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Damaged Lymphocytes and Cancer Risk in Medical Nuclear Workers

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ABSTRACT

Aim: Cytogenetic analysis of peripheral blood lymphocytes is the most sensitive test for detecting a clinical biologic response to ionizing radiation. Damaged lymphocytes, containing chromosomal aberrations and cancer risk in medical nuclear workers doe to contamination with radioiodine may indicate a thyroid gland tumor while it was operable and curable may lead to immune disorder, a susceptibility to certain cancers, and/or other chronic disorders.

Method: Workers are periodically reviewed to check the contamination (by measuring the radioactivity in urine) and received doses, analysis of the frequency of chromosome aberrations. The 24 h urine samples were measured by gamma-spectrometry methods. Chromosomes were observed in peripheral blood lymphocytes. Moorhead's method and conventional cytogenetic technique were used for preparation of lymphocytes.

Results: Workers in nuclear medicine had a chromosomal abnormality. Significantly higher probability to occur chromosome aberrations, λ=0.62, have been found on periodical examination PE_3. Higher probability to occur chromosome lesions, have been found on each periodical examination, λ_1=0,12/λ_2=0,14/λ_3=0,31.

Conclusion: Consequently, the probability (λ) that a greater number of lymphocytes is damaged due to aberrations and lesions of chromosomes, significantly increases with exposure; p=0.01. Their sensitivity for measuring exposure to low dose and their role as early predictors of cancer risk have contributed to this success.

Key words: Nuclear medical workers, urine radioactivity, chromosomal abnormalities

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INTRODUCTION

Chromosomal aberrations in peripheral blood lymphocytes are recognized as biomarkers of exposition to ionizing radiation and can be predicting factor for risk of cancer in medical workers [1]. Occupational exposure to radioactive and chemical toxic substances may results in binding or DNA break, which may lead to chromosome alterations and could be an initial event in the carcinogenesis [2]. The induction of geno-toxicity such as chromosomal lesions may lead to further problems of mutagenic and carcinogenic activity, because chromosome damage is the underlying cause of mutations which lead to cancer [1, 2]. Many forms of damaged lymphocytes can be detected: chromatid (one chain) and chromosome (double chain) breaks and exchanges, acentric, dicentric and ring chromosomes, after double breaks [3]. The broken ends of chromosomes can combine with broken ends of different chromosomes. The frequency of chromosomal aberrations (F-ca) in peripheral circulating lymphocytes (Ly) correlates with the received dose. The minimum dose that can be detected by peripheral lymphocyte analysis is about 100 mSv to 200 mSv total-body exposure. Medical nuclear workers are exposed to ionizing radiation by radio-nuclides, during work time [4]. Radio-nuclides are used for laboratory research and for various tests in vitro, and the treatment and diagnosis of disease, in vivo. Medical nuclear workers can be doctors, specialists, senior medical technicians, technicians, nurses, laboratory technicians, physics, and engineers. Effective time shedding the organism of radioactivity is correlated with physical and biological half-life of elimination, and depends on the metabolism of radionuclide and health condition of workers [5].

METHODS

Nuclear medicine workers are periodically checked once a year (by measuring the radioactivity in urine) and received doses, analysis of the frequency of chromosome aberrations (F-ca).

The 24 h urine samples of nuclear medical workers (NMW) and control subjects were measured by gamma-spectrometry methods. Natural and artificial radionuclide (RN) from environmental and working place are detected by Gamma-spectrometry method and presented by Bq per liter of urine, all on purpose to calculate individual absorbed dose, according to AIL annual intake limits [6, 7].

To detect chromosomal aberrations, peripheral lymphocytes from venous blood are used by Moorhead's method and conventional cytogenetic technique, according to a standard protocol[8].

Fixed cells were spread on slides and dried over a flame. The slides were aged for the next 5-7d. Giemsa stained slides were coded and scored blind under a light microscope. Two hundred well-spread metaphases per subject were screened for chromosome damage.

The most characteristic aberration was dicentric chromosome. Ring chromosome and acentric fragment 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 (ca and/or cl) were marked as damaged cells[8, 9].

Analyses of health condition of NMW have been for long-term as a longitudinal study. They were working with radioactive iodine isotopes 131(^{131}I) and technetium 99 metastabile (^{99}Tc$_m$) - mostly the gamma emitters.

The study includes 51 workers (doctors, technicians, laboratory technicians and nurses)[2]. The average age was 33.78 ± 7.82, and 19.61 % were male and 80.39 % female. Hematological parameters, chromosomal changes and damaged cells after different duration of occupational exposure (DOE) have been analyzed. After previous examination (until 5 years), next check-up have been after 10, 15 and 20 years of DOE (periodical examination PE_1; PE_2; PE_3, respectively).

Workers in the laboratories of nuclear medicine have been compared with control group, the 28 healthy medical workers without the risk of radioisotope`s contamination.

Control group include doctors, technicians and nurses, work experience 9.86 ± 8.23 years. The average age of control group was 32.36 ± 9.30 (17.85 % were male and 82.14 % female). The only risk of internal contamination in the control group was from the environment via ingestion (radio cesium ^{137}Cs, gamma emitter, as well).

Statistical methods

Investigated parameters had been observed using the estimated Poisson distribution and linear regression correlation analysis. The significance of the difference was tested using Mann-Whitney U test and Wilcoxon rank sum test with 95 % confidence interval and significance threshold at the level of 0.05. The probability of characteristic biomarkers appearance was specially analyzed using logarithmic function of the Poisson regression and it was quantified by lambda parameter (λ). Lambda parameter (λ) shows relatively risk (RR). Relatively risk can be expressed as the probability to occur DNA changes and the probability of damaged cells.

RESULTS

Gamma-spectrometry analyses of 24-h urine have presented radioactive iodine isotopes 131(^{131}I) ranged: 0.1 – 18.7±1.5 Bq/l urine and technetium 99 metastabile (^{99}Tc $_m$), ranged from 0.1 to 1.0 Bq/l urine in 26 (50 %) NMW; and radio cesium ^{137}Cs in 5 NMW (10 %) ranged 0.01 – 0.1 Bq/l urine. NMW's urine was contaminated with ^{131}I, but the contamination have been under the AIL (Annual Intake Limit) in the urine during previous examination there was only ^{137}Cs <1 Bq/l urine.

[2] Human ethics procedure has approved according to the legal requirements of the Republic of Serbia

Statistical analyses (Poisson regression) of the frequency (F) and density of chromosome aberration`s and lesion`s probability (λ) during 20 years occupationally exposition to RN in nuclear medicine (with 95 % confidence interval and significance threshold at the level of 0.05) have been shown in the Table 1.

Previous examination did not define ca in any NMW on 10,200 lymphocytes. Two of the 51 workers had non-specific chromosome lesions. Therefore, damaged cells frequency cl was found 0.03% and probability (λ) was 0.09 %.

Significantly higher frequency and probability (λ) of appearance chromosome aberrations have been found on periodical examination PE_1, after 5 to 10 years. Frequency of ca was 0.21 %, and probability for ca appearance was 0.33. On periodical examination PE_2, after 11-15 years of duration of occupationally exposure (DOE), probability to occur chromosome aberrations is increased ($\lambda = 0.38$) but not significantly. However, the exact number of ca was decreased and frequency has been 0.16 %. Number and frequency of chromosomal aberrations have been significantly increased after 16-20 years *($p=0.04<0.05$)*. Frequency ca has been 29 %.

Significant higher probability for chromosome aberrations appearance (λ=0.62) have been found on periodical examination PE_3 (Table 1 and Table 2; Graph 1). Frequency of chromosomal aberrations is not linear correlated with the time of service (coefficient correlation is 0.10 *($p>0.05$)* (Graph 2). Probability for ca appearance (λ), are increased with the time of work in irradiations zone.

During long term exposition to ionizing radiation appearance of aberrations can increase. After the cessation of exposure, the aberrations disappear. For example, when NMW are return to work, CA can arise again (Graph 2).

Number and frequency of chromosomal lesions (cl) have been increased for all the time, on each periodic examination. However, compared to the previous examination (P_0), was not significantly different after 5-10 years of exposure (P_1).

Higher probability of chromosome lesion`s appearance, have been found on each periodical examination, λ_1=0,12/λ_2=0,14/λ_3=0,31 (Graph 1).

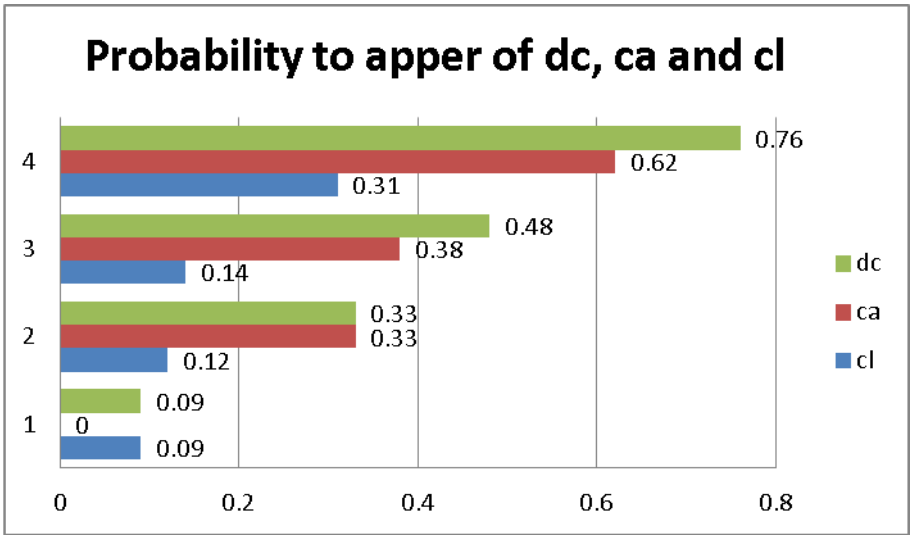
Significantly higher values of all investigated parameters were on the third periodic examination, significantly different from the previous check-up (Table 1). The correlation coefficient with exposure time is 0.37; *$p<0.05$* (significance with confidence 95%). Consequently, the probability (λ) that a greater number of cells (lymphocytes) is damaged due to aberrations and lesions of chromosomes, significantly increases with exposure; *$p=0.01$* (Table 1, Table 2 and Graph 1). The correlation coefficient between damaged cells and exposure time is 0.40; *$p<0.05$.*

Table 1. Chromosomal changes in blood lymphocytes of Nuclear Medical Workers on periodical versus previous examinations

Change on DNA and damaged cell			Nuclear Medical Workers			
			Periodical and Previous Examinations **(PE)** Time: Years *(y)*			
			$PE_{5\text{-}10y}$	$PE_{11\text{-}15y}$	$PE_{16\text{-}20y}$	**$PE_{0\text{-}4y}$**
Nº workers			51	51	51	**51**
Nº cells			10200	10100	9700	**10200**
Chromosomal aberrations ca	Workers with ca		14	9	18	**0**
	Percents (%)		27.45	17.64	35.29	**0**
	Total ca		21	16	29	**0**
	Frequency of ca (%)		**0.21%**	**0.16%**	**0.29%**	**0**
	Puason α_ci = 0.05	λ	**0.33**	**0.38**	**0.62**	**0**
		ci_ up	0.15	0.18	0.35	**0**
		ci_ down	0.64	0.70	1.00	**0**
Chromatid lesions cl	Workers with cl		4	5	11	**2**
	Percents (%)		7.84	9.80	21.57	**7.00**
	Total cl		5	7	15	**2**
	Frequency of cl (%)		**0.05**	**0.07**	**0.15**	**0.03**
	Puason α_ci = 0.05	λ	**0.12**	**0.14**	**0.31**	**0.09**
		ci_ up	0.02	0.04	0.13	**0.01**
		ci_ down	0.34	0.37	0.61	**0.30**
Damaged cells dc	Workers with dc		15	14	21	**2**
	Percents of workers with dc (%)		29.41	27.45	41.17	**7.00**
	Total dc		21	21	32	**2**
	Frequency of dc (%)		**0.21**	**0.21**	**0.33**	**0.03**
	Puason α_ci = 0.05	λ	**0.33**	**0.48**	**0.76**	**0.09**
		Ci_up	0.15	0.25	0.46	**0.01**
		Ci_down	0.64	1.83	1.18	**0.30**

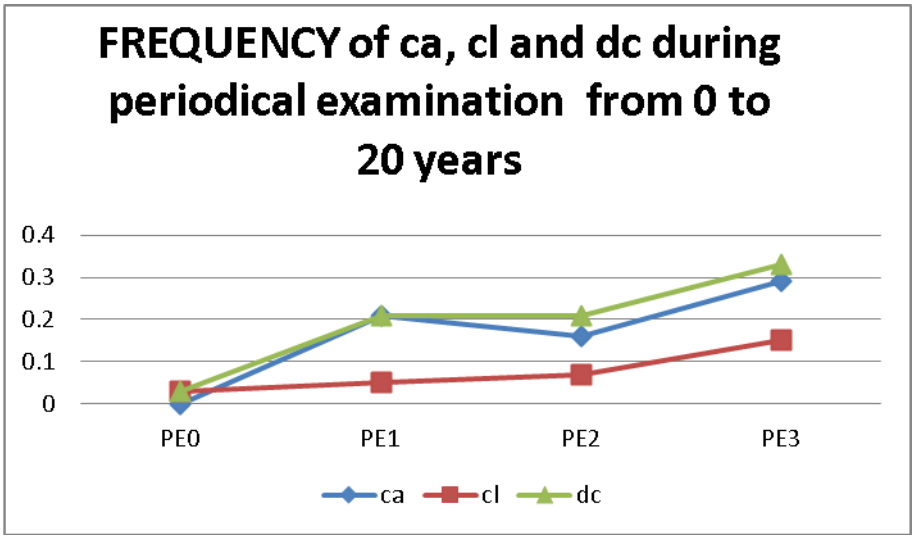
Table 2. Significance of difference of change on DNA and damaged lymphocytes

Change on DNA and damaged cells		Periodical examinations 3	
		Significance with Confidence 95%	**p**
Periodical examinations 2	Chromosomal aberration	Yes	0.04<**0.05**
	Chromosomal lesion	Yes	0.05=**0.05**
	Damaged Lymphocytes	Yes	0.01=**0.01**



Graph 1. Comparison probability (λ) between the changes in the cells depending on the time

dc = damaged cells; ca=chromosomal aberration; cl=chromosomal lesion;
1 = previous examinations
2 = periodical examinations
3 = periodical examinations 11-15years on 5-10years
4 = periodical examinations 16-20years

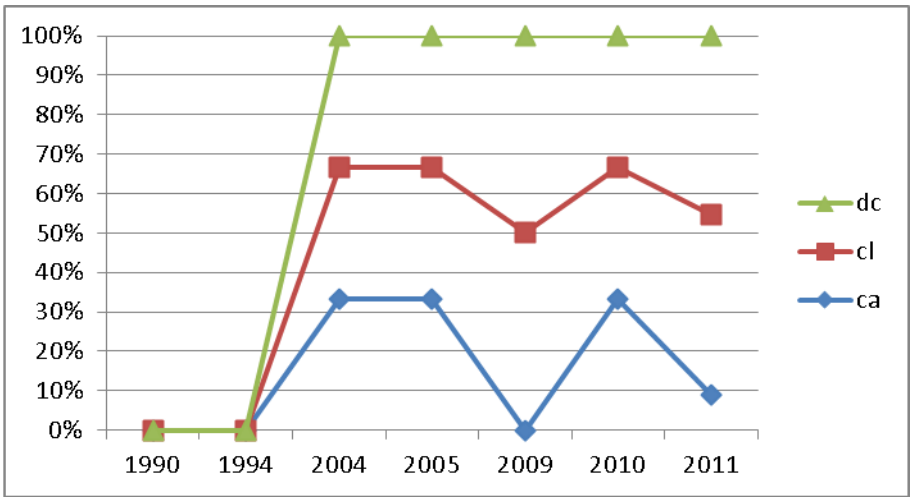


Graph 2. Frequency of chromosomal aberation (ca), lesion (cl) and damaged cells – lymphocytes (dc) during previous (PE0) and periodical examinations (PE1;PE2;PE3) from 0 to 20 years

PE = Periodical Examination after 5-10 (PE1); 11-15 (PE2) and 16-20 years (PE3) of occupational exposures;
PE0 = Previous Examination: 0-4years

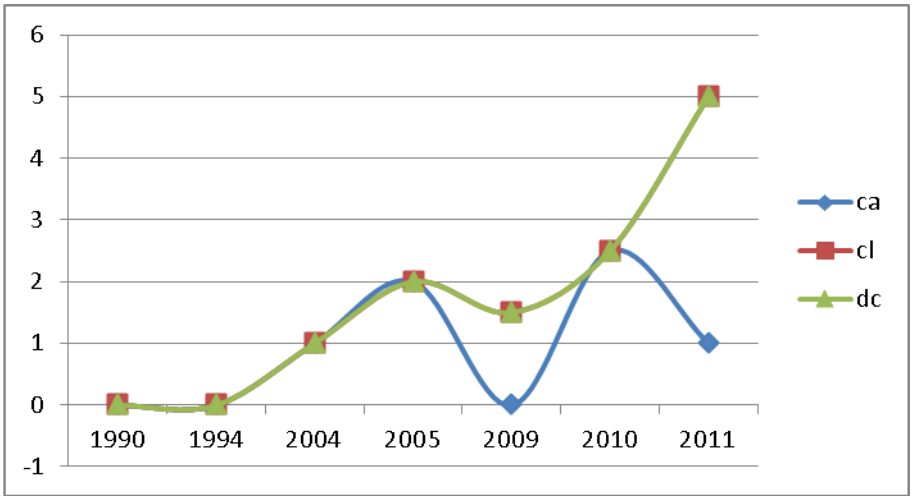
Relative risk of cancerogeneses, expressed as a probability of damage karyotype Ly (Graph 1), significant in relation to the initial value of 0.09 versus 0.33, 0.48 and 0.78 respectively.

Among damaged lymphocytes, chromosomal lesions, like as double breaks, contributes 68%, versus chromosomal aberrations, like as dicentric, which contribute to damage 32 % (Graph 3).



Graph 3. Chromosomal alterations in damaged lymphocytes during 20 years working with I^{131} *(32%ca versus 68%cl in 100%dc)*

Medical technician, female, after 21 years of occupational work with radioactive iodine, had thyroid gland cancer (Graph 4). It can be explained by disturbances in DNA damaged cells and lack of immunity. After 10 years of exposition to ^{131}I a mitotic index of cells was decreased. TLD and gamma spectrometry analysis of urine are not indicative of an increased dose. Frequency of ca varied before and after the cessation of exposure, while lesions (cl) were present, and damaged Ly increased (Graph 4).



Graph4. Chromosomal alterations in Patient with thyroid cancer after 21 years occupationally exposure to I^{131}

DISSCUSION

Chromosomal changes and disruption of cell division are potential risk of carcinogenesis. Low doses of ionizing radiation in medical laboratory workers in nuclear medicine, due to internal contamination with radionuclide and external irradiation, can cause malignant diseases. Absorbed radiation energy accumulates in the cell in the form of various alterations in chromosome. The probability of malignancy increases with the absorbed energy (dose) of radiation, latent time, the frequency of chromosomal lesions in lymphocytes - the power of immunity against tumors[7, 8, 9]. The relative risk for carcinogenesis, reported damaging cells, increasing the duration of exposure to radio-nuclides, from 33 % after 10, and 76 % after 20 years amount nuclear medical workers, who are worked with radioactive iodine and technetium. One worker developed thyroid cancer. The method of chromosome damage analysis is sensitivity for measuring exposure to low dose and damage cells as early predictors of cancer risk. Genome instability is related with individual cancer development risk. Our results support the idea that the chromosomal alterations may act as predisposing factors in carcinogenesis[10, 11].

Workers who are exposed to radionuclide such as radioactive iodine could develop thyroid cancer because of its accumulation in thyroid tissue. Thyroid tumors develop in people exposed to large amounts of environmental thyroid radiation, as occurs from atomic bomb blasts, nuclear reactor accidents, or incidental thyroid irradiation due to radiation therapy. Tumors may be detected 10 years after exposure, but risk remains increased for 30 to 40 years[12, 13, 14, 15].

Large international cohort study on 6718 individuals from 10 countries showed a significant association between chromosomal abnormalities, such as micronuclei formation, in healthy subjects and cancer risk[16]. Cancers incidence was significantly higher in groups with high micronuclei frequency in peripheral blood lymphocytes[16]. Relative cancer risk was 1.53. Case control study on lung cancer suggested that high lymphocyte nuclear damaged were predictor to cancer patients.

Chromosomal aberration and micronuclei are formation in peripheral blood lymphocytes use to genotoxicity biomarkers influence with ionizing radiations[17]. Cytogenetic biomarkers in peripheral blood lymphocytes predict the risk of cancer in humans on different tissues[16, 17].

About 1% of a population can develop cancer in their lifetime as a result of ionizing radiation from background levels of natural and man-made sources. Small doses of ionizing radiation may cause cancer in occupational workers exposed to chronic low levels, above normal background, below about 10mSv. The effect of occupational long term radiation exposure on the lymphocyte, which are especially sensitive to radiation after the contamination and accumulation in tissue, is screening test for risk assessment. This is because the lymphocyte may be precursors of immunity disorders, cancer susceptibility or other chronic conditions.

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