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A cytogenetic study of nuclear power plant workers using the micronucleus-centromere assay

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Abstract

A cytogenetic study was performed in 215 nuclear power plant workers occupationally exposed to radiation using the micronucleus-centromere assay for peripheral blood lymphocytes. As control population served administrative staff with yearly doses below 1 mSv. The increase of the micronucleus frequency with age, observed in the non-smoking control population, is mainly due to an enhanced number of centromere-positive micronuclei, pointing to an increased chromosome loss. No differences in the number of micronuclei, centromere-positive and centromere-negative micronuclei between smokers and non-smokers are observed. An analysis of the micronucleus data vs. the dose accumulated over the 10 years preceding the venepuncture shows no significant clastogenic or aneuploidogenic effects of the exposure in the studied population which is representative for workers in the nuclear industry at present. According to the linear fits to our data an increase of the micronucleus frequency pro rata 0.5 per 1000 binucleated cells per year, related to the centromere-negative micronuclei, may be expected for workers with the maximal tolerable dose of 20 mSv/year.

Keywords: Occupational clastogen; Cytogenetic monitoring; Human lymphocyte

1. Introduction

Several cytogenetic studies of radiation workers occupationally exposed in the nuclear industry revealed an enhanced baseline level of chromosomal aberrations compared to control populations [1–9]. Although cytogenetic analyses for dicentrics and translocations are the state of the art techniques for biological dosimetry, application of these techniques for biomonitoring or screening of relatively large groups of radiation workers is difficult as chromosome analyses are time-consuming and require highly skilled personnel. The cytokinesis-block micronucleus assay for peripheral blood lymphocytes [10] is a valuable and less laborious alternative for large scale studies [11]. Satisfactory dose relationships were established for different radiation qualities [12–14] and studies of patient groups exposed medically showed the applicability of the micronucleus assay as a biological indicator for radiation damage after in vivo exposures [12,15–19]. Our previous cytogenetic

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study of nuclear power plant workers showed a small statistically not significant increase of micronucleus frequency in the group of workers with the highest radiation burden [20]. On the contrary, in a recent study Vaglenov et al. [9] found a significant correlation between the radiation burden and the micronucleus frequency in nuclear power plant workers.

Application of fluorescence in situ hybridization with a pancentromeric probe on the micronucleus preparations allows to distinguish between the clastogenic and aneuploidogenic actions of radiation [21]. Scoring of micronuclei (MN) with and without centromeres has increased the sensitivity of the technique substantially [22]. According to Streffer et al. [23], the micronucleus-centromere assay is able to detect the effect of chronic exposure of uranium miners a long time after exposure.

Using the micronucleus-centromere assay we performed a large scale cytogenetic study of the radiation damage in nuclear power plant workers occupationally exposed to radiation. The doses received over the past 10 years preceding the venepuncture amounted up to 100 mSv. As control population served administrative staff with a cumulative radiation burden less than 10 mSv over the last 10 years according to their dosimetry records. The effect of donor age, smoking and radiation burden on the number of centromere-positive and centromere-negative MN was studied. This study was performed in the framework of the Programme of Scientific Support to the Protection of Workers in the Area of Health of the Services of the Prime Minister Science Policy Office of the Belgian Government.

2. Materials and methods

2.1. Examined subjects and screening

The studied group comprised 215 nuclear power plant workers (Electrabel Doel, Belgium). Written informed consent was obtained from all donors. A heparinized blood sample was taken at the occasion of the periodical medical examination. The volunteers were asked to fill in a questionnaire to obtain the information necessary for the study: age, smoking or non-smoking, previous exposures to diagnostic X-rays as patient and nuclear medical examinations. All volunteers were males. The regular occupational medical examinations revealed no abnormal findings for all studied subjects and none had received chemotherapeutic drugs. The mean age of the studied population at the time of the blood sampling was 44.0 years. Almost three quarters of the workers considered in the study (166) were non-smokers.

For each individual, the occupational radiation burden received over the past 10-year period preceding the venepuncture (1989–1998) was adopted from the official personal dosimetry records. The film badge doses included the radiation burden due to natural background radiations. About half of the studied population (104), members of the administrative staff, received a very low exposure over the past 10 years (< 10 mSv) and could serve as reference control population regarding the effect of the exposure level on the number of centromere-positive and centromere-negative MN. For the remaining group of 111 individuals the mean dose accumulated over the past 10 years was 30.3 mSv (range 10–104). The estimation of the radiation dose induced by the medical exposures was adopted from Bennett [24] and the ICRP Publication 53 [25]. Statistical analyses of the comparison between groups of donors were carried out using the Wilcoxon test.

2.2. Micronucleus-centromere assay

The same day of blood-sampling whole blood cultures containing 0.3 ml blood in RPMI 1640 medium with L-glutamine and 25 mM HEPES buffer (Gibco Laboratories, Ghent, Belgium) supplemented with 10% fetal calf serum (Gibco) were initiated. A protein purified phytohaemagglutinin (PHA P, 30 μg/ml, Sigma, St. Louis, MO, USA) was added as mitogen. Cytochalasin B (6 μg/ml; stock solution 2 mg/ml in dimethylsulphoxide; Sigma) was added 42 h after culture initiation to block cytokinesis. After an incubation period of 70 h the cells were collected, treated with a hypotonic solution of 0.075 M KCl, and fixed with a mixture of methanol-glacial acetic acid as described elsewhere [26]. After fixation the cells were dropped on clean slides, allowed to dry and stored at -20°C before in situ hybridization with the centromeric DNA probe.

In situ hybridization was performed as described by Nederlof et al. [27] with some modifications. A

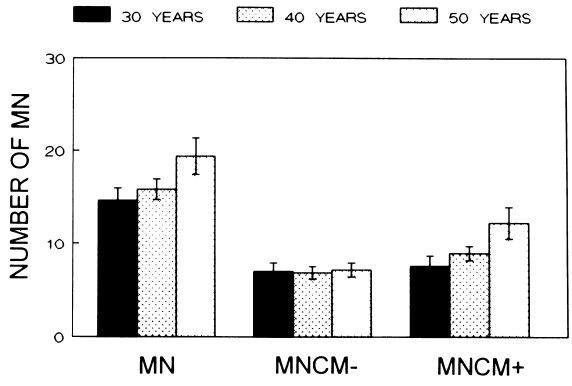

Fig. 1. Comparison of the average number of MN, MNCM+ and MNCM− per 1000 binucleated cells for the age groups 30–39, 40–49 and 50–59 years in the non-smoking control population. The error bars represent the standard deviation of the mean.

centromeric DNA probe ''p82H'' (provided by A.R. Mitchell), which is a cloned alphoid sequence, present at the centromeric region of all human chromosomes, was used [28]. The probe was biotinylated (biotin-16–dUTP; 0.4 mM, Boehringer Mannheim) by standard nick-translation. The slides, stored at −20°C, were pretreated with RNase (4 μg/100 μl, 30 min at 37°C) and pepsin (7.5 μg/ml, 5 min at 37°C; both from Sigma), followed by a postfixation in 4% paraformaldehyde (UCB, Vel, Leuven, Belgium). A probe concentration of 4 ng/μl (in 50% deionized formamide, UCB) was added to the slides. Probe and target DNA were denaturated simultaneously (5 min, 80°C). Following overnight hybridization, immunofluorescence detection of the probe was performed by means of the tyramide signal amplification method (NEN Life Science products). The tyramide signal amplification technology uses streptavidin-horseradish peroxidase to catalyse the deposition of biotin labeled tyramides at the hybridization sites. These labeled tyramides are then visualised by means of streptavidin–fluorescein isothiocyanate (FITC) with significant enhancement of the signal. The slides were counterstained with propidium iodide. As validation of the centromere detection method, FISH treatment of metaphase spreads showed that the p82H probe hybridizes to the centromeres of all chromosomes.

For the scoring of MN and analysis for the presence of centromeres in the MN the preparations were examined under a Leitz fluorescence microscope with 400× magnification. For the scoring an FITC-Texas Red (Chroma) filter set was used. The MN frequencies were scored according to the criteria proposed by Fenech [29]: the diameter of the MN is less than one third of the diameter of the macronuclei, they are non-refractile and not linked to the macronuclei via a nucleoplasmic bridge. MN partly overlapping with the nuclei or with each other were also taken into account. Thousand binucleated cells were scored on two slides per individual. The results are expressed as the total number of MN per 1000 BN cells, the number of MN containing a centromere (MNCM+) per 1000 binucleated cells and the number of MN without a centromere (MNCM−) per 1000 binucleated cells.

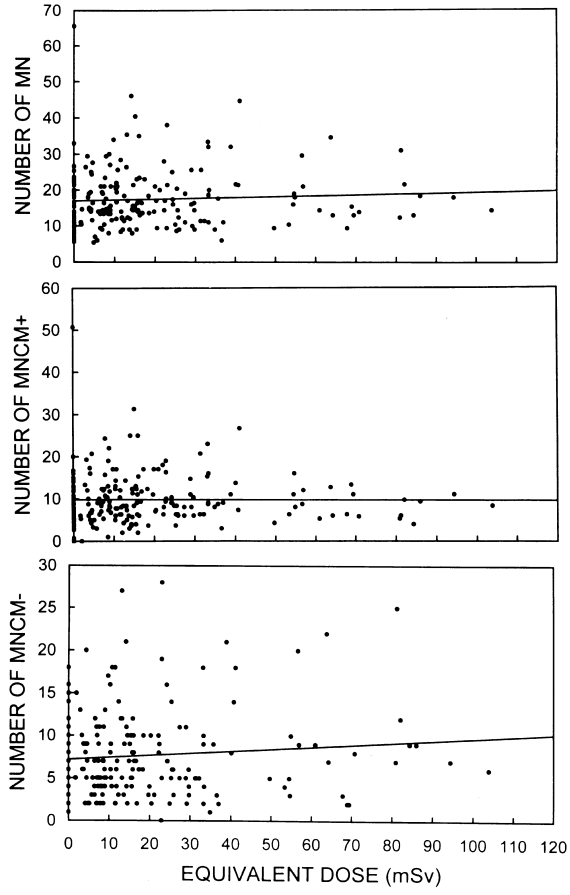

Fig. 2. The individual MN, MNCM+ and MNCM− frequencies vs. the accumulated dose over the 10 years preceding the venepuncture. The full lines represent the linear regression fits of the data.

3. Results

For the study of the age dependence of MN frequency, the data of the non-smoking individuals of the control population were selected ($n = 91$). A comparison of the number of MN, MNCM + and MNCM − per 1000 binucleated cells for the age groups 30–39, 40–49 and 50–59 is presented in Fig. 1. This figure shows that the increase of the total number of MN with age (0.24 per year) is almost completely due to the increase of MNCM + (0.23 per year).

In Fig. 2, the individual MN, MNCM + and MNCM − yields are plotted vs. the accumulated dose over the 10 years preceding the venepuncture, H_{cum}. The full lines represent linear regression fits to the data:

$$MN = 16.98 + 0.023 H_{cum}$$

correlation coefficient 0.03

$$MNCM+ = 9.79 - 0.002 H_{cum}$$

correlation coefficient 0.05

$$MNCM- = 7.19 + 0.025 H_{cum}$$

correlation coefficient 0.10

A slight increase of the total number of MN with H_{cum} is observed due to the increase of the MNCM − yield, while the MNCM + yield remains constant.

For statistical analysis, the studied population was grouped into four classes according to the equivalent

Table 1
Number of MN, MNCM − and MNCM + per 1000 binucleated cells vs. the dose accumulated over the past 10 years for the different exposure classes of radiation workers considered in the study. The data for the exposure classes subdivided into the non-smoking and the smoking population are also given

	Non-smokers A		Smokers B		A + B	
Low exposure level I	MN	16.68 (8.40)	MN	16.69 (6.49)	MN	16.68 (8.16)
$H_{cum} < 10$ mSv	MNCM −	6.99 (4.16)	MNCM −	7.00 (2.80)	MNCM −	6.99 (4.00)
	MNCM +	9.69 (6.88)	MNCM +	9.69 (4.82)	MNCM +	9.69 (6.64)
	H	3.01 (3.52)	H	3.55 (3.54)	H	3.08 (3.52)
	$n = 91$,	Age 45.9	$n = 13$,	Age 43.5	$n = 104$,	Age 45.6
Exposure level II	MN	18.42 (8.33)	MN	16.06 (4.54)	MN	17.62 (8.12), $p = 0.52$
10 mSv $< H_{cum} <$ 20 mSv	MNCM −	8.55 (6.15)	MNCM −	7.56 (2.85)	MNCM −	8.21 (5.25), $p = 0.16$
	MNCM +	9.87 (6.65)	MNCM +	8.50 (4.46)	MNCM +	9.41 (5.98), $p = 0.79$
	H	14.16 (2.48)	H	13.92 (2.85)	H	14.08 (2.59)
	$n = 31$,	Age 41.9	$n = 16$,	Age 43.1	$n = 47$,	Age 42.8
Exposure level III	MN	17.58 (8.79)	MN	18.23 (9.04)	MN	17.77 (9.75), $p = 0.52$
20 mSv $< H_{cum} <$ 50 mSv	MNCM −	7.39 (5.57)	MNCM −	9.00 (7.15)	MNCM −	7.86 (6.04), $p = 0.38$
	MNCM +	10.19 (6.45)	MNCM +	9.23 (4.80)	MNCM +	9.91 (5.97) $p = 0.85$
	H	29.26 (5.80)	H	29.81 (8.49)	H	29.42 (6.61)
	$n = 31$,	Age 42.4	$n = 16$,	Age 44.2	$n = 44$,	Age 42.9
Exposure level IV	MN	17.23 (9.05)	MN	17.21 (6.89)	MN	17.22 (8.17), $p = 0.79$
50 mSv $< H_{cum}$	MNCM −	8.23 (6.48)	MNCM −	10.29 (6.65)	MNCM −	8.95 (6.44), $p = 0.20$
	MNCM +	9.00 (4.39)	MNCM +	6.92 (3.44)	MNCM +	8.27 (4.11), $p = 0.20$
	H	71.25 (16.14)	H	67.82 (12.07)	H	70.51 (14.65)
	$n = 13$,	Age 43.5	$n = 7$,	Age 40.7	$n = 20$,	Age 42.5
I + II + III + IV	MN	17.22 (8.86)	MN	16.97 (6.99), $p = 0.84$	MN	17.16 (8.46)
	MNCM −	7.46 (5.04)	MNCM −	8.18 (4.91), $p = 0.36$	MNCM −	7.62 (5.01)
	MNCM +	9.76 (6.55)	MNCM +	8.79 (4.48), $p = 0.24$	MNCM +	9.54 (6.15)
	H	15.39 (20.15)	H	23.08 (21.85)	H	17.14 (20.75)
	$n = 166$,	Age 44.3	$n = 49$,	Age 43.2	$n = 215$,	Age 44.0

dose accumulated over the past 10 years. The low exposure class (I), control population, refers to an accumulated dose less of than 10 mSv. The exposure classes II and III refer to accumulated doses with ranges 10–20 and 20–50 mSv, respectively. The exposure class IV comprises the radiation workers with the highest radiation burden: accumulated doses exceeding 50 mSv. In Table 1, the number of MN, MNCM + and MNCM − per 1000 binucleated cells, the dose over 10 years and the age of the donors, averaged over the considered class are given together with the number of subjects. The values of these quantities obtained by subdivision of the exposure classes in smokers and non-smokers are also included in the table. The values within brackets represent the standard deviations. The *p*-values resulting from the Wilcoxon test are also given. No significant differences in the number of MN, MNCM + and MNCM − per 1000 binucleated cells between the control population and the other exposure classes at the 95% confidence level are observed. A comparison of the MN data between smokers and non-smokers independent of the radiation burden (I + II + III + IV in Table 1) leads to the same conclusion regarding the effect of smoking.

An analysis of the data was performed in which the accumulated doses were corrected for the lymphocyte half life of 3 years [30] as in our previous study [20]. Sorting the studied population in four classes according to the corrected accumulated doses showed also no effect of the radiation burden on the micronucleus frequency at the 95% confidence level.

4. Discussion

Detection of centromeres in the MN with a pan-centromeric probe and in situ hybridization allows to distinguish between the clastogenic and aneuploidogenic actions of radiation The results of present work show that the systematic increase in the MN frequency with donor age is almost completely due to an increase in MNCM +, reflecting an increased chromosome loss with age. The slope of 0.24 MN per year, obtained from the analysis of the non-smoking control population in the present study based on FISH stained slides, is close to the value of 0.32 MN per year, obtained in previous work [20] based on Giemsa stained slides. Our result is also in agreement with the value of 0.19 MN per year reported by Ban et al. [31] from a large scale study of about 1000 atomic bomb survivors. Fenech [32] reported in his study of the same size a slope value of 0.314 MN per year for the male population. In our study one non-smoking individual, 50 years old, belonging to the control population, has a very high MN yield (67) related to the number of centromere-positive MN (53). Apart from an exposure to an aneuploidogenic agent this high yield may be the result of a clone of aberrant cells, resulting from the age-dependent accumulation of genetic errors.

No differences in the MN, MNCM + and MNCM − frequencies between smokers and non-smokers were observed in the present study. Concerning the effect of tobacco smoking on the micronucleus frequency the data in the literature are contradictory. Our negative result is supported by the large-scale studies of Ban et al. [31] and Migliore et al. [33]. On the other hand, according to other authors [34–37] the micronucleus frequency is elevated in a smoking population.

To investigate the effect of the radiation burden on the MN, MNCM + and MNCM − frequencies, the population of nuclear power plant workers was sorted in four classes according to the dose accumulated over the 10 years preceding the venepuncture (period 1989–1998). The upper dose limit for the lower level exposure class, serving as control population, 10 mSv over 10 years, corresponds to a mean radiation burden of 1 mSv per year, which is the upper limit recommended for public exposure by the International Commission for Radiation Protection (ICRP) [38]. A comparison of the data between the control population and the three classes of exposed radiation workers showed no significant effect of the accumulated dose on the MN, MNCM + and MNCM − yields, not even for the highest exposure class with a mean yearly dose of 7 mSv (Table 1). In view of the dose limit of 20 mSv per year for radiation workers according to the ICRP [38] the latter group is representative for the working population with the highest dose in the nuclear industry nowadays. An analysis with correction of the MN data for the age effect as in our previous study [20] was not performed as the radiation classes are nearly age-matched.

A linear regression fit to the individual MN data shows an increase of the centromere-negative micronucleus yield with accumulated dose, 0.025 MN/mSv, although with a low correlation coefficient value (0.10). This enhanced centromere-negative micronucleus yield is responsible for the increase of the total number of MN with dose, pointing to the clastogenic action of ionizing radiation. According to Streffer et al. [23] uranium miners with high exposures after the inhalation of radon show only slightly higher micronucleus yields than healthy subjects but a significant difference in centromere-negative MN. The slope of the dose dependence of the total number of MN obtained in the present work, 0.023 MN/mSv is in agreement with the value 0.03 MN/mSv reported by Vaglenov et al. [9] in his study of 142 nuclear power plant workers with accumulated doses up to 700 mSv. Furthermore, the linear regression fit of the micronucleus frequency vs. accumulated dose over 10 years, obtained in the present work based on FISH stained slides ($MN = 16.98 + 0.023\ H_{cum}$), is close to the relationship reported in our previous paper ($MN = 15.74 + 0.0175\ H_{cum}$) based on Giemsa stained slides [20].

In conclusion, for the studied population with radiation burden representative for workers in the nuclear industry at present and below the dose limit of 20 mSv per year of the ICRP, no significant clastogenic or aneuploidogenicc effects of the exposure could be observed. According to the linear regression fits to our data, an increase of the MN yield pro rate 0.5 MN per year, related to centromere-negative MN, may be expected for the workers with the maximal tolerable dose.

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