Arh hig rada toksikol 1998;49:1–8

ORIGINAL SCIENTIFIC PAPER

CHROMOSOMAL ABERRATIONS IN MEDICAL STAFF OCCUPATIONALLY EXPOSED TO X-RAYS: A FOLLOW-UP STUDY

VILENA KAŠUBA[1], RUŽICA ROZGAJ[1] AND KARMELA ŠENTIJA[2]

Institute for Medical Research and Occupational Health, Zagreb[1], Clinical Hospital Merkur, Zagreb[2], Croatia

Received 24 October 1997

The decrease of unstable chromosomal damages (dicentrics, rings and acentric fragments) was observed in circulating peripheral blood lymphocytes after exposure to ionizing radiation. The study comprised 100 subjects, all medical staff occupationally exposed to X-rays, who were re-examined for unstable chromosomal aberrations after 3 months (12 subjects), 6 months (31 subjects), 9 months (13 subjects), 12 months (21 subjects), over 24 months (14 subjects), and over 36 months (9 subjects) controls were 60 subjects who were not exposed to ionizing radiation or chemical mutagens at their workplaces. The results did not show time-dependent recovery from chromosomal aberrations in all cases.

Key words:
chromosomal damages, ionizing irradiation, occupational exposure

The harmful effects of ionizing radiation were first recognized in occupationally exposed workers. Chromosomal aberrations are considered to be a sensitive biological indicator of the effects of ionizing radiation. They are a manifestation of DNA damage. They can also indicate alterations in the cell homeostasis which cause genome instability (1). Chromosomal abnormality (i.e. dicentric chromosomes) is formed soon after irradiation and before the DNA replication (2). It is well known that X-rays produce double strand breaks in DNA molecule (1). Strands rejoin rapidly while most breaks are repaired accurately (3–5). A small number of breaks remains unrepaired and becomes visible in metaphase chromosomes. *In vivo* analyses of chromosomal aberrations in peripheral blood lymphocytes in persons occupationally exposed to ionizing radiation have shown various aberration frequencies. It has been well documented that some lymphocytes containing aberrations continue to exist in the peripheral circulation for many years after exposure (6, 7). In this study, we followed the recovery of DNA damage manifested as chromosome breakage in human peripheral blood lymphocytes in medical staff occupationally exposed to X-rays who were re-examined at various intervals.

SUBJECTS, MATERIALS, AND METHODS

We examined 100 subjects for structural chromosomal aberrations (one or more dicentric and/or ring chromosome), all of them medical staff occupationally exposed to X-ray radiation. The examinees were divided into six groups classified according to the period of re-examination, ranging from 3 to 36 months. A questionnaire designed to obtain relevant details with health history, current health status, lifestyle, and exposure to ionizing radiation was completed for each subject. None of the subjects had previously been exposed to chemical mutagens. The control group of 60 subjects was comparable in age and smoking habit. After the first examination of the exposed subjects, analyses for chromosomal aberrations were repeated at intervals of 3 months (Group 1, N=12), 6 months (Group 2, N=31), 9 months (Group 3, N=13), 12 months (Group 4, N=21), < 24 months (Group 5, N=14), and < 36 months (Group 6, N=9).

The presence of chromosomal aberrations was examined in the lymphocytes from whole peripheral blood. We used a standard 48-hour culture technique; the cells were cultivated in F-10 medium (Gibco, GB), supplemented with PHA (PHA-Murex, GB), 20% fetal calf serum (Biological Industries, Israel) and antibiotics (penicillin–10000 IU/ml and streptomycin–10000 μg/ml). The slides were stained by Giemsa solution. The analysis of at least one hundred first metaphases per subject (100–200 cells) was limited to structural aberrations such as chromatid and chromosome breaks, dicentrics, rings, chromatid exchanges (tetraradial) and acentric fragments.

Intragroup differences in the number of chromosomal aberrations (acentric fragments, dicentric and ring chromosomes) between the first and second sampling periods were evaluated by χ^2-test at the level of significance $P<0.05$.

RESULTS

The results obtained at first and second examination (ranging in the period from 3 to 36 months) are given in Figure 1 and Table 1. Table 2 summarizes all results of the exposed group. Table 3 shows the results of the control group.

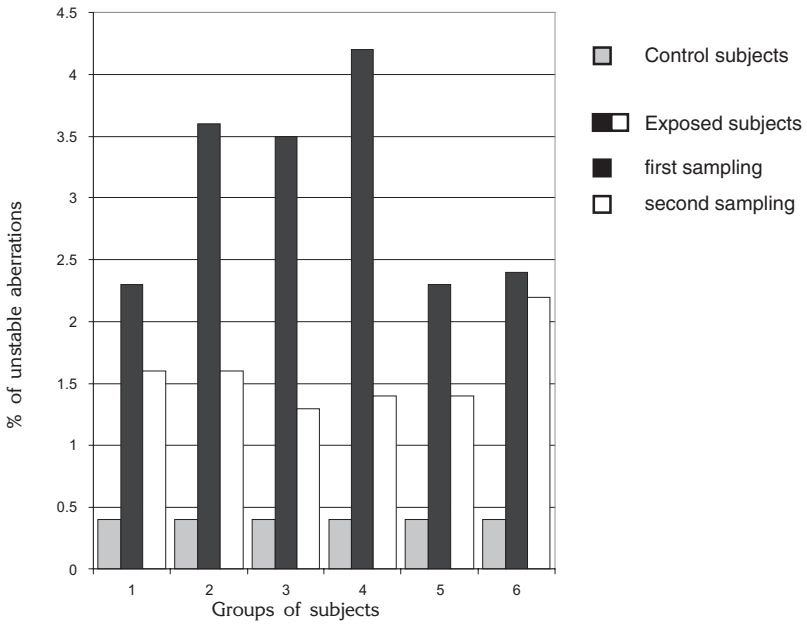

Figure 1 *Percentage of unstable chromosomal aberrations in control subjects and in exposed subjects at first and second sampling times: after 3 months (Group 1), 6 months (Group 2), 9 months (Group 3), 12 months (Group 4), <24 months (Group 5) and <36 months (Group 6).*

In comparison to the number of chromosomal aberrations observed on the first examination, the findings for 25% of examinees remained unchanged after the re-examination three months later, whereas 8.3% of subjects maintained an increase, and 66.7% a decrease in chromosomal aberrations (Table 1 and 2).

Table 1 *Chromosomal aberration analysis in six groups of subjects occupationally exposed to X-rays*

Group	N		Chromatid break %	Chromosome break %	Acentric fragment %	Dicentric %	Ring %	Chromatid interchanges (tetraradial) %
1	12	First sampling	1.35 (0.5 – 3.8)	0.83 (0.5 – 3.6)	2.23 (0.5 – 11.3)	0.93 (0.5 – 3.8)	0.04 (0.5)	0.04 (0.5)
		Re-examination 3 months later	1.26 (0.5 – 2.5)	0.57 (0.5 – 2.0)	1.19 (0.5 – 2.5)	0.33 (0.5 – 1.0)	0.08 (1.0)	0.04 (0.5)
2	31	First sampling	0.93 (0.5 – 3.0)	1.5 (0.5 – 4.8)	2.39 (0.5 – 13.0)	1.05 (0.5 – 5.5)	0.20 (0.5 – 1.0)	0.03 (1.0)
		Re-examination 6 months later	0.83 (0.5 – 4.0)	1.33 (0.5 – 3.8)	1.51 (0.5 – 4.2)	0.43 (0.5 – 2.5)	1.19 (0.5 – 1.8)	0.02 (0.1 – 0.5)
3	13	First sampling	1.05 (0.5 – 4.0)	1.03 (0.5 – 2.5)	3.37 (1.0 – 16.0)	1.27 (0.5 – 3.3)	– –	1.15 (0.5 – 1.4)
		Re-examination 9 months later	0.64 (0.5 – 1.5)	2.35 (0.5 – 9.0)	1.09 (0.5 – 2.0)	0.19 (1.0 – 1.5)	– –	0.08 (0.5)
4	21	First sampling	0.86 (0.5 – 2.0)	2.74 (0.5 – 9.0)	2.69 (0.5 – 11.9)	1.49 (0.5 – 5.5)	0.09 (0.5 – 1.3)	0.08 (0.6 – 1.0)
		Re-examination 12 months later	1.32 (0.5 – 3.0)	1.64 (0.5 – 5.0)	0.99 (0.5 – 4.5)	0.3 (0.5 – 2.0)	0.02 (0.5)	0.02 (0.5)
5	14	First sampling	1.29 (0.7 – 3.5)	2.04 (1.6 – 5.0)	1.69 (1.0 – 7.2)	0.94 (0.5 – 1.8)	0.04 (0.5)	0.06 (0.8)
		Re-examination < 24 months later	1.39 (0.7 – 6.3)	1.45 (0.5 – 4.5)	1.03 (0.6 – 2.0)	0.27 (0.5 – 1.1)	0.13 (1.8)	– –
6	9	First sampling	1.73 (1.0 – 2.0)	1.19 (1.0 – 3.3)	1.41 (1.0 – 3.0)	0.79 (0.7 – 1.5)	0.24 (0.9 – 1.3)	0.1 (1.0)
		Re-examination < 36 months later	1.51 (0.5 – 3.5)	1.24 (0.5 – 3.0)	1.42 (0.5 – 3.5)	0.54 (0.5 – 1.9)	0.2 (1.8)	0.1 (1.0)

Results are presented as percentages (range in brackets).

Table 2 *Number of changed findings of chromosomal aberrations in the exposed subjects*

Group	N	Increase	Decrease	Unchanged
1	12	1 (8.3)	8 (66.7)	3 (25)
2	31	8 (25)	22 (68.8)	2 (6.2)
3	13	0	12 (92.3)	1 (7.7)
4	21	2 (9.5)	17 (81)	2 (9.5)
5	14	1 (7.1)	8 (57.2)	5 (35.7)
6	9	2 (22.2)	6 (66.7)	1 (11.1)

Results are presented as absolute numbers (percentage in brackets).

Table 3 *Chromosomal aberration analysis in the control subjects*

Sex	Age (range)	No. of persons	No. of cells	Chromatid break %	Chromosome break %	Acentric fragment %	Dicentric %	Chromatid interchanges (tetraradial) %
Men	25–36	32	4036	0.5	0.8	0.4	0.05	–
Women	19–46	28	4679	0.7	1.3	0.4	–	0.02
Total	19–46	60	8715	0.6	1.0	0.4	0.02	0.01

Regardless of the results, all examined subjects, except nine, have continued to work. As indicated by film dosimeter, three subjects received the exposure dose that exceeded currently recommended dose limits (the current whole-body dose limit in Croatia is 50 mSv for persons occupationally exposed to radiation).

We also observed multiply damaged cells containing more than one dicentric or ring, even some tricentrics, and nearly 20 acentric fragments.

DISCUSSION

The estimation of chromosomal aberrations in human peripheral blood lymphocytes is an important technique for risk assessment in occupational exposure (8). Most cells repair radiation induced damages shortly after the occurrence (5, 9). However, some breaks remain unrepaired or repair wrongly. These changes may be observed as chromosomal aberrations in metaphase cells.

Figure 2 shows the chromosomes from a single lymphocyte taken from a radiologist who worked for 12 years in a radiation zone. It displays a selection of types of aberrations encountered in cytogenetic analyses. All these aberrations are termed unstable because they hinder the normal separation of genetic material during cell division. These aberrations are likely to be lethal within a few cell cycles and would be selectively eliminated from a naturally proliferating population of cells.

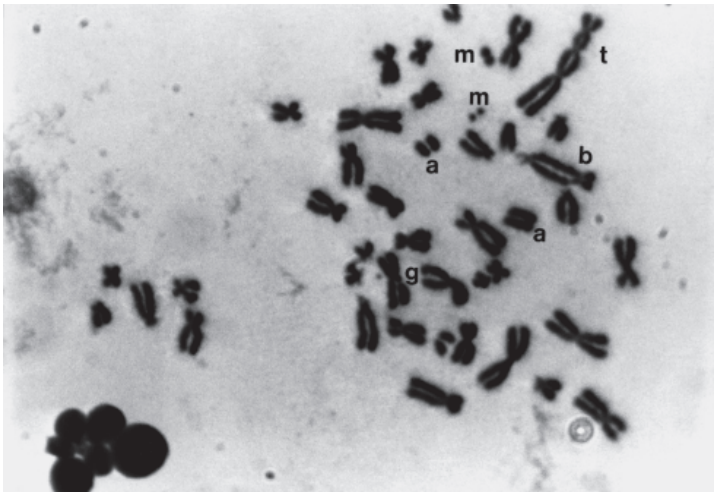

Figure 2 *A metaphase spread from a human lymphocyte exhibiting unstable chromosomal aberrations. b – dicentric, t – tricentric, a – acentric fragment, m – minute, g – gap. (Not all the aberrations have been labeled in this figure).*

It is well established that cells containing aberrations slowly disappear from the circulating lymphocyte pool (10, 11). The results of our study show that the recovery from chromosomal aberrations observed at various intervals of blood sampling is not significantly time-dependent. Summarizing all results, Table 2 shows a decrease of chromosomal aberrations in 92.3% and 81% of subjects reexamined after 9 and 12 months respectively. The number of dicentrics or ring chromosomes was not always followed by a corresponding number of acentric fragments.

We analyzed and compared individual differences in the number of chromosomal aberrations within each group and between the groups. Individual differences in

radiosensitivity, as well as different repair ability were also considered. The cell has a number of mechanisms to repair DNA damage. Rapid and flawless repair systems can compensate for much higher irradiation doses than the slow and error-prone ones can. *Oesch and co-workers* (12) showed that the same individual has a different repair capacity at different times, which is a consequence of differences in endogenous physiological status or changing exposure to exogenous compounds. It is also known that human lymphocytes consist of cell sub-populations with different radiosensitivity (13). There is evidence to suggest differences in sensitivity as a function of cell-cycle position. Using the comet assay in different mammalian cell lines, *Olive and Banath* showed that damage repair also depended on cell-cycle position (4).

After a partial or random exposure, cells from non-irradiated areas or areas irradiated with low doses might reduce the average aberration frequency (14). It is therefore difficult to define the period for which the unstable aberration frequency observed in lymphocytes can be used directly for dosimetry.

The results point out the importance of chromosomal aberration analysis as a means of the detection of radiation induced damage, as well as the significance of implementation of protective measures. It is also important to stress the necessity of pre-employment analysis for every person who is to start to work in an ionizing radiation zone. The individual differences in radiosensitivity as well as the frequency of cytogenetic aberrations depend on physical condition of each subject and on the variability in DNA repair processes. As time-dependent changes in dicentrics may be subject to individual variations, it may be difficult to extrapolate from one case to another (14). The present results do not allow definitive conclusions about low-dose ionizing radiation during the occupational exposure, but are intriguing enough to motivate further investigation.

REFERENCES

1. *Galloway SM*. Chromosome aberrations induced *in vitro*: mechanisms, delayed expression, and intriguing questions. Environ Mol Mutag 1994;23(Suppl 24):44–53.
2. *Natarajan AT*. Mechanisms for induction of mutagenesis and chromosome aberrations. Environ Health Persp 1993;101(Suppl. 3):227–31.
3. *Borek C*. Molecular mechanisms in cancer induction and prevention. Environ Health Persp 1993;101(Suppl. 3):237–45.
4. *Olive PL, Banath JP*. Induction and rejoining of radiation-induced DNA single-strand breaks: »tail moment« as a function of position in the cell cycle. Mutation Res DNA Repair 1993;294:275–83.
5. *Fortini P, Raspaglio G, Falchi M, Dogliotti E*. Analysis of DNA alkylation damage and repair in mammalian cells by the comet assay. Mutagenesis 1996;11:169–75.
6. *Awa AA*. Review of thirty years study of Hiroshima and Nagasaki atomic bomb survivors. II. Biological effects. Chromosomal aberrations in Somatic Cells. J Radiat Res 1975;16(Suppl):122–31.
7. *Leonard A, De Knudt Gh, Leonard ED*. Persistence of chromosomal aberrations in an accidentally irradiated subject. Radiat Protect Dosim 1988;22:55–7.
8. *Bauchinger M*. Cytogenetic effects in human lymphocytes as a dosimetry system. In: Eisert WG, Mendelsohn ML, eds. Biological Dosimetry. Berlin: Springer. 1984:15–24.

9. *Olive PI, Banath JP, Durand R*. Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells measured using the »comet« assay. Radiat Res 1990;122:86–94.
10. *Guedeney G, Grunwald D, Malabert JL, Doloy MT*. Time dependence of chromosomal aberrations induced in human and monkey lymphocytes by acute and fractionated exposure to ^{60}Co. Radiat Res 1988;116:254–62.
11. *Ramalho AT, Curado MP, Natarajan AT*. Lifespan of human lymphocytes estimated during a six year cytogenetic follow-up of individuals accidentally exposed in the 1987 radiological accident in Brazil. Mutat Res 1995;331:47–54.
12. *Oesch F, Aulmann W, Platt KL, Doerjer G*. Individual differences in DNA repair capacities in man. Arch Toxicol 1987;(suppl 10):172–9.
13. *Wuttke K, Streffer C, Muller W-U*. Radiation induced micronuclei in subpopulations of human lymphocytes. Mutat Res 1993;286:181–8.
14. *Doloy MT, Malabert JL, Guedeney G, et al*. Use of unstable chromosomal aberrations for biological dosimetry after the first postirradiation mitosis. Radiat Res 1991;125:141–51.

Sažetak

PRAĆENJE KROMOSOMSKIH ABERACIJA U MEDICINSKOG OSOBLJA PROFESIONALNO IZLOŽENOG DJELOVANJU RENDGENSKIH ZRAKA

Pad broja nestabilnih oštećenja kromosoma (dicentrični kromosomi, prstenasti kromosomi i acentrični fragmenti) praćen dulje vrijeme u limfocitima periferne krvi osoba koje su bile izložene ionizirajućem zračenju upozorio je na to da limfociti mogu preživjeti više godina nakon izloženosti. Ovim istraživanjem obuhvaćeno je 100 osoba profesionalno izloženih djelovanju rendgenskih zraka. Praćene su nestabilne kromosomske aberacije u izloženim osobama u vremenskim intervalima 3 mjeseca (12 osoba), 6 mjeseci (31 osoba), 9 mjeseci (13 osoba), 12 mjeseci (21 osoba), <24 mjeseca (14 osoba), <36 mjeseci (9 osoba) i u 60 osoba koje nisu bile izložene djelovanju ionizirajućeg zračenja ili kemijskim mutagenima na svojim radnim mjestima.

Dobiveni rezultati nisu pokazali popravak oštećenja ovisan o vremenu u svim slučajevima, što upućuje na individualne razlike u osjetljivosti na zračenje, kao i na različitu sposobnost za popravak oštećenih molekula.

Ključne riječi:
ionizirajuće zračenje, oštećenja kromosoma, profesionalna izloženost

Requests for reprints:

Vilena Kašuba, Ph.D.
Institute for Medical Research and Occupational Health
2 Ksaverska St., P.O. Box 291,
HR–10001 Zagreb, Croatia
E-mail: *vkasuba@mimi.imi.hr*