Assessment of chromosomal aberrations in workers chronically exposed to ionising radiation

Birutė Gricienė[1, 2*],

Gražina Slapšytė[1, 3]

[1] Vilnius University, Department of Botany and Genetics, M. K. Čiurlionio 21/27, LT-03101 Vilnius, Lithuania

[2] Radiation Protection Centre, Kalvarijų 153, LT-08221, Vilnius, Lithuania

[3] Institute of Oncology, Vilnius University, Santariškių 1, LT-08660, Vilnius, Lithuania

Chromosome aberration analysis was performed in blood lymphocytes of 29 Ignalina Nuclear Power Plant workers and 41 medical workers employed in diagnostic radiology. Their radiation exposure doses did not exceed the annual dose limit (20 mSv of the effective dose). The control groups comprised age- and sex- matched donors, 26 females and 64 males. Chromosome aberration frequencies in the Ignalina Nuclear Power Plant workers did not differ from those in controls (1.62 ± 0.25 *versus* 1.65 ± 0.15 CA/100 cells, P > 0.05). However, a significant increase in the total chromosome aberration (2.57 ± 0.24 *versus* 1.74 ± 0.22 CA/100 cells, P < 0.05) and total chromosome-type aberration frequencies (1.40 ± 0.19 *versus* 0.82 ± 0.18 per 100 cells, P < 0.05) was found in the medical personnel.

Key words: occupational exposure, ionising radiation, chromosome aberrations, human lymphocytes

INTRODUCTION

More than 23000 ionising radiation sources are used in about 1200 medical, industrial and research institutions of Lithuania [1]. The Ignalina Nuclear Power Plant (INPP) workers constitute the largest group of occupationally exposed persons in the country. About 4000 INPP workers are monitored for the radiation exposure every year since 1984. The majority of monitored workers receive annual doses less than 1 mSv [2, 3]. Though exposure doses are generally low and don't exceed the annual dose limit (20 mSv of the effective dose), somewhat higher doses are obtained during outages that contribute up to 80% of the annual occupational collective dose [2]. The highest external exposure doses are received by workers employed in the reactor, centralized repair, metal and technical control departments (2–4 times higher than the average dose of all workers). The highest annual doses in the range of 15–20 mSv were measured for 28 radiation workers in 2006 [3].

Another large group of persons occupationally exposed to low doses of radiation comprises medical personnel. Up to 50% of about 2600 medical workers annually monitored for radiation exposure are employed in diagnostic radiology [4, 5]. During the last ten years, the average annual effective dose of these workers decreased from 1.95 mSv in 1995 to 1.03 mSv in 2004 and now is very close to the natural background for the large majority of employees.

During the last three decades, numerous studies have been performed to examine the frequency of chromosome aberrations in radiation workers employed in medicine [6–14] and nuclear industry [15–27]. Most studies reported the presence of elevated frequencies of chromosome damage in the lymphocytes of the exposed workers compared to those in the general population. Moreover, recent studies have confirmed the usefulness of biomonitoring chromosome damage in groups exposed to genotoxic agents by finding an increased risk of cancer in subjects with high levels of chromosome aberrations and thus proving chromosome aberration assay as a reliable indicator of cancer risk [28, 29]. Despite the large body of literature and taking into account that sometimes results are quite inconsistent, the monitoring of chromosome damage in radiation-exposed workers remains important to estimate the genotoxic risk associated with chronic exposure to low doses.

The aim of the present study was to assess chromosome aberration frequencies in diagnostic radiology and nuclear industry workers chronically exposed to low doses of ionising radiation.

MATERIALS AND METHODS

Study subjects

The study comprised 29 Ignalina NPP male workers (mean age 45.6 ± 2.7 years) employed in the reactor, centralized repair, metal and technical control departments. Their radiation exposure resulted from external radiation. The registered total cumulative doses for 29 INPP workers were in the range 1–632 mSv (mean 197.7 ± 32.4 mSv) during the employment periods of 1–21 years.

Physical dosimetry of radiation exposure is carried out at the Personal Dosimetry Laboratory of the INPP since 1984 when the INPP came into operation. The external exposure doses are measured with Rados thermoluminescence dosimeters (TLD) consisting of 3 Li_2B4O_7 pellets covered with a 1 mm Al filter in

a standard holder. The monitoring periods vary from one day to one month depending on the type of the work performed. The natural background radiation is subtracted from the occupational dose records. The used operational dose quantity is personal dose equivalent Hp(10) – the dose equivalent at a body depth of 10 mm at the point of application of the personal dosimeter. In routine personal monitoring when dose values are far below the dose limit, the measured Hp(10) value is regarded as the value of the effective dose.

Another study group comprised 41 nurses and physicians employed in diagnostic radiology (all females, mean age 49.1 ± 1.5 years) with employment periods from 1 to 40 years. Their radiation exposure resulted from external X-rays. Physical dosimetry of radiation exposure of medical personnel has been carried out at the Radiation Protection Centre since 1991 [30]. Thus, the official personal dose records for this study group were available, and cumulative doses were calculated only since that year. The mean cumulative dose for this period was 8.6 ± 0.9 mSv (range 1.1–17.4 mSv). The occupational exposure doses of medical personnel are measured with Rados TLD dosimeters. Dosimeters are worn on the protective aprons with a monitoring period of three months. The used operational dose quantity is Hp(10). The natural background radiation wasn't subtracted from the occupational dose records. The expanded uncertainty of measurements with the coverage factor k = 2, ensuring a 95% level of confidence, is below 25%. Summarised information on age, period of potential exposure and cumulative doses in both study groups is listed in Table 1.

The control groups consisted of 26 female and 64 male healthy donors who had not been exposed to either ionising radiation or chemical mutagens at their workplaces. None of the radiation workers and the control subjects had undergone radiotherapy or chemotherapy, or had any serious viral infection at least three months prior to blood sampling. A written informed consent to be included into the study and a detailed questionnaire on personal data and life style (health status, occupational and medical history, involvement in radio-diagnostic procedures and smoking habits) was obtained from all subjects. The Lithuanian Bioethics Committee approved the study.

Cytogenetic methods

Peripheral blood samples were collected by venipuncture into heparinised BD Vacuteiner blood collection tubes. For lymphocyte cultivation the standard technique was used. In brief, phytohaemagglutinin (7.8 µg/ml) stimulated cultures were incubated at 37 °C for 72 h in RPMI 1640 medium supplemented with 12% heat-inactivated newborn calf serum, 40 µg/ml gentamycin, 0.25 µg/ml colchicine. All reagents were purchased from Sigma (St. Louis, MO, USA). Thus, colchicine was present during the entire culture period. In such cultures, even cultivated for 72 h, the majority (95–97%) of metaphases are in the first-mitotic division. Besides, they represent lymphocytes with a fast and slow proliferation rate, and thus a non-random selection of cell populations may be avoided. This methodology was described by Chen and Zhang [31] and successfully used in cytogenetic studies of human populations [6, 7, 18, 32]. The harvested lymphocytes were treated with hypotonic KCl (0.075 M) and then fixed in methanol-glacial acetic acid (3 : 1). Flame-dried slides were prepared and stained using the conventional Giemsa staining procedure. All slides were coded and scored blind. Generally, 500 cells per subject were analysed. The chromosome aberrations were recorded according to An International System for Human Cytogenetic Nomenclature [33] and for statistical analysis were grouped as chromosome-type (acentric fragments, dicentrics, rings) and chromatid-type (chromatid breaks, quadri-radials and tri-radials) aberrations. Rogue cells (i. e. multi-aberrant cells with a large number of chromosome-type rearrangements including dicentric, polycentric and ring chromosomes, acentric fragments and double minutes) and gaps were registered but not included into the analysis.

Statistical analysis

Statistical tests were performed using the SPSS statistical software package (for Windows, Version 13.0, Chicago, IL, USA). One-way analysis of variance (ANOVA), post-hoc LSD test, Poisson regression analyses, Pearson correlation test were applied for detection of differences between the groups and correlations among the variables. P > 0.05 was considered as the level of significance.

Table 1. **Summarised information on age, period of potential exposure and cumulative doses of occupationally exposed individuals and controls**

Group	Medical personnel (females)	NPP workers (males)	Controls (females)	Controls (males)
Number of donors	41	29	26	64
Age, mean ± SEM	46.7 ± 1.5	45.6 ± 1.8	44.9 ± 1.7	36.2 ± 1.5
(range)	(27–63)	(24–58)	(22–65)	(18–60)
Years of employment, mean ± SEM	17.8 ± 1.9	13.2 ± 1.0	–	–
(range)	(1–40)	(1–21)		
Total cumulative dose, mSv, mean ± SEM	8.6 ± 0.9*	197.7 ± 32.4	–	–
(range)	(1.1–17.4)	(1–632)		
Cumulative dose over the last 3- year period,	3.02 ± 0.15	33.3 ± 4.1	–	–
mSv, mean ± SEM (range)	(1.1–6.1)	(0.1–58.0)		
Dose over the last year prior blood sampling,	0.97 ± 0.04	9.7 ± 1.3	–	–
mSv, mean ± SEM (range)	(0.6–1.5)	(0.1–18.0)		

* For the medical workers official personal dose records were available and cumulative doses were calculated for the last 11-year period preceding blood samples collection.

RESULTS

Mean aberration frequencies between exposed and control individuals from the pooled data are compared in Table 2. Chromosome aberration analyses did not reveal significant differences between the nuclear power plant workers and the adequate controls, i. e. the male control group (1.62 ± 0.25 versus 1.65 ± 0.15 CA/100 cells, $P > 0.05$). The differences were significant neither for dicentrics, acentrics or chromatid-type aberrations (breaks and exchanges). Differences were not found when nuclear power plant workers were divided into two groups according to the level of the cumulative dose received over the last 3-year period (Fig. 1). One group comprised 11 workers with cumulative doses less than 20 mSv (mean 5.4 ± 1.8 mSv, range 1–19 mSv), and the other group consisted of 18 workers with cumulative doses higher than 20 mSv (mean 47.6 ± 1.9 mSv, range 35–58 mSv). Aberration frequencies in these two groups did not differ and were 1.59 ± 0.32 and 1.79 ± 0.40 per 100 cells, respectively. However, it should be noted that from 7 workers for whom dicentrics were determined, 5 workers had cumulative doses close to 50 mSv.

A significant increase in the total chromosome aberration (2.57 ± 0.24 versus 1.74 ± 0.22 CA/100 cells, $P < 0.05$) and total chromosome-type aberration (1.40 ± 0.19 versus 0.82 ± 0.18 CA/100 cells, $P < 0.05$) frequencies was observed for the medical personnel when compared with the adequate control group, i. e. the female control group. The increase of chromosome aberration frequency was mainly due to a statistically significant increase of excess acentrics (1.33 ± 0.19 versus 0.74 ± 0.17 acentrics/100 cells, $P < 0.05$). No differences were found in aberration frequencies between male and female control groups.

No correlation between aberration frequency and estimated cumulative doses and the duration of employment was found when all study subjects were included in the test (Pearson's correlation test). Though no correlation between chromosome aberration frequency and cumulative dose was determined, a significant correlation at the level of 0.01 was found between the duration of employment and the total chromosome aberration

Table 2. **Group mean yields of various categories of chromosomal damage (chromosome aberrations /100 cells ± S.E.M) in control and occupationally exposed individuals**

| Group	Medical personnel (females)	NPP workers (males)	Controls (females)	Controls (males)
Dicentrics	0.07 ± 0.04	0.09 ± 0.04	0.08 ± 0.05	0.08 ± 0.02
Excess acentrics	1.33 ± 0.19*	0.38 ± 0.07	0.74 ± 0.17	0.59 ± 0.08
Total chromosome-type aberrations	1.40 ± 0.19*	0.47 ± 0.09	0.82 ± 0.18	0.67 ± 0.09
Chromatid breaks	1.16 ± 0.16	1.04 ± 0.15	0.89 ± 0.10	0.92 ± 0.09
Chromatid exchanges	0.02 ± 0.02	0.11 ± 0.04	0.04 ± 0.04	0.06 ± 0.04
Total chromatid-type aberrations	1.18 ± 0.17	1.17 ± 0.18	0.93 ± 0.15	0.98 ± 0.11
Total chromosome aberrations	2.57 ± 0.24*	1.62 ± 0.25	1.74 ± 0.22	1.65 ± 0.15

*$P < 0.05$ as compared with controls.
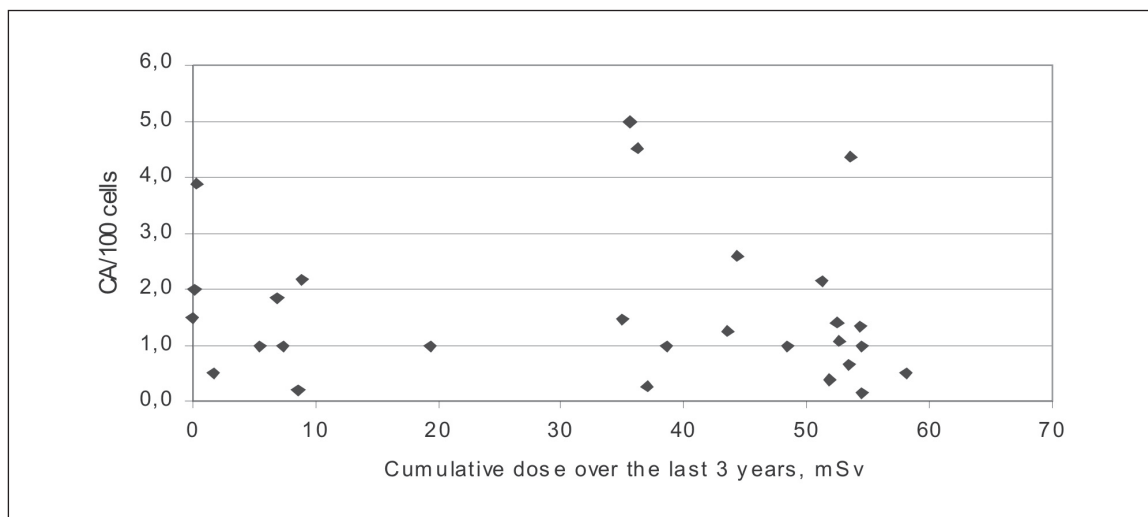
Figure. Cumulative doses (mSv) over the last 3 years prior blood sampling and chromosome aberration frequencies (CA/100 cells) in the Ignalina NNP workers

frequency (r = 0.452), chromosome-type (r = 0.492) and chromatid-type aberrations (r = 0.463) when the group of diagnostic radiology workers with the adequate female controls were considered. Also, a significant correlation between the duration of employment and the cumulative dose over the last 3 years was determined (r = 0.683).

DISCUSSION

The aim of our study was to investigate the chromosomal damage in the lymphocytes of nuclear power plant workers and medical personnel exposed to low levels of ionising radiation. Occupational exposure monitoring of the study groups was performed according to the Lithuanian regulatory requirements. The legal basis for radiation protection of radiation workers was established according to the IAEA, ICRP, EC and other international requirements and recommendations [34–37]. The INPP personal dosimetry service is authorized by the Radiation Protection Centre (RPC). The RPC personal dosimetry laboratory has been accredited according to the requirements of ISO / IEC 17025 Standard in 2005.

Numerous studies indicate that ionising radiation at doses far below annual dose limits can increase chromosome aberration frequencies in lymphocytes of occupationally exposed workers [6–27]. Most authors indicate an increase of chromosome-type aberrations, i.e. dicentric and ring chromosomes and/or excess acentric fragments. In the present study, we found a significant increase of acentric fragments in the lymphocytes of medical personnel with mean cumulative doses of 8.6 ± 0.09 mSv over the last 11-year period prior to blood sampling. Our data are in agreement with findings of other authors who reported a more pronounced increase in acentric fragments as compared to dicentrics in workers exposed to very low doses (between 0.25–42.7 mSv) [38, 39]. Barquinero et al. [39] consider acentric fragments to be the best indicators of irradiation for doses below 50 mSv.

As compared with controls, no significant increase in the frequency of dicentric chromosomes, known as typical ionising radiation exposure indicators, was determined in either of our radiation-exposed groups. The background level for the total dicentrics determined in our controls (0.08 ± 0.03 per 100 cells) is in accordance with the findings of other authors who reported the mean frequency of dicentrics to be between 0.035 and 0.15 per 100 cells [8, 9, 11]. The spontaneous yield of excess acentrics is usually higher than of dicentrics. The range of aberration frequencies for acentric fragments varies from 0.3 to 0.7 per 100 cells. Though acentrics are more variable and cannot serve as a dosimeter for radiation exposure alone, the presence of high frequency acentrics along with dicentrics supports radiation exposure to a low dose rate at a low LET exposure [12, 39]. Chromatid-type aberrations are less widely reported in literature, probably because they are not induced by ionising radiation in peripheral (Go) lymphocytes. The reported background values for chromatid-type aberrations are usually in the range of 0.81 per 100 cells and are comparable with those determined in our study (0.93 and 0.98 in female and male control groups, respectively). Frequencies of chromatid-type aberrations in both of our study groups were only slightly higher than in controls, with no significant differences. Generally, the baseline frequency of chromosome aberration from a normal population has a wide range of variation. Some authors have reported the effect of age and smoking habit on the aberration yields, while others found no such effect [15, 40]. In our study group, no correlation between chromosome aberration frequency and age was found.

It is interesting to note that significantly increased aberration frequencies were determined only in medical personnel (females) with radiation exposure doses considerably lower (up to 10 times) than those determined for nuclear power plant workers (males). Aberration frequencies in nuclear power plant workers did not differ from the controls. Numerous studies show no differences in background chromosome aberration frequencies in males and females [41]. We found no differences in aberration frequencies in our female and male controls groups, either. However, recent studies suggest that there are real differences in radiosensitivity among individuals [42]. Roberts et al. [42] have shown that females have a greater variability in their radioresponse, and that this variability is related with progesterone which has a profound effect upon radiosensitivity as measured by cytogenetic end-points (chromosome aberrations). The study of Kanda and Hayata [43] provides a direct evidence that radiosensitivity in humans may vary in relation to hormonal conditions. They demonstrated the effect of estradiol on the yield of radiation-induced chromosome aberrations in cultured lymphocytes *in vitro* and suggested that estradiol reduces the repair of radiation-induced damage in lymphocytes. Besides, chromosome radiosensitivity was demonstrated to increase during pregnancy and the variation in the radiation sensitivity of the mothers to parallel that of the pregnancy hormones [44].

The results of numerous studies on the effect of cumulative dose and/or exposure duration on chromosome aberration yield are quite inconsistent. In the present study, no correlation between radiation doses recorded as a cumulative dose, a 3-year dose prior to blood sampling, and a dose over the last year prior to blood sampling and chromosome aberration frequency was found. This observation is in agreement with other cytogenetic studies among radiation workers [11, 15, 16, 22, 23]. The absence of a correlation between aberration frequency and cumulative radiation dose may be attributed to several factors. According to most authors, the main reason is the limited life span of lymphocytes and the slow disappearance of cells with aberrations from the circulation [11, 45]. Variable half-lives in the range of 130–1600 days were reported in circulating lymphocytes [46–48]. Balakrishnan and Rao [12] found a good agreement between the biological dose and the corrected physical dose based on a half-life of 6.9 years for lymphocytes. Similar results were obtained by Braselmann et al. [27]. The dose–response relationship may also be influenced by interindividual differences in the proliferation rate of PHA-responsive lymphocytes [49], the variability of individual radiosensitivity and adaptive response to a very low level of ionising radiation [50].

In conclusion, the results of our investigation indicate that chromosome aberration frequencies in the Ignalina Nuclear Power Plant workers did not differ from those in controls. However, we determined increased frequencies of chromosome aberrations in lymphocytes of medical personnel occupationally exposed to a low level of radiation. Under exposure conditions

where medical personnel received mean cumulative doses of 8.6 ± 0.09 mSv over the last 11-year period prior to blood sampling, the observed damage was almost exclusively due to chromosome-type aberrations (acentric fragments).

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Birutė Gricienė, Gražina Slapšytė

CHROMOSOMŲ ABERACIJŲ NUOLAT JONIZUOJANČIĄJA SPINDULIUOTE VEIKIAMŲ DARBUOTOJŲ LIMFOCITUOSE TYRIMAI

S a n t r a u k a

Lietuvoje yra per 1200 įstaigų, turinčių 23 tūkstančius jonizuojančiosios spinduliuotės (JS) šaltinių. Didžiausias darbuotojų, dirbančių su JS šaltiniais ir gaunančių profesinės apšvitos dozes, skaičius yra Ignalinos atominėje elektrinėje bei asmens sveikatos priežiūros įstaigose. Šio darbo tikslas – ištirti chromosomų aberacijų dažnius diagnostinės radiologijos ir atominės energetikos darbuotojų limfocituose. Tirtų darbuotojų apšvitos dozės neviršijo leistinos metinės apšvitos dozių ribos (20 mSv). Medicinos darbuotojų grupėje nustatytas statistiškai patikimas chromosomų aberacijų kiekio padidėjimas palyginus su kontrole (2,57 ± 0,24 vs. 1,74 ± 0,22 CA/100 ląst., P < 0,05). Ignalinos atominės elektrinės darbuotojų, gaunančių išorinę jonizuojančiosios spinduliuotės apšvitą, chromosomų aberacijų kiekis nuo kontrolės nesiskyrė (1,62 ± 0,25 vs. 1,65 ± 0,15 CA/100 ląst., P > 0,05).