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EXPERIMENTAL / LABORATORY STUDIES

Chromosomal Aberrations in Radiation Waste Repository Workers Detected by FISH Painting and Giemsa Staining

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Abstract: We aimed to assess chromosomal aberration frequency by means of FISH chromosome painting and conventional Giemsa staining in peripheral blood lymphocytes of workers at a nuclear waste repository. The results obtained from the exposed group were compared with those from a control group from the administrative staff of the company. Data on health and social status were collected using a questionnaire. Conventional chromosomal analysis after Giemsa staining did not indicate any significant difference between radiation exposed and control workers. Analysis after FISH chromosome painting revealed a 2-fold increased in genomic frequency of stable chromosomal translocations in the exposed group over the control group. The difference is of marginal statistical significance.

Key Words: Occupational radiation exposure, Human lymphocytes, Chromosomal aberrations, Fluorescent in situ hybridization

Introduction

Ionizing radiation is a well known classical mutagen capable of inducing various kinds of stable and unstable chromosomal alterations. Analysis of chromosomal aberrations in the lymphocytes of subjects professionally exposed to ionizing radiation is one of the most reliable biological indicators of radiation risk. Mutational events are key steps in a multistage process of cancerogenesis and many neoplasms have been proved to be associated with chromosomal rearrangements (1-3). An increased frequency of chromosomal aberrations in a population may be associated with increased cancer risk (1).

All radioactive waste in Bulgaria, except that generated at NPP Kozlodui, is stored at Novi Han nuclear waste repository (NWR). The disposal facility is situated near the village of Novi Han, 35 km from Sofia (4). The total activity stored by now is over 60,000 Ci, which requires health monitoring and radiation protection precautions for the personnel. Dicentric chromosomes are well known biological dosimeter for the estimation of radiation exposure (5-8). The assessment of radiation exposure based on dicentric chromosomes scored is restricted to recent, acute exposures, due to the removal of the dicentrics over time (2,3,7). Occupational exposure is mainly protracted over a period of years. The development of the fluorescent in situ hybridization technique (FISH) has allowed the scoring of the so-called stable chromosomal aberrations (translocations and inversions) that persist with time after exposure and can show the accumulation of cytogenetic effects of radiation exposure under fractionated, chronic occupational exposure condition (8-12).

The aim of the present study was the assessment of chromosomal aberration frequency in peripheral blood lymphocytes of nuclear waste repository workers by means of FISH chromosome painting and standard Giemsa staining.

Materials and Methods

Investigated subjects

Conventional cytogenetic analysis for chromosomal aberrations was performed on 27 workers at the nuclear waste repository. Molecular cytogenetic analysis for chromosomal translocations was performed on 15 workers. The subjects were exposed at low level doses and dose rates to alpha, beta and gamma rays for periods ranging between 1 and 35 years. Regular whole body radiation counts on the workers showed that ionizing radiation doses are incurred exclusively through external exposure. The absorbed dose accumulated during the total period of radiation employment measured by TLD and film badges ranged between 0.50 and 30.40 mSv, and was well below the permissible limit of 50 mSv/year. Regular use of personal protection devices is reported.

The control group was assembled from the administrative staff of the same company, mainly new employees. It consisted of 9 individuals, who had not been exposed to ionizing radiation, 6 of whom were also analyzed by FISH painting. Blood samples were collected during their yearly medical examinations.

All investigated subjects, exposed and controls, answered a personal questionnaire from which a profile of each group was obtained. Informed consent documents were obtained after an explanation of the study had been given. The characteristics of the study groups are presented in Table 1.

Blood samples and culture conditions

Venous blood was collected in vacutainers containing Li-herapin, and lymphocyte cultures were incubated at 37 ^{o}C in 4.25 ml of RPMI + 0.75 ml of fetal bovine serum

Table 1. Characteristics of the study groups.

		Number of subjects analyzed by conventional Giemsa staining		Number of subjects analyzed by chromosome painting technique	
		NWR workers	Control workers	NWR workers	Control workers
Examined subjects		27	9	15	6
Duration of employment	1-10- years	13	9	8	6
	11-40- years	14	-	7	-
Accumulated dose	0-5 mSv	16	-	11	-
	6-10 mSv	5	-	3	-
	11-30, 44 mSv	6	-	1	-
Gender	Male	21	4	14	2
	Female	6	5	1	4
Age	21-40 years	6	7	4	4
	41-60 years	21	2	11	2
Smoking habit	never	7	3	2	0
	former	4	-	3	-
	current (cigarettes/day):	16	6	10	6
	1- 10	1	4	1	4
	11- 21	9	2	6	2
	21- 31	4	-	2	-
	>31	2	-	1	-
Radiological diagnostic examination (within the last 3 years)		-	3	-	1

and phytochemagglutinin P (5). Two cultures were set up from each donor. Colcemid was added to the cultures 46 h after incubation. The cells were harvested at 48 h. After hypotonic treatment with KCl (0.075 M) the cells were fixed with methanol–acetic acid (3:1) and spread onto clean slides.

Analysis of unstable chromosomal aberrations

In Giemsa stained preparations unstable aberrations were examined. Between 100 and 500 cells were scored per person for conventional analysis of structural chromosomal aberrations (fragments, dicentrics, rings) after staining with 5% Giemsa. Some of the slides were frozen at –20 °C for subsequent FISH staining.

Analysis of stable chromosomal aberrations

FISH was applied to detect stable aberrations (translocations). Chromosome specific DNA libraries (Cambio), FITC labeled, for chromosomes #1 and #4 were used, representing 14.3% of the whole genome. In brief, DNA probes were denatured for 5 min in 70 °C water bath, and then incubated for 2 h at 37 °C to allow the competition of repetitive sequences. Denaturation of in situ DNA was achieved at 80 °C incubation for 3 min in the presence of 70% formamide, 2xSSC and 10 mM phosphate buffer under the coverslip. The hybridization in situ was allowed to occur overnight at 37 °C in a moist chamber. After hybridization the slides were washed in 50% formamide, 2xSSC and 4xSSC, and Tween 20. The preparations were mounted in PI/DABCO, containing antifade. Between 1000 and 4040 metaphases were analyzed to detect reciprocal, terminal and intersticial translocations for each individual under a Zeiss fluorescence microscope.

Statistical analysis

The genomic translocation frequencies were calculated using the formula for correction of the painted fraction chromosomes for the whole genome (2). Significant differences between the exposed and control group were tested by using the chi square test.

Results

The results of the conventional cytogenetic analysis of total number of cells with chromosomal aberrations and their types in the peripheral blood lymphocytes each individual in the group of occupationally exposed workers and for the control group are illustrated in Table 2. The frequency of chromosomal aberrations in the peripheral lymphocytes of storage workers, is higher than that in the controls, although the difference is not statistically significant ($P > 0.05$). The cytogenetic damage is dominated by a single chromatid and chromosomal fragments. Chromosomal and chromatid type exchanges were found only in the exposed workers. In one of the exposed subjects an acentric ring and 1 cell with multiple aberrations were also found, although these are not included in the table.

FISH for translocations scoring was performed simultaneously with conventional chromosomal aberration analysis. In this way, in addition to unstable structural chromosomal aberrations, stable translocations were also recorded. Fifteen subjects, randomly selected from the exposed workers at the NWR, were investigated by means of FISH chromosome painting assay and the results were compared to those of 6 individuals from the control group. Table 3 presents the frequency of different type chromosomal exchanges (complete and incomplete) for the labeled chromosomes #1 and #4 in exposed radioactive waste storage workers and the controls. Recent experiments using telomeric probes have demonstrated that most of the apparently incomplete-type exchanges are actually complete, but the reciprocal fragments are too small to be detected (3,8). For this reason, the pooled results of incomplete and complete exchanges are presented in Table 3 and are used for calculating translocation frequencies over the whole genome. A 2-fold increase in genomic translocation frequency compared to the controls was recorded. A statistical comparison of the mean values of total genome translocations showed a marginal statistical difference between the 2 groups ($X^2 = 4.061$; $P < 0.05$); ($X^2 = 3.84$; $P = 0.05$). Only one complex exchange (3 breaks in 2 chromosomes) involving #1 was documented in the exposed workers group, which is not included in the table.

Discussion

The mean values of the aberration frequencies detected by solid Giemsa staining and FISH staining in exposed and control workers at the NWR are shown in the Figure. The average frequency of dicentrics in the exposed group was 0.02%, and the average genomic translocation frequency was 1.01%. In our internal

Table 2. Frequency and spectrum of chromosomal aberrations in NWR workers detected by classical Giemsa staining.

Groups	Age	Gender	Smoking habits	Years of employment	Accum. dose/ mSv	Cells no.	Cells with CAs	CAs					Total CAs
								Chromosomal type			Chromatide type		
								fragm.	dic	sym. ex.	Fragm.	exch.	
Exposed workers	56	f	no	14	23.96	500	1.4	1	-	-	0.4	-	1.4
(n = 27)	50	m	20	5	5.94	1000	0.7	0.4	-	-	0.3	-	0.7
	55	m	20	5	8.24	500	1.2	0.8	-	-	0.2	0.2	1.2
	47	f	former 20	22	10.29	500	0.4	0.2	-	-	0.2	-	0.4
	43	f	no	17	12.37	500	1	0.6	-	-	0.4	-	1
	39	f	20	13	12.73	600	2.5	1.8	-	-	0.66	-	2.5
	52	f	no	7	1.6	384	1.04	0.7	-	-	0.26	-	1.04
	24	m	no	2	2.95	500	1.8	1	-	-	0.8	-	1.8
	51	m	former	23	3.41	500	1.8	1	-	-	0.8	-	1.8
	50	m	40	11	7.42	1000	1.5	1	0.1	-	0.4	-	1.5
	52	m	30	2	1.85	1000	1.4	1	-	-	0.3	0.1	1.4
	46	m	20	1	0.5	500	2.6	2	-	-	0.8	-	2.8
	57	f	10	21	20.35	500	2.6	1.6	-	-	0.8	-	2.6
	59	m	7	25	30.44	1000	2.3	1.5	0.1	0.1	0.3	0.1	2.3
	25	m	no	2	0.98	200	1.5	0.5	-	-	1	-	1.5
	24	m	20	1	1.55	500	1	0.6	-	-	0.4	-	1
	58	m	no	35	1.47	500	2.4	1	0.2	0.2	0.6	0.4	2.4
	52	m	30	2	2.48	500	0.2	-	-	-	0.2	-	0.2
	49	m	former 20	20	2.65	500	1.8	1	0.2	-	0.6	-	1.8
	55	m	40	7	6.9	200	1.5	1	-	-	0.5	-	1.5
	27	m	30	2	1.98	200	0.5	-	-	-	0.5	-	0.5
	56	m	former	14	2.1	500	0.8	0.6	-	-	0.2	-	0.8
	52	m	20	30	6.61	400	1.5	0.75	-	-	0.75	-	1.5
	66	m	20	40	1.58	1000	1.5	1	-	-	0.4	0.1	1.5
	58	m	no	26	3.23	200	1	0.5	-	-	0.5	-	1
	55	m	30	1	1.39	350	0.85	0.28	-	-	0.57	-	0.85
	36	m	20	1	1.64	100	2	-	-	-	1	1	2
Total						14,134							
Mean ±S.D. Controls	47.9			12.92	6.54 ± 7.59	523	1.43 ± 0.67	0.81 ± 0.52	0.02 ± 0.05	0.01 ± 0.04	0.51 ± 0.24	0.07 ± 0.2	1.44 ± 0.68
(n = 9)	24	f	no	2	-	500	0.6	0.2	-	-	0.4	-	0.6
	25	m	no	3	-	100	2	-	-	-	2	-	2
	33	f	20	1	-	500	0.8	0.4	-	-	0.4	-	0.8
	26	m	10	1	-	500	1	0.4	-	-	0.6	-	1
	49	f	7	7	-	500	1.4	0.4	-	-	0.6	-	1.4
	29	m	no	1	-	600	0.66	0.16	-	-	0.5	-	0.6
	33	m	20	6	-	500	1.2	0.4	-	-	0.8	-	1.2
	56	f	7	4	-	500	1	0.4	-	-	0.6	-	1
	27	f	10	1	-	500	1.2	0.6	-	-	0.6	-	1.2
Total						4200							
Mean ± S.D.	33.5				2.88	466	1.09 ± 0.43	0.32 ± 0.18			0.72 ± 0.49		1.08 ± 0.44

Legend: fragm., fragment; exch., exchange; sym.ex., symmetric exchange

Table 3. Frequency and spectrum of chromosomal aberrations in NWR workers detected by FISH staining.

Groups	Age	Gender	Smoking	Years of employment	Cumulative dose/mSv	No. cells scored	# 1 translocations (tr)				# 4 translocations (tr)				Total no. tr. for # 1 and # 4	Fg/100 ± S.D.
							Total no. tr.	rcp	tt	it	Total no. tr.	rcp	tt	it		
workers	51	m	former	23	3.41	1166	3	-	3	-	1	1	-	-	4	1.23
(n = 15)	50	m	40	11	7.42	2768	4	1	2	1	4	2	1	1	8	1.04
	52	m	30	2	1.85	2387	2	1	1	-	1	-	1	-	3	0.45
	46	m	20	1	0.5	4040	2	1	1	-	2	1	1	-	4	0.35
	57	f	10	21	20.35	1455	3	1	2	-	3	2	1	-	6	1.489
	32	m	20	1	0.71	2794	1	1	-	-	2	1	1	-	3	0.387
	59	m	7	25	30.44	1017	4	1	3	-	2	1	1	-	6	2.13
	24	m	20	1	1.55	1220	3	1	1	1	-	-	-	-	3	0.88
	58	m	no	35	1.47	1195	6	2	3	1	3	2	1	-	9	2.719
	52	m	30	2	2.48	1000	1	-	1	-	2	2	-	-	3	1.083
	49	m	former 20	20	2.65	1723	-	-	1	-	-	-	-	-	1	0.209
	56	m	former	14	2.1	1020	2	1	1	-	1	-	1	-	3	1.062
	46	m	no	7	7.82	1790	2	1	1	-	2	1	1	-	4	0.807
	33	m	10	1	0.5	1210	2	-	2	-	1	-	1	-	3	0.895
	31	m	10	1	0.5	1605	1	1	-	-	1	1	-	-	2	0.45
total					83.75	26390									62	
mean	46.4			11.0	5.55	1759									4.13	1.012 ± 0.68
controls	24	f	no	2	-	3354	2	1	1	-	2	-	2	-	4	0.431
(n = 6)	33	f	20	1	-	1256	-	-	-	-	1	-	1	-	1	0.287
	26	m	10	1	-	1364	-	-	-	-	2	-	2	-	2	0.529
	49	f	7	7	-	1220	1	-	1	-	1	1	-	-	2	0.592
	33	m	20	6	-	1797	-	-	-	-	1	-	1	-	1	0.2
	27	f	10	1	-	1524	2	1	1	-	1	-	1	-	3	0.71
total					-	10515									13	
mean	32.0			3.0	-	1752									2.16	0.458 ± 0.19

Legend: tr., translocation. Rcp, reciprocal, Tt, terminal translocation. It, intersticial translocation, Fg, genomic translocation frequency

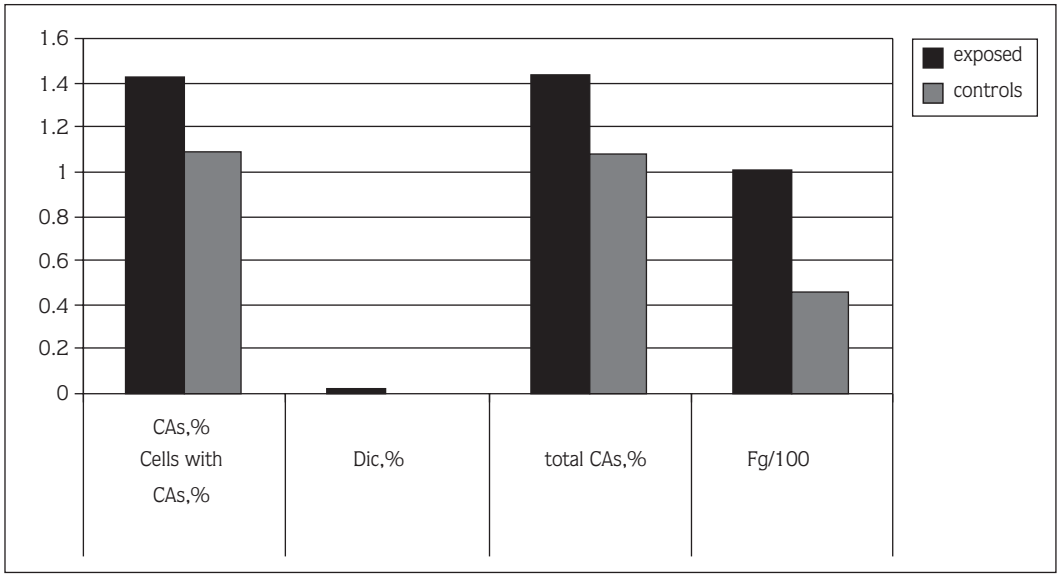

Figure. Characteristics of the study groups.

control group of 9 workers we did not determine any dicentrics in the 10,515 cells examined. When we referred to our laboratory rural control group we found the same frequency of dicentrics (0.02%) in 10,000 cells of the 49 control subjects analyzed (6). Comparison with a historic control (1 dic/1000 cells) did not show any increase either (7).

Comparing the average frequency of translocations over the whole genome in exposed workers to the level in the control group, a 2-fold increase was determined. The mean values were 1.01% translocations for exposed workers and 0.46% translocations for control workers. The difference is significant, although on the margin of statistical significance ($P < 0.05$). The same statistical significance was found when compared to historic control of 5 translocations per 1000 cells (9).

The frequency of dicentric chromosomes is considered a sensitive indicator and dosimeter of exposure to ionizing radiation. They are widely used for biological dosimetry and biomonitoring (3-10). However, they are unstable type aberrations because they are not transmissible forms and they are eliminated with time. The mean half-life of dicentrics is estimated to be about 130 days (8). In the case of chronic occupational exposures to low doses and low dose rates chromosomal aberrations induced in peripheral lymphocytes are accumulated only in the long-life lymphocyte population.

FISH painting of chromosomes allows reciprocal translocations that are stable and persist with time to be distinguished. Consequently, they represent the chromosomal damage that accumulates in occupationally exposed individuals. FISH was applied in our study for the estimation of chronic radiation exposure to low doses and low dose rates. The higher frequency of stable aberrations detected in the occupationally exposed group suggests historic exposure. Some uncertainties are associated with the age-related variables of translocations (3,9,13). It should be noted, however that in our study the mean age in the exposed group was 46.4 with 8 persons over the age of 50. The control group consisted of younger persons (mean age 32.0), mainly new employees.

There have been few studies assessing the genetic effects of human occupational radiation exposure analyzing stable and unstable chromosomal aberrations in peripheral blood lymphocytes (3,8,10-12). Lindholm found a significant association between translocation frequency and cumulative dose among 20 nuclear power plant workers investigated (10). Tucker et al. (12) also reported a significant dose-effect relation among 58 nuclear power plant workers applying FISH (12). In

another study, FISH, applied to a population of 50 persons living in the area radioactively contaminated by the Chernobyl fallout revealed no increase in the mean translocation yield (11).

In conclusion, the results of this study indicate that prolonged occupational exposure to radiation can be detected using FISH chromosome painting and analysis of chromosomal translocations.

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