# Stable and Unstable Chromosome Aberrations Measured after Occupational Exposure to Ionizing Radiation and Ultrasound

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**Aim** To evaluate chromosome aberration and fluorescent in situ hybridization (FISH) assays as a method to estimate of health risk, we monitored 9 male subjects occupationally exposed to low doses of both ionizing radiation and ultrasound during a period of over 3 years.

**Methods** Sampling was performed at 6-month intervals during a three-year period. First we used conventional chromosomal aberrations analysis. When the aberration frequency for a particular subject reached the background, we measured translocations in the final sample, using fluorescence in situ hybridization. Chromosome painting probes for chromosomes 1, 2, and 4 were used simultaneously.

**Results** Dicentric and ring chromosomes were eliminated within a year. Translocations persisted and deviated from control values in all examinees. Translocations were detected long after unstable aberrations decreased to the background level.

**Conclusion** Fluorescence in situ hybridization-based translocation detection was a reliable method for monitoring chronic occupational clastogen exposure. Chromosome aberration assay correlated with translocation frequency. Stable chromosomal aberrations reflected cumulative genome damage during job exposure.

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For the last 30 years, chromosome aberration assay and detection of unstable aberrations, dicentrics and acentric fragments, have been used for the estimation of genome damage caused by physical and chemical clastogens (1). Since physical dosimetry provides only limited information when it comes to complex exposures, biodosimetry has increasingly gained in importance. The introduction of ultrasound in medicine and industrial technology has made the evaluation of genome damage more complicated due to absence of personal dosimetry and still undefined mechanisms of its clastogenic and aneugenic potentials.

The scientific importance of dicentric chromosome detection is significant. Biodosimetry based on dicentric calculations improved radiation protection and supplied data on the correlation between genome damage and other biomarkers related to ionizing radiation exposure, such as hematological parameters or development of neoplasms (1,2). However, a false perception was created that a decrease in the frequency of dicentrics means that the health-risk has disappeared.

There is a need for reliable methods to assess past exposure to clastogens and the related risk. This is particularly the case for a large number of individuals exposed to various levels of ionizing radiation caused by nuclear accidents such as Chernobyl, atmospheric nuclear testing prior to the early 1960s, the atomic bombing at Hiroshima and Nagasaki, various medical radiological procedures, and occupational exposures for which dosimetric information may be poor or absent (3,4). Our study focused principally on the application and evaluation of a technology referred to as "chromosome painting" for investigating human exposure at workplace and its risk assessment. This technology employs the method of fluorescence in situ hybridization (FISH) with whole chromosome probes, which rapidly and accurately detect stable chromosome abnormalities, such as translocations, in individuals exposed in the past (5-8).

The aim of our study was to compare the use of chromosomal aberrations and FISH method in the evaluation of health risk in individuals occupationally exposed to low doses of ionizing radiation and ultrasound in industry over long periods time, by biomonitoring the results of unstable and stable chromosome aberrations

## Participants and methods

### Subjects

Nine male subjects occupationally exposed to radioactive irridium ($^{192}$Ir) and ultrasound were followed up for a period of 3 years. The sampling period lasted for 18 months. They were specialists in industrial radiography and were working with $^{192}$Ir with the activity of 1.85 TBq as a source of ionizing radiation. Physical dosimetry showed that the annual dose was below 50 mSv over the study period. The frequency of the ultrasound equipment used in non-destructive methods was 1-5 MHz (mean ± standard deviation, 3 ± 6.8 MHz). The ultrasound frequency rarely exceeded the range between 1 kHz and 15 MHz. Subjects were simultaneously exposed to both ionizing radiation and ultrasound during their work hours (approximately 6 hours per day, 5 days per week).

Subjects were not exposed to ionizing radiation or ultrasound for 6 months before blood sampling. Over the sampling period, subjects took no drugs and were not vaccinated.

Subjects manifesting dicentric or ring chromosomes were suspended from work with ionizing radiation for 6 months. During that period they worked exclusively with ultrasound. Subjects had average duration of employment of 20.7 ± 9.6 years (range between 7 and 37 years, the average age of 46.8 ± 8.9 years, range 37-58); they were all non-smokers with no records of exposure to any other physical or chemical agent that might have interfered with the results of the study.

The control group consisted of 9 males doing administrative jobs in the government and county offices, living in the city of Zagreb. They were chosen from a convenience sample consisting of subjects from the entire population of voluntary blood donors in Zagreb area, chosen to match the exposed subjects by age and lifestyle charcteristics. They had to be healthy non-smokers, with no recorded chronic or acute diseases and radiochemical therapy and diagnostics (Table 1). They completed the questionnaire about their recent exposure to physical and chemical agents, nutritional, and smoking habits.

**Table 1.** Demographic characteristics of exposed and control group

| | Participants | | |
|---|---|---|---|
| Characteristics | exposed (n = 9) | control (n = 9) | P |
| Age/years (mean±SD)* | 46.8 ± 8.9 | 47.2 ± 7.1 | 0.92 |
| Years of exposure (mean±SD) | 20.7 ± 9.6 | | |
| Gender (n, %): | | | |
| female | 0 | 0 | 1.00 |
| male | 9 (100.0) | 9 (100.0) | |
| Smoking (>5 cigarettes per day, n, %): | | | |
| smokers | 0 | 0 | 1.00 |
| non-smokers | 9 (100.0) | 9 (100.0) | |
| Annual dose during 18 mo (mean±SD, mSv) | <50 ± 1.2 | | |
| Ultrasound frequency during 18 months of the sampling period (mean±SD, MHz) | 3 ± 6.8 | | |

*SD - standard deviation.

All subjects were informed about the purpose and procedure of this study and they signed an informed consent. The study was performed within the national project and approved by ethical committee of the Institute for Medical Research and Occupational Health and Ministry of Science, Education, and Sports of the Republic Croatia.

### Assays

Chromosome aberration assay was performed every 6 months until the number of dicentric chromosomes decreased to that of the controls. At that point, no further sampling was conducted and FISH was performed on the last sample only. Subjects who had dicentric, ring, or tetraradial chromosomes were suspended from the

work with ionizing radiation for a period of 6 months, in accord with the regulations of occupational medicine and radiation protection of the Republic of Croatia.

Whole blood samples of were collected from each subject. The cell cultures were incubated in F10 medium (Sigma-Aldrich, St. Louis, MO, USA) with 20% calf serum (Biological Industries, Kibbutz Beit Haemek, Israel) and stimulated with phytohaemagglutinin (Remel, Lenexa, KS, USA). The slides were prepared, as described previously (9). Two hundred well spread metaphases were analyzed for each subject. Chromosome aberrations were scored according to Bender et al (10), and translocation frequencies were converted into genomic frequencies with the formula for correction established by Lucas et al (6).

Fluorescence in situ hybridization was performed using painting probes for chromosomes 1, 2, and 4 (Cytocell, Cambridge, UK). Probes were applied according to manufacturer's specification. At least 1000 metaphases were analyzed per subject.

### Statistical analysis

Possible differences in the frequency of stable and unstable chromosomal aberrations were tested by $\chi^2$ test, using Statistica 5.5 (StatSoft, Tulsa, OK, USA).

### Results

With chromosome aberration assay, we observed significantly higher frequencies of chromosome breaks and dicentrics in the exposed group than in the control group (Table 2, $P=0.035$ for both). In 4 subjects, we observed significantly higher dicentric frequencies than in controls. However, all of these frequencies decreased during the follow-up time.

The translocation frequencies were significantly increased in all exposed subjects compared with controls (Table 2, $P=0.027$), whereas for the same time point (final sampling), the fre-

**Table 2.** Percentage of unstable and stable chromosomal aberrations in lymphocytes of subjects simultaneously exposed to ionizing radiation and ultrasound*

| Subject† | Sample period (months) | Chromosome aberration assay (%) | | | | FISH translocations (%) |
|---|---|---|---|---|---|---|
| | | chromosome breaks | dicentric chromosomes | ring chromosomes | tetraradius | |
| 1 | 0 | 0.5‡ | 0.0 | 0.0 | 0.0 | N/A |
| | 12 | N/A | N/A | N/A | N/A | 2.1‡ |
| 2 | 12 | 0.5‡ | 1.0‡ | 0.0 | 0.0 | N/A |
| | 18 | N/A | N/A | N/A | N/A | 2.8‡ |
| 3 | 0 | 1.0‡ | 0.0 | 0.0 | 0.0 | N/A |
| | 12 | 0.5‡ | 0.0 | 0.0 | 0.0 | 1.9‡ |
| 4 | 0 | 0.0 | 0.0 | 0.5 | 0.5 | N/A |
| | 6 | 1.5‡ | 0.0 | 0.0 | 0.0 | N/A |
| | 12 | N/A | N/A | N/A | N/A | 2.5‡ |
| 5 | 0 | 0.0 | 1.0‡ | 0.0 | 0.0 | N/A |
| | 6 | 0.5‡ | 0.0 | 0.0 | 0.0 | N/A |
| | 12 | N/A | N/A | N/A | N/A | 2.2‡ |
| 6 | 0 | 0.0 | 0.5‡ | 0.0 | 0.0 | N/A |
| | 6 | N/A | N/A | N/A | N/A | 1.9‡ |
| 7 | 0 | 1.0‡ | 0.5‡ | 0.0 | 0.0 | N/A |
| | 6 | 0.5‡ | 0.0 | 0.5 | 0.0 | N/A |
| | 18 | N/A | N/A | N/A | N/A | 2.6‡ |
| 8 | 0 | 2.0‡ | 0.5‡ | 0.0 | 0.0 | N/A |
| | 6 | 0.0 | 0.0 | 0.0 | 0.0 | 1.0‡ |
| 9 | 0 | 2.0‡ | 1.0‡ | 0.0 | 0.0 | N/A |
| | 6 | 0.5‡ | 0.0 | 0.0 | 0.0 | N/A |
| | 12 | N/A | N/A | N/A | N/A | 1.6‡ |
| Controls (mean±SD) | | 0.1±01 | 0.02±0.01 | 0.0 | 0.0 | 0.4±0.1 |

*Abbreviations: FISH – fluorescent *in situ* hybridization; N/A – not analyzed, SD - standard deviation.
†The FISH analysis was performed only in the samples taken 6 months after the sampling period in which dicentrics and ring chromosome reached zero level. For each subject blood samples were collected in the course of 3 y with 1-y intervals. For the control population mean frequencies are presented.
‡Statistically significant vs control, *P*<0.05.

quency for unstable aberrations, including dicentrics, decreased to the control levels (Table 3). In 2 subjects, the frequency of unstable aberrations was the same as in controls. The percentage of dicentric chromosomes detected in the exposed subjects varied between 0% and 1.0% and the percentage of translocations between 1.0% and 2.8%. Ring chromosomes were detected only in two subjects with the same frequency (0.5%), whereas tetraradius was present in a single sampling (Table 2). Unstable aberrations were less frequent relative to translocations. The baseline data were taken from samples six months after the workers had stopped working with radiation.

**Table 3.** Mean values of chromosome aberrations frequencies measured over 18 months*

| Sample period | Percentage of (mean±SD) | | |
|---|---|---|---|
| | chromosome breaks | dicentric + ring chromosomes | FISH translocations |
| 0 | 0.357±0.5† | 0.429±0.3† | no data |
| 6 | 0.250±0.5† | 0.125±0.1† | 1.9±0.7† |
| 12 | 0.417±0.2† | 0.017±0.3† | 2.1±1.2† |
| 18 | 0 | 0 | 2.7±1.2† |
| Control | 0 | 0 | 0.4±0.1 |

*Abbreviations: FISH – fluorescent *in situ* hybridization, SD - standard deviation.
†Statistically significant vs control *P*<0.05.

## Discussion

Our study confirmed the importance of fluorescence in situ hybridization in evaluating stable genome damage caused by long-term occupational exposure to low doses of both ionizing radiation and ultrasound. We confirmed our hypothesis that this type of exposure could induce significant number of translocations in peripheral blood lymphocytes, persistence of which is much higher than that of dicentric chromosomes. The rate of elimination, persistence, and the accumulation of genetic damage are of great importance in epidemiological studies of occupationally exposed population in industry, as the induction of such damage occurs under different conditions than in medicine or nuclear plants. Working conditions in industrial radiography, which occasionally entail a working day longer than 8 hours, field work, and often combined exposure to ultrasound and chemical substances pose a high risk for the employees. The results of physical dosimetry are not informative since we

cannot confirm with certainty that subjects wear dosimeters all the time during the exposure (6 hours/d, 18 months). The number of subjects included in the present study was limited because we attempted to analyze only the subjects working under similar exposure conditions. Translocation frequencies detected in our exposed group (1.9-2.1%) and control group (0.4%) are comparable with those detected in a similar study by Livingston et al (11).

Data collected during the last 25 years of the application of chromosome aberration assay make it possible to evaluate chromosome aberrations as a biomarker of increased health risk of developing a neoplasm (1,12). However, the ability of FISH to detect stable aberrations such as balanced translocations and inversions opened new perspectives in investigating consequences of long-term exposure to low doses of physical and chemical agents.

Occupational exposure in industry and medicine has become more complex due to application of new sources of ionizing radiation, ultrasound, and electromagnetic fields, which makes ever-greater demands on biodosimetry. At the same time, biodosimetry is still unable to establish causal relation between the type of radiation and specific damage to DNA molecule. However, contrary to unstable dicentric and ring chromosomes analysis (13), the use of FISH in detecting stabile translocations offered an answer to cumulative effects of radiation for the first time. Even more, beside stable aberrations, it also enabled simultaneous evaluation of unstable chromosome aberrations as a biomarker in estimation of health risk (6,8,14-16). In contrast to the majority of similar studies based on accidental overexposures (17), our study showed translocation accumulation after low dose exposure.

In this follow-up study, we confirmed transient nature of dicentric and ring chromosomes. Detected dicentric and ring chromosomes were eliminated during a period of one year, confirming that detected aberrations were not constitu-

tive. Our results indicated that translocations remained elevated, while unstable chromosome aberrations decreased. Translocation frequencies in all subjects significantly deviated from control values. These findings are in agreement with the results obtained by Sevan'kaev et al (18). In the cytogenetic follow-up study, the authors showed that the number of dicentric chromosomes declined significantly faster than the translocation frequency, with a half-life of 4 months, followed by a half-life of 2-4 years. Further, in the study by Atanasova et al (19), the frequency of translocations in the lymphocytes of nuclear repository workers was 50 times higher than that of dicentrics. However, Natarajan et al (20) reported that the initial frequency of dicentrics was two to 3-fold higher than the frequencies of translocations observed years after the exposure to $^{137}$Cs in the Goiania accident in Brazil, where an old radiation source was scavenged from an abandoned hospital caused serious radioactive contamination, resulting in a number of deaths. Tucker et al (21,22) reported that the frequency of translocations significantly declined following the irradiation of peripheral blood lymphocytes in vitro with doses lower than 1 Gy. However, they noted that if the initial translocation yield was low, the decline would be harder to detect. They were also not able to conclude whether translocations induced by occupational exposure exhibited similar declines since most of it occurred chronically and involved total absorbed doses below 0.2 Gy, which was the lowest dose in their study. According to health risk estimation, there is no possibility to make extrapolations or predict the curve of accumulation of translocations, as unstable aberrations showed unequal distribution of exposure due to individual activity at workplace.

We were not able to estimate the fraction of genome damage caused by ultrasound, although ultrasound in combined exposure with ionizing radiation increases genome damage (23,24). Further, Garaj-Vrhovac and Kopjar (25,26) showed that long-term exposure of medical personnel

to ultrasound is able to increase the DNA damage, measured by comet and micronucleus assay. Study groups described in these studies were exposed to similar frequencies of ultrasound (4-6 MHz) as those used in industry. However, frequencies used in medicine (1-50 mW/cm²) are lower than dose used in industry (1-5 kW/cm²). The DNA damaging potential of ultrasound was also proved in vitro (27).

Contrary to ultrasonic medical equipment, industrial equipment is not provided with specification such as power density, which is a relevant parameter for the biological effect of ultrasound. Ultrasonographic equipment is capable of delivering substantial levels of acoustic energy into the body. The main mechanism by which ultrasound produces irreversible cell damage is cavitation, which occurs when ultrasound interacts with bodies of gas in liquids or tissue. These gas bodies concentrate ultrasonic energy in its local vicinity, creating a zone of mechanical perturbations. In this process, cytotoxic or mutagenic free radicals are produced (28-31). Some bioeffects like inhibition of cell proliferation, DNA repair, and cell dependent apoptosis are found to be similar to those produced by gamma-irradiation (32). The evaluation of biological effects produced by medical ultrasound showed that ultrasound could produce intercellular space widening (33) and alter the cell membrane, resulting in deviations from normal cell uptake (34). These changes could be connected with the bystander effect, which implies that genetic alterations can occur in cells that receive no direct radiation at all.

In conclusion, chromosome aberration assay did not correlate with translocation frequency so an evaluation of health risk by chromosome aberration assay could underestimate the health risk. Translocation frequency obtained by FISH facilitates data on genome damage caused by physical agents cumulated over time. In spite of the limited number of subjects comprised by the study, stable chromosomal aberrations reflect cumulative genome damage during employment, while unstable ones may lead to underestimation of health risk. However, this was one of the first studies trying to evaluate low dose occupational exposure to both ionizing radiation and ultrasound, indicating the need for further research in this area on a larger number of subjects.

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