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Comparison of chromosome aberrations in peripheral blood lymphocytes from people occupationally exposed to ionizing and radiofrequency radiation.

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Abstract

The genotoxic effects of occupational exposure to ionizing and non-ionizing radiation were investigated in 25 physicians and nurses working in hospitals and in 20 individuals working at radio-relay stations. Examination was conducted by chromosome aberration analysis of peripheral blood lymphocytes. The data showed that total number of chromosome aberrations in people exposed to ionizing and radio-frequency radiation (4.08 +/- 0.37 and 4.35 +/- 0.5 on 200 scored metaphases, respectively) were almost equally higher than those of non-irradiated subjects. The increase was in proportion to the number of individuals having more that 5-aberration/200 metaphases. Acentric fragments comprised the most frequently seen type of aberration. The average numbers in examined groups (11.8 x 10(-3) and 14.8 x 10(-3) per cell, respectively), were significantly higher than 4.2 x 10(-3), which was observed in controls, unexposed individuals. Dicentric fragments were also frequent (4.8 x 10(-3) and 6.25 x 10(-3), respectively, vs. 0.52 x 10(-3) in control). In contrast, the frequency of chromatid breaks increased only after ionizing radiation (3.8 x 10(-3) vs. 0.26 x 10(-3) in control). A positive correlation between the total number of chromosome aberrations and cumulative 6-years dosage was also found. The data emphasized the dangerous effects of prolonged exposure to both types of radiation and indicated that chromosomal aberration analysis should be obligatory for individuals working at radio-relay stations.

KEYWORDS: chromosomal aberrations, ionizing radiation, radiofrequency radiation

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Original Article

Comparison of Chromosome Aberrations in Peripheral Blood Lymphocytes from People Occupationally Exposed to Ionizing and Radiofrequency Radiation

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The genotoxic effects of occupational exposure to ionizing and non-ionizing radiation were investigated in 25 physicians and nurses working in hospitals and in 20 individuals working at radio-relay stations. Examination was conducted by chromosome aberration analysis of peripheral blood lymphocytes. The data showed that total number of chromosome aberrations in people exposed to ionizing and radio-frequency radiation (4.08 ± 0.37 and 4.35 ± 0.5 on 200 scored metaphases, respectively) were almost equally higher than those of non-irradiated subjects. The increase was in proportion to the number of individuals having more that 5-aberration/200 metaphases. Acentric fragments comprised the most frequently seen type of aberration. The average numbers in examined groups (11.8×10^{-3} and 14.8×10^{-3} per cell, respectively), were significantly higher than 4.2×10^{-3}, which was observed in controls, unexposed individuals. Dicentric fragments were also frequent (4.8×10^{-3} and 6.25×10^{-3}, respectively, *vs.* 0.52×10^{-3} in control). In contrast, the frequency of chromatid breaks increased only after ionizing radiation (3.8×10^{-3} *vs.* 0.26×10^{-3} in control). A positive correlation between the total number of chromosome aberrations and cumulative 6-years dosage was also found. The data emphasized the dangerous effects of prolonged exposure to both types of radiation and indicated that chromosomal aberration analysis should be obligatory for individuals working at radio-relay stations.

Key words: chromosomal aberrations, ionizing radiation, radiofrequency radiation

It is well known that exposure to radioactive sources such as X and gamma rays, neutrons, electrons ("beta" particles) and alpha particles (helium nuclei) is dangerous, as is exposure to background radiation primary emitted from cosmic sources. Such irradiation induces damage of DNA chains and significantly high frequencies of chromosomal aberrations [1-5] in health care professionals and those living near sources of radiation. However, a limited number of contradictory reports have appeared that discuss the ability of non-ionizing radiation to induce similar changes in different biological systems. In this regard a particular attention has been given to radiation of an extremely low frequency range (50 to 60 MHz) and radiofrequency radiation (with frequencies ranging from 30 kHz to 30,000 MHz). Other wavelengths on the non-ionizing electromagnetic spectrum are also suspected of inducing damage, including wavelengths emitted from microwave appliances, radar, video-display

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terminals, and even cellular telephones [6, 7]. It has been generally concluded that the evidence does not indicate any direct genotoxic risk from exposure to all of these types of radiation [8-11], but there remains some controversy about their toxicological effects [12, 13].

In an attempt to correlate the genotoxic effects of non-ionizing and ionizing radiation, we performed a chromosome aberration analysis of peripheral blood lymphocytes in 20 individuals, who were working in Croatian telecommunications and relay stations. We also studied cells from 25 medical professions who had been regularly exposed to ionizing radiation. Analysis of structural chromosomal aberrations in peripheral blood lymphocytes (chromatid breaks, acentric fragments and dicentric fragments) demonstrated that both groups of examinees have a higher incidence of acentric and dicentric fragments than do controls. The number of such abnormalities per cell was significantly greater in those exposed to radiation than that reported for controls, who were unexposed individuals. The data also revealed that in both groups, the proportion of individuals with more than 5 aberration in 200 metaphases was greater. Among individuals exposed to such irradiation, there was a greater frequency of the combination of 3 acentric and 2 dicentric fragments in the cells from the group exposed to non-ionizing radiation, than in the cells of the other experimental group. However, prolonged periods of working under non-ionizing radioactive conditions, in contrast to the cumulative effect of ionizing radiation, were not positively correlated with the total number of chromosomal aberrations.

Materials and Methods

This study included blood samples from 25 medical workers (*e.g.*, engineers of medical radiology and X-ray technicians and nurses in the angioroom), who had been exposed to ionizing radiation used at the in Clinical Hospital Center of Rijeka. Twenty Croatian telecommunication workers employed to maintain relay stations and telecommunication centers were also included; these individuals worked in non-ionizing zones.

In the group exposed to ionizing radiation, 14 individuals were males and 11 were females. Their mean age was 44.56 year old (range 27-63). For all subjects, film dosimeters were used to calculate both the average annual dose of ionizing radiation and the cumulative dose of radiation received in the last 6 years.

In group exposed to non-ionizing radiation 18 individuals were male, and 2 were females. Their mean age was 48.6 years old (range 31-60). The bulk of their work takes place at relay stations, where as radio transmitters, they spend the entire day. The mean power of the electromagnetic waves in these fields can reach 10 W/m^2, with frequencies reaching 8 GHz. Data, concerning the duration of exposure in both groups were collected from a questionnaire designed to obtain relevant detail about the health of the examinees.

Chromosome aberration analysis. A genotoxic analysis was performed by conventional metaphase analysis of peripheral blood lymphocytes, which were stained by Giemsa staining techniques [14]. Briefly, short-term lymphocyte cultures were prepared using Gibco F10 medium, which was supplemented with 20% fetal calf serum, antibiotics and phytohaemagglutinin (Murex, Biotech ltd., Dartford, England). Two cultures of each sample were prepared. The cells were harvested at 48 h following stimulation. Colchicine (0.004%) (Sigma, Chemical Co., St. Louis, MO, USA) was added 3 h before harvest. The cultures were centrifuged and subjected to a hypotonic shock (20 min, 0.075 M KCl) at 37 °C. The lymphocytes were then fixed in acetic-methanol (1:3) and air-dried with 5% aqueous Giemsa solution for 10 min. Only structural aberrations such as chromatid and chromosome breaks (CB) and acentric (AC) and dicentric (DIC) fragments were analyzed. In each person, 200 metaphases were analyzed.

Statistical analysis. Distribution of aberrations among the cells was analyzed by Mann-Whitney *U* test, Person-product moment correlation, Poisson distribution, Kolmogorov-Smirnov, and Chi-Square tests. The computer program StatSoft was used for these analyses.

Results

The influence of ionizing and non-ionizing radiation on the frequency of chromosomal aberrations. The results showed that working in both ionizing and non-ionizing radiation zones led to the enhanced appearance of chromosomal aberrations; such radiation affected mainly the larger chromosomes (Fig. 1). Incidence of spontaneous aberration ranged from 0 to 5.5 % (Tables 1 and 2). However, in both groups, 40-50% of the examinees had more than 5 aberrations in 200 metaphases (Fig. 2). The total number of aberrations in

the ionizing group was 4.08 ± 0.37 and in the non-ionizing group the number was 4.35 ± 0.5.

Acentric fragments comprised the dominant type of aberration in both groups. Among 200 metaphases, 2.36 ± 0.3 and 2.95 ± 0.5 aberrations were found in the ionizing group and the non-ionizing group, respectively (Fig. 3). Both values were markedly higher than that of chromatid breaks, which up on 200 metaphases, were 0.76 ± 0.2 and 0.15 ± 0.1 respectively ($P < 0{,}001$), and that of dicentric fragments, found in frequencies of 0.96 ± 0.2 and 1.25 ± 0.3 respectively ($P < 0{,}001$). Different effects of ionizing and non-ionizing radiation were observed only as regards the frequency of chromatid breaks, which were more frequent in individuals who had been exposed to ionizing radiation ($P < 0{,}01$; Fig. 3).

These data, collected during 200 metaphases, were then expressed per cell. The findings were compared with the average values for chromosome aberrations, found in the controls, who were unexposed individuals discussed in previous reports [3, 14–16]. As presented in Table 3, the frequency of all fragments in the group exposed to ionizing radiation (chromatid breaks 3.8×10^{-3}, acentric fragments 11.8×10^{-3}, and dicentric fragments 4.8×10^{-3}), as well as, these in the non-ionizing radiation group (chromatid breaks 0.7×10^{-3}, acentric 14.8×10^{-3} and dicentric fragments 6.25×10^{-3}) were markedly higher than that of the controls (control referent values: 0.26×10^{-3}, 4.2×10^{-3}, and 0.52×10^{-3}, respectively).

Distribution of chromosomal aberrations in relation to type and sex. These findings, expressed as the average number of chromosome aberrations observed during 200 metaphases were then subjected to a Poisson distribution analysis in order to visualize the frequency of persons with 1, 2, 3, or more chromosomal aberrations. As shown on Fig. 4, in the group exposed to ionizing radiation, more individuals than expected had one chromatid break and either 2 acentric or 2 dicentric fragments. However, in individuals exposed to non-ionizing radiation, more than the expected number of persons had 2 or 3 acentric fragments, or 2 dicentric fragments (Fig. 5).

In the group exposed to non-ionizing radiation, there were only 2 females; thus, subclassification according to sex was performed only in the group exposed to ionizing

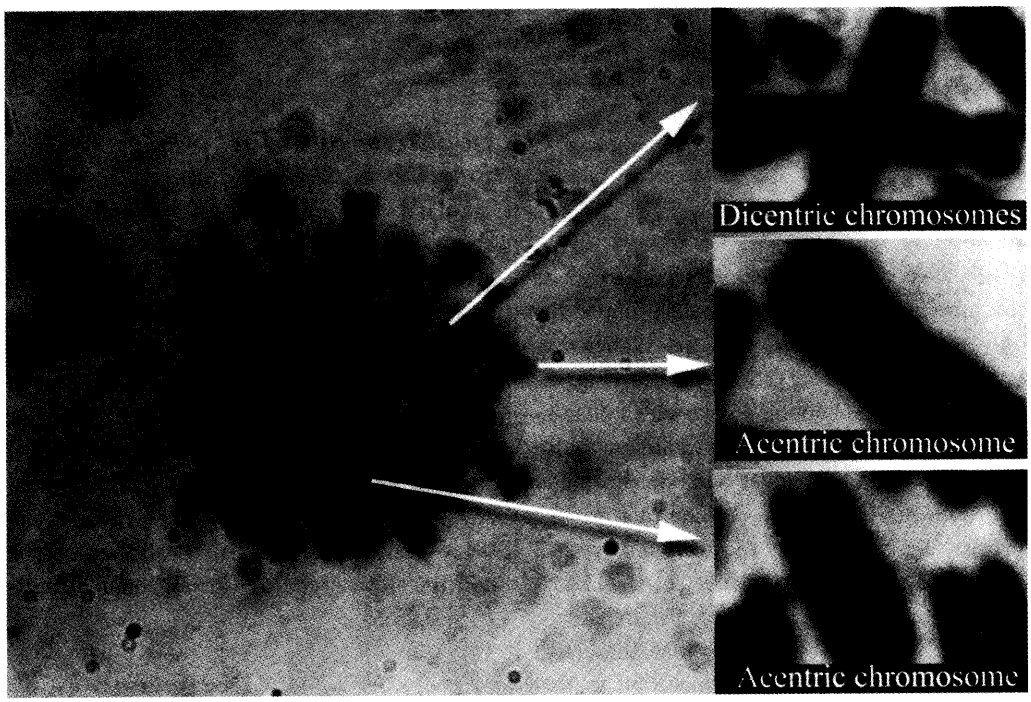

Fig. 1 Typical chromosome aberrations found by conventional metaphase analysis of peripheral blood lymphocytes, stained by Giemsa, after exposure to ionizing or non-ionizing radiation.

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Table 1 Individual data from persons exposed to X-radiation

NO.	Sex	Years of exposure	Dose/μSv Years One	Six	CB	AC	DIC	All/ 200 met.	% all
1	M	19	3750	14100	1	4	0	5	2.5
2	M	17	2670	13820	0	3	0	3	1.5
3	M	27	3850	13140	0	0	0	0	0
4	M	20	4080	24090	0	2	1	3	1.5
5	F	24	3500	18570	0	4	0	4	2
6	M	18	3860	12820	0	3	1	4	2
7	M	26	3920	14200	1	2	0	3	1.5
8	F	1.5	3560	-	0	3	0	3	1.5
9	F	2	3850	-	1	1	0	2	1
10	M	10	3430	39040	0	8	2	10	5
11	F	3	2900	-	1	2	4	7	3.5
12	F	9	3950	8450	1	2	0	3	1.5
13	M	11	3870	13500	0	2	0	2	1
14	F	3.5	4030	-	0	2	1	3	1.5
15	M	23	3410	12910	0	3	2	5	2.5
16	M	10	4620	14180	2	1	1	4	2
17	F	26	4130	14050	1	2	2	5	2.5
18	M	11	3720	13870	1	1	1	3	1.5
19	M	23	3820	12370	3	2	0	5	2.5
20	F	1.5	3900	-	1	3	1	5	2.5
21	F	23	3620	12200	3	1	1	5	2.5
22	F	10	3520	11350	0	2	2	4	2
23	M	10	3620	13140	1	3	1	5	2.5
24	M	32	-	-	1	1	2	4	2
25	M	30	3520	12580	1	2	2	5	2.5

AC, acentric fragments; CB, chromatid break; DIC, dicentric fragments. Shadowed boxes-5 or more chromosome aberrations found on 200 metaphases. % all = percent of aberrations found in 100 metaphases.

Table 2 Individual data from persons exposed to non-ionizing radiation

NO.	Sex	Years of exposure	CB	AC	DIC	All/200 metaphases	% all
1	M	35	0	3	2	5	2.5
2	M	22	0	3	2	5	2.5
3	M	14	1	2	2	5	2.5
4	M	25.5	0	3	2	5	2.5
5	M	27	0	3	2	5	2.5
6	M	38	0	0	2	2	1
7	M	35	0	3	1	4	2
8	M	35	1	2	2	5	2.5
9	M	34	0	2	2	4	2
10	M	30	0	2	2	4	2
11	M	16	0	1	1	2	1
12	M	30	0	4	4	8	4
13	M	37	0	10	1	11	5.5
14	M	5	0	2	0	2	1
15	M	26	0	4	0	4	2
16	F	6	0	5	0	5	2.5
17	F	22	0	5	0	5	2.5
18	M	32	1	0	0	1	1
19	M	29	0	2	0	2	1
20	M	21	0	3	0	3	1.5

AC, acentric fragments; CB, chromatid break; DIC, dicentric fragments. Shadowed boxes-5 or more chromosome aberrations found on 200 metaphases. % all = percent of aberrations found in 100 metaphases.

radiation (Fig. 6).

Although the frequency of all examined aberrations differed slightly among the female and male groups, the only statistically significant change was the higher frequency of chromatid breaks observed in females (Fig. 6; $P < 0{,}01$). The Poisson distribution analysis also demonstrated that in females exposed to ionizing radiation, more than the expected number of examinees had one chromatid break and 1–2 acentric fragments, in contrast to the males, who had greater a frequency of individuals with 2 or 3 acentric and 1–2 dicentric fragments (Fig. 7). The data imply the potential influence of sex on the sensitivity of cells to X-rays. It should be noted that the small sample size does not permit any final conclusion in this regard.

The effects of received dosage of X-ray radiation and the period spent in a non-ionizing radiation zone. To elucidate the effect of dose and duration of non-ionizing exposure on chromosomal aberrations, a correlation analysis of the annual dose *vs.* the 6 year absorption dose, as well as, an analysis of entire duration of exposure (in years) spent at a telecommunication center and the total frequency of aberrations were performed. As shown in Fig. 8, the received radiation dose in one year, and the duration of employment under non-ionizing conditions did not correlate with the total number of chromosomal aberrations. However, significant positive correlation was found between the dose of ionizing radiation received in 6 years and the appearance of chromosomal damage ($r = 0.616$; $P < 0{,}05$).

Discussion

The data emphasize that a significant number of

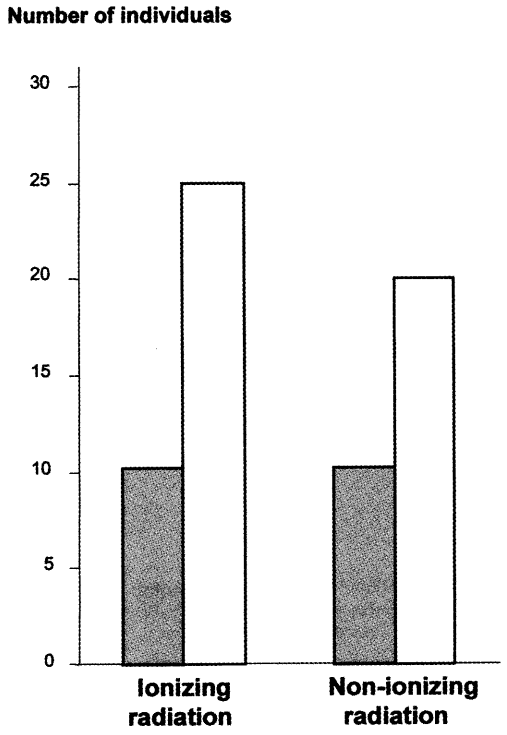


Fig. 2 The number of individuals with 5 of more chromosomal aberrations in 200 metaphases, observed after exposure to ionizing or non-ionizing radiation. ■, over 5 aberrations in 200 metaphases; □, sample.

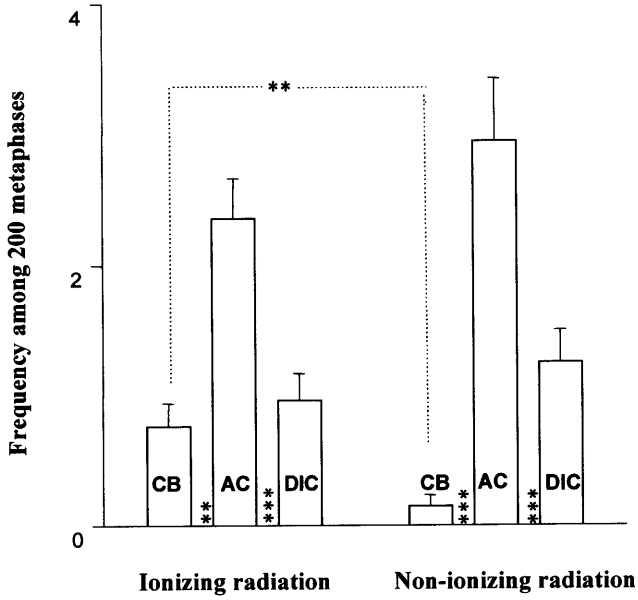
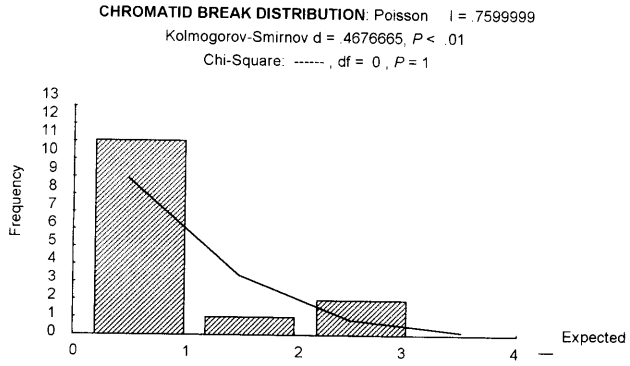


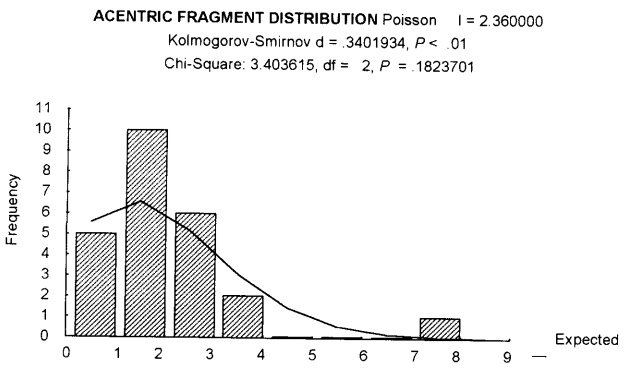
Fig. 3 The frequency of structural chromosomal aberrations after exposure to ionizing or non-ionizing radiation. CB-chromatid breaks, AC-acentric fragment, DIC-dicentric fragment. **, $P < 0{,}01$; ***, $P < 0{,}001$.

Table 3 Frequency of chromosomal aberrations expressed per cell

Group	Chromatid breaks $\times 10^{-3}$	Acentric fragments $\times 10^{-3}$	Dicentric fragments $\times 10^{-3}$
Ionizing radiation	3.8	11.8	4.8
Non-ionizing raiiation	0.7	14.8	6.25
Control	0.26	4.2	0.52

chromosomal aberrations may be induced not only by ionizing radiation, but also by non-ionizing radiation. The incidence of spontaneous chromosomal aberrations ranged from 0–5.5%, a value that is not higher than that observed in the non-exposed population [14]. However, in both groups, almost half of the individuals had more than 5 aberrations in 200 metaphases (Fig. 2). The dominant type of aberration in both groups was the acentric fragment (value expressed per cell 11.8×10^{-3} and 14.8×10^{-3}, for ionizing and non-ionizing group, respectively). These values were significantly greater than that calculated from the literature as an average value for the incidence of acentric fragments, observed in controls (4.2×10^{-3}), who were unexposed individuals [3, 14–16]. Similarly, in both groups a greater frequency of dicentric fragments was also observed (4.8×10^{-3} and 6.25×10^{-3} *vs.* 0.52×10^{-3}). These data show the presence of similar changes in peripheral blood lymphocytes in individuals working in the hospital and in those working at radio-relay stations. The findings imply that professional exposure not only to ionizing radiation, but also to radiofrequency radiation, may have certain cytogenetic effects. The genotoxic risk among those exposed to non-ionizing radiation appears to be smaller than among those exposed to ionizing radiation, since only in the later group, an additional increase in the frequency of chromatid breaks was found (Table 3), as well as a positive correlation between a 6-year exposure dose and the total number of chromosomal aberrations (Fig. 8). However, due to the fact that people working in low-frequency electromagnetic fields (EMFs) are not





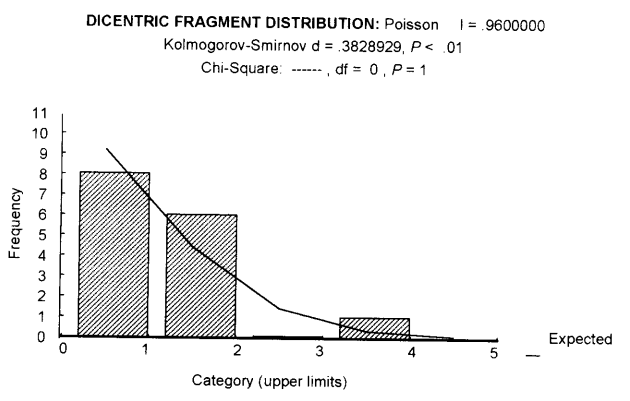
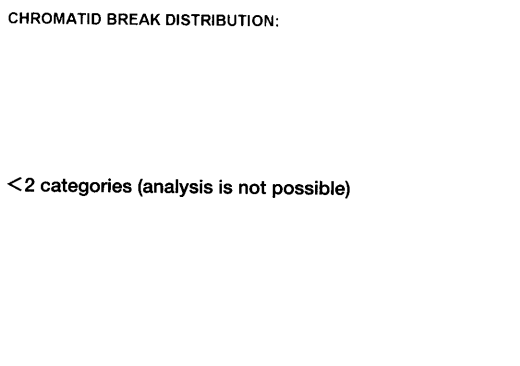
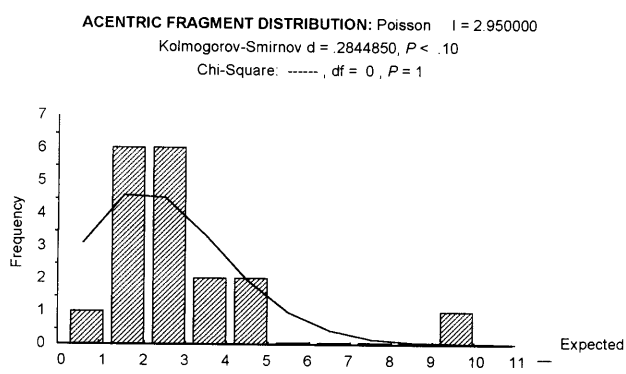


Fig. 4 Distribution analysis of chromatid breaks, acentric fragments and dicentric fragments in individuals occupationally exposed to ionizing radiation.





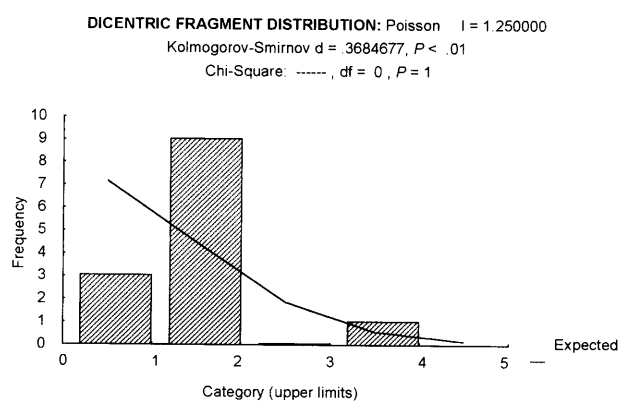


Fig. 5 Distribution analysis of acentric and dicentric fragments in individuals occupationally exposed to non-ionizing radiation. Chromatid break distribution is not given, due to the existence of less than 2 categories.

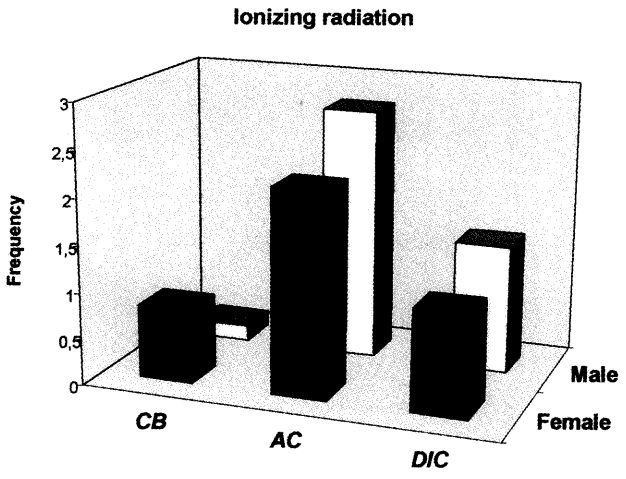


Fig. 6 Distribution of chromosomal aberrations according to sex in persons exposed to ionizing radiation.

under legal obligation to record received doses of irradiation, we were not able to perform real dose-response data analysis on data from this group of examinees. The lack of a relationship between the duration of the working period and the total number of chromosome aberrations thus suggests the absence of a cumulative effect of radiation (Fig. 8). However, it should be noted that in this group, one examinee, which had worked under conditions of exposure for 37 years, had the greatest number of chromosomal aberrations (Table 2; No 13).

The data obtained by Poissonian test also point to some differences in the distribution of determined types of chromosome aberrations observed after exposure to ionizing radiation, (Fig. 4) and non-ionizing radiation (Fig. 5). This demonstrated that in examinees exposed to radiofrequency radiation, more persons than expected had 2 or 3 acentric fragments and 2 dicentric fragments (Fig. 5). The findings in this study are particularly interesting, because both groups of workers were exposed to doses of ionizing and non-ionizing radiation, that were well below the accepted standards for exposure to radiation. According to the results of the dosimeter analysis, those exposed to ionizing radiation received on average an annual radiation dose of 3,000–4,000 μSv and 10,000–15,000 μSv during the last 6 years. Only one examinee received a total dose of 39,040 μSv in 6 years, which was still far below the established border line toxic dose of 0,05 Sv. Similarly, the examinees working in radio-relay stations were exposed to the power and frequency of electromagnetic waves that usually did not exceed permitted values of 10 W/m^2. The analysis was made by blind assessment of exposure, and appears to address the genotoxic effects of low doses of both ionizing and non-ionizing radiation. However, it should be noted that the present study was conducted on the a small sample of examinees and in order to be verified, would need to be replicated, in particular as regards the effect of radiofrequency radiation. An extensive review of all published studies on the potential genotoxicity of electric and magnetic fields that have appeared in the published literature until 1997 strengthens the conclusion that electric or magnetic fields do not have any genotoxic potential [9, 10]. However, the results of some epidemiological studies still might be interpreted as suggesting that living close to high-voltage transmission (HVT) lines appears to slightly increase the risk of childhood leukaemia [8, 12, 13]. Preece *et al.* [17], reported that group working under the auspices of the US National Institute of Environmental Health Sciences interpreted the findings in the literature as insufficient to warrant aggressive regulatory concerns; instead, passive regulatory action, such as continued emphasis on educating both the public and the regulated community about ways to reduce exposure, is considered warranted, because virtually everyone is routinely exposed to extremely low frequency EMFs. Our initial data concerning the similar incidence of chromosomal aberrations in professionals exposed to ionizing and nonionizing radiation, emphasizes the necessity that persons working in radio-relay stations perform continuos cytogenetic analysis, similar to that performed in the case of occupationally exposed medical staff. Currently, there is no obligation to perform such physical examinations more frequently than every 5 years; this is the same rate as that of examinations of employees working in other, unexposed settings. This also accounts why we have only data obtained by conventional techniques; such data are usually used for studies of persons professionally exposed to ionizing radiation. More sophisticated technologies, like fluorescence *in situ* hybridization (FISH) or spectral and multi-color FISH, are less-often used. However, these newer techniques are the methods of choice to visualize structural chromosomal aberrations and obtain a more detailed and informative picture of effects of the radiation. Eventually, such techniques will also allow for the identification of genes involved in radiation tumorigenesis and phenotype-genotype correlation on a cell-by-

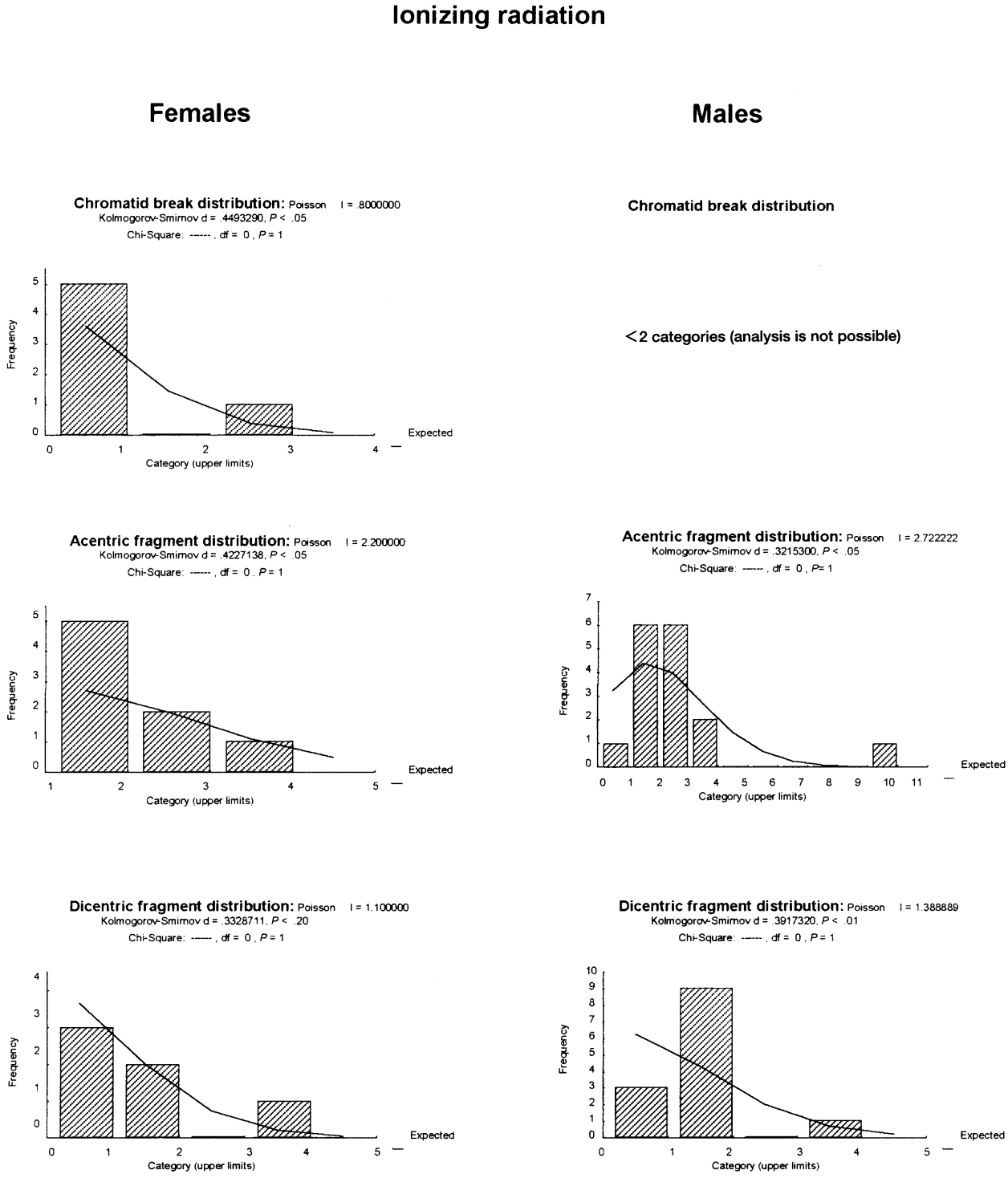


Fig. 7 Comparison between the acentric and dicentric fragment distribution found in females and males who were exposed to ionizing radiation. Chromatid break distribution in males is not presented, due to the small number of categories (< 2).

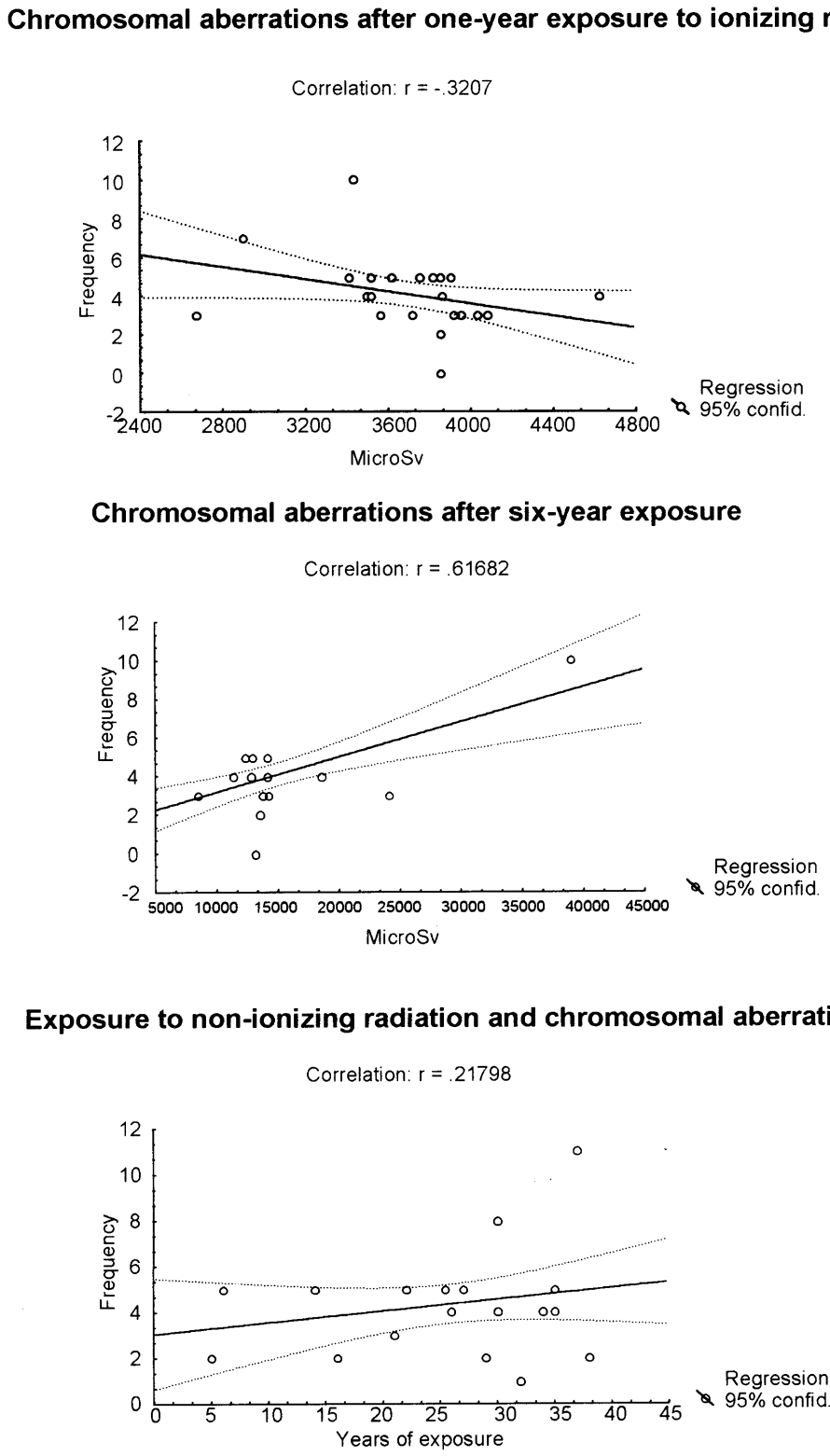


Fig. 8 Correlation between the total number of chromosomal aberrations found in 200 metaphases and received doses of ionizing radiation (annual and 6-year dose) or duration of work period under conditions involving exposure to non-ionizing radiation.

cell basis [18, 19]. However, it should be emphasized that the data obtained by conventional banding analyses, such as staining with quinacrine (Q-band) or Giemsa (G-band), are often comparable with those visualized by new methods [20-22]. Thus, our observation that particularly the larger chromosomes were affected during radiation (Fig. 1), seems to be supported by data obtained by chromosome painting; in other words, the frequencies of radiation-induced aberrations might correlate with chromosome size [23]. Similarly, the X-rays administrated *in vitro*, in a dose-dependent manner, increased the frequency of cells with translocations, dicentrics, insertions, tricentrics, and fragments of chromosomes 1, 3 or 4 [20, 24]. In our prospective study we will try to obtain more information about the life span of lymphocytes carrying chromosomal aberrations. Available cytogenetic follow-up studies, made on individuals accidentally exposed to radiation, point to dose-dependent, fast disappearance of unstable aberrations (dicentrics and rings) during the first year after exposure. This is in contrast to the disappearance of translocations, which in all subjects after whole-body exposure, remained relatively more stable [25, 26]. The life span of chromosomal aberrations induced by exposure to EMFs or microwaves is less well understood, although in several reports, their *in vivo* and *in vitro* clastogenic effects were emphasized [27-29]. Our data demonstrate that individuals exposed to radiofrequency radiation have an increased number of chromosomal aberrations, which obviously suggests that conventional analysis might be sensitive enough and thus helpful test for the detection of the potential genotoxicity of EMFs, as well as of other DNA damaging factors linked with specific job conditions at radio-relay station. Other factors include stress, changes in circadian rhythm, and disturbance of neuroendocrine homeostasis. Under these conditions the effect of continuous exposure to light may be particularly important; light deprivation may induce the physiological pinealectomy, resulting in a disturbance of melatonin secretion [30]. Such effects probably potentiate the toxic effects of radiofrequency radiation. In support of these findings, it was recently discovered that melatonin, given before the exposure of brain cells to a 60-Hz magnetic field, at an intensity of 0.5 mT, may prevent magnetic field-induced DNA damage; thus melatonin may act as an efficient free radical scavenger [31], or as an immunostimulating substance [32].

Keeping in mind the limitations of such a study, we would like to summarize our finding of comparable, increased incidence of chromosomal aberrations in persons working in hospitals and radio-relay stations. Our findings point out the necessity of performing continuous cytogenetic analysis, not only of occupationally exposed medical staff, but also of professionals working during the 24 h cycles under conditions which might include the exposure of radiofrequency radiation.

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