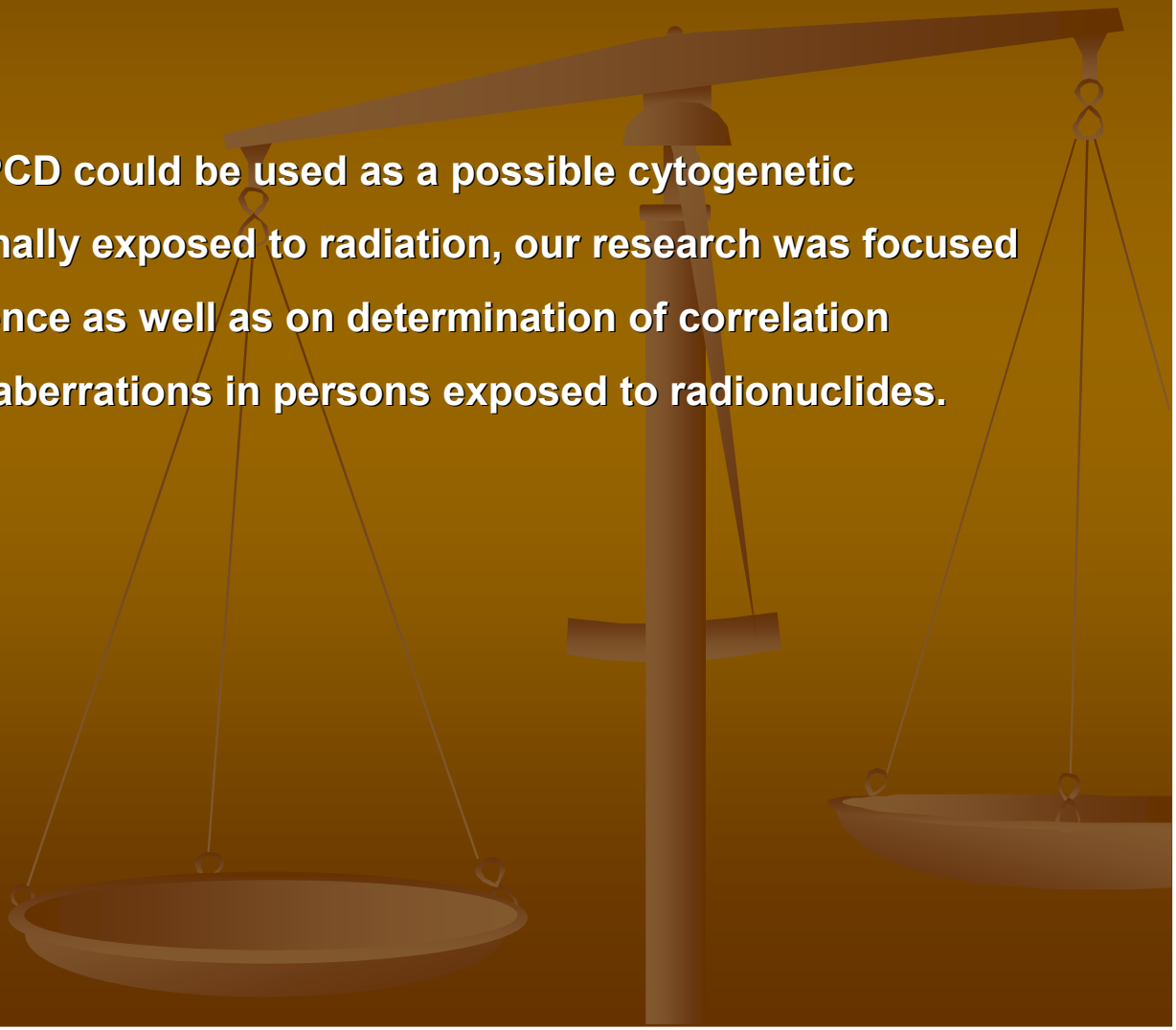


Figure 4. Interphase nucleus with two bipartitive signals indicates of PCD in both chromosome 18



PURPOSE

With aim to test hypotesis that PCD could be used as a possible cytogenetic biomarker in persons occupationally exposed to radiation, our research was focused on determination of PCD prevalence as well as on determination of correlation between PCD and chromosome aberrations in persons exposed to radionuclides.



MATERIAL AND METHODS

Our research included a group of 50 patients employed by Clinical Center of Serbia, who are occupationally exposed to radionuclides (C) and 40 patients from the control group (K), who have never been exposed to physical or chemical agents in their workplaces (Table 1). The effective radiation doses were measured by thermoluminiscent dosimeter (TLD) once a month during their occupational exposure. Biological dosimetry was carried out by the means of modified micromethod for lymphocytes of peripheral blood and conventional cytogenetic technique for the analysis of chromosome aberrations. The analysis of CA and PCD was examined in 200 metaphase cells. In order to confirm the results of premature segregation of centromere regions, FISH was applied. The results in this paper were processed by applying Statistics 5 (StatSoft, Inc) and SAS 6.12 software for PS.

Table 1. General characteristics of the exposed and control patients groups

		Control (N=40)	Exposed (N=50)	*P*-value
Age	Mean±SE	44.40±0.98	45.24±1.18	0.599
	Min-Max	34-60	27-59	
Sex				
Male	N	16	23	0.568
Female	N	24	27	
Smoking status				
Smokers	N	12	22	0.173
Non-smokers	N	28	28	
LT- labour time	Mean±SE	19.67±0.98	21.94±1.13	0.144
	Min-Max	13-36	5-35	
ERS – exposition	Mean±SE	/	17.96±1.15	
	Min-Max	/	5-35	
Effective dose (2008) (mSv)	Mean±SE	/	2.19±0.27	
	Min-Max	/	0-10.10	
Effective dose (2004-2008) (mSv)	Mean±SE	/	9.87±1.31	
	Min-Max	/	0.16-47.38	

RESULTS AND CONCLUSION

The results obtained in analysis of CA and PCD in persons occupationally exposed to the low doses of ionizing radiation are provided in Table 2.

On the basis of statistic data (Mann-Whitney U test), we have reached the conclusion that the frequency of CA and PCD is more significant in lymphocytes of peripheral blood in persons from the exposed group than in the control one ($p>0.05$). The frequency of PCD is far lower in the control than in the exposed group.

Frequency of tPCD (PCDs in more than 10 chromosomes per methaphase) among the occupationally exposed people was positively correlated with the dicentric frequency, acentric fragments and chromatide breaks (Spearman's test, $r=0.49, 0.54, 0.29$, $P<0.05$)

There was no positive correlation between the measured effective doses among patients and frequency of chromosome breaks and PCD.

Applying the FISH method we have established that centromere division occurs already in the cell cycle interphase, i.e. in G2 phase of the cell cycle. In addition, we have confirmed the presence of PCD in metaphases and interphase nuclei of patients exposed to radionuclei (Table 3).

Table 2. The frequency of CA and PCD in limphocytes of the exposed groups and control ones (ACF - acentric fragments, HB – chromatide breaks, iHB – isochromatide breaks, tPCD – metaphases with more than 10 PCD)

		Control	Exposed	*P*-value
No of aberrated cells	Mean±SE	0.85±0.16	4.06±0.15	0.000
	Min-Max	0-4	2-7	
Dicentric	Mean±SE	0.13±0.05	0.66±0.11	0.001
	Min-Max	0-1	0-2	
Ring	Mean±SE	/	0.12±0.05	0.334
	Min-Max	/	0-1	
ACF	Mean±SE	0.37±0.09	2.64±0.17	0.000
	Min-Max	0-2	0-5	
HB	Mean±SE	0.33±0.07	0.68±0.07	0.004
	Min-Max	0-1	0-1	
iHB	Mean±SE	0.17±0.06	0.5±0.07	0.008
	Min-Max	0-1	0-1	
tPCD	Mean±SE	0.25±0.09	2.12±0.25	0.000
	Min-Max	0-2	0-7	
PCD 1-5	Mean±SE	4.05±0.25	8.5±0.41	0.000
	Min-Max	1-7	2-14	
PCD 5-10	Mean±SE	1.72±0.22	4.64±0.23	0.000
	Min-Max	0-5	2-9	

Table 3. Distribution of fluorescent signals for centromere region of chromosome 18 in metaphases and in interphase nuclei by applying DNA repetitive probe, both to the exposed and control groups

	INTERPHASE NUCLEI			METAPHASES		
Patients	Number of analysed nuclei	Without PCD	With PCD	Number of analysed metaphases	Without PCD	With PCD
1.	83	70 (84.337%)	13 (15.663%)	28	14 (50%)	14 (50%)
2.	102	95 (93.137%)	7 (6.863%)	24	24 (100%)	0 (0%)

Our researches suggest the possibility to use PCD as cytogenetic biomarker in persons occupationally exposed to radionuclides. It is necessary to emphasize that these results include preliminary researches in relatively small sample. Our future researches will include larger number of patients in combination with *in vitro* researches, which will surely contribute to better understanding of this phenomenon.