

Assessment of chromosomal aberrations induced in patients undergoing vertebroplasty and workers occupationally exposed to low-dose ionizing radiation

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ABSTRACT

► Original article

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Received: November 2024

Final revised: May 2025

Accepted: June 2025

Int. J. Radiat. Res., January 2026;
24(1): 63-68

DOI: 10.61186/ijrr.24.1.10

Keywords: Low-dose irradiation, chromosomal aberrations, vertebroplasty, NPP workers.

Background: The Chromosomal aberrations (CAs) are among the most crucial biological effects resulting from ionizing radiation. The aim of the current study is to investigate cytogenetic effects after both short- and long-term exposure of low irradiation doses (less than 100 mSv). **Materials and Methods:** In this study, two groups were included: 1) eighteen patients (age 54 - 90 years) undergoing fluoroscopic X-ray guided vertebroplasty for a short time (0.63 - 2.07 min) and 2) eighteen workers (age 40 - 66 years) employed in Bulgarian Nuclear Power Plant (NPP) (19 - 42 working years). Blood samples were taken from all subjects followed by culturing in RPMI-1640 medium. After cells harvesting and standard samples processing, chromosomal aberrations frequency was analyzed in Giemsa stained metaphase spreads. For patients group, blood samples were collected before and after the medical procedure. Nine healthy volunteers (from NPP administrative staff) served as reference controls in workers group. **Results:** The mean frequency of total CAs after vertebroplasty (0.0213 ± 0.0016 per cell) is slightly but significantly increased compared to the baseline level before the medical procedure (0.0143 ± 0.0013 ; $P = 0.013$). However, variabilities at the individual level were found. In the workers group, the total CAs yield raised 4 times relative to controls (0.0113 ± 0.0017 vs. 0.0028 ± 0.0012 ; $P = 0.001$). At the same time, no correlation was found between aberrations frequency and accumulated dose in both patients and workers. **Conclusion:** Low doses exposure whether acute or chronic results in deoxyribonucleic acid (DNA) damages and consequent CAs.

INTRODUCTION

Ionizing radiation (IR) induces deoxyribonucleic acid (DNA) damage and various chromosomal abnormalities related to mutagenesis, genome instability and cancer ⁽¹⁾. Its biological effects depend on both the irradiation dose and dose rate. Low linear energy transfer (LET) radiation <100 mGy and dose rate < 6 mGy/h are defined as low-dose and low-dose rates, respectively ⁽²⁾. According to linear non-threshold model, low-dose radiation exposure could cause late stochastic consequences, such as cancer; cardiovascular disease etc. ^(3, 4).

Over the past few decades, the use of doses lower than 100 mSv for medical purposes has expanded. This results in a higher health risk for many patients undergoing either diagnostic or therapeutic procedures regardless of short-term irradiation. Low-dose-induced effects remain unclear and depend on many factors, including individual radiosensitivity. Several reliable biomarkers are known to detect DNA damages and respectively to assess low dose irradiation effects ⁽⁵⁾. Thus, some authors have

observed that low doses delivered to patients led to increased chromosomal aberrations (CAs) frequency after interventional radiology procedures ⁽⁶⁻¹⁰⁾.

In contrast to short-term medical irradiation, occupational exposure to low-dose IR can also be protracted. This results in cumulative effects that increase with the duration of workers' employment. Among the personnel affected by low-dose irradiation are physicians and medical staff, nuclear power plant (NPP) workers, and workers in industry. Based on assessment of CAs, micronuclei and 53BP1 DNA repair foci it has been reported that irradiation with doses below accepted standards induced cytogenetic effects in nuclear medicine workers ^(11, 12) or radiologists ⁽¹³⁾. Conversely, Basri *et al.* ⁽¹⁴⁾ did not find a statistical difference in both γ -H2AX foci and micronuclei frequency between medical radiation workers and controls. Although occupational exposures are regulated and monitored to ensure the safety of employed individuals, the accumulated doses could be related to increased cancer risk, cataracts, cardiovascular disease, and dysfunction of the central nervous system ⁽¹⁵⁻¹⁸⁾. Increased cases of

malignancy among interventionists who performed fluoroscopically guided procedures were observed ⁽¹⁹⁾. On the other hand, Kim *et al.* ⁽²⁰⁾, demonstrated that chromosome damage can be induced in NPP workers occupationally exposed to doses below the permissible dose limit.

The purpose of the present study was to investigate IR-induced cytogenetic effects after medical and occupational low-dose irradiation. The two groups were the subjects of interest: 1) patients undergoing percutaneous vertebroplasty under short-term X-ray fluoroscopic guidance and 2) NPP workers exposed to low irradiation doses. Vertebroplasty is a minimally invasive procedure that is used to treat painful vertebral compression fractures. Supporting cement is routinely injected into weakened vertebral bodies, mainly due to osteoporosis or tumor growth. Fluoroscopic guidance is needed to control needle progression and cement injection, which increases the risk of IR exposure for surgeons and treated patients ^(21, 22). It is well known that either short-term or chronic low-dose irradiation may cause cytogenetic effects, that can be detected in peripheral blood lymphocytes ^(13, 23). In the current study, a metaphase analysis was performed to assess unstable chromosomal aberrations after low-dose irradiation.

Data concerning the effects of radiation exposure on patients undergoing spinal surgical procedures are scarce, although such procedures are widespread. Accordingly, the IAEA (International Atomic Energy Agency) has started to create a database through the SAFRAD (SAFety in RADiological procedures) system for patients undergoing fluoroscopically guided diagnostic and interventional procedures. To our knowledge, there are no studies regarding radiation-induced cytogenetic effects in patients undergoing vertebroplasty. Our study contributes to the literature. Furthermore, the current investigation is comparative, including both short- and long-term irradiation effects of low doses.

MATERIAL AND METHODS

Chromosomal aberrations in peripheral blood lymphocytes were analyzed after either medical or occupational exposure to low irradiation doses.

Medical exposure after fluoroscopy-guided Vertebroplasty: Eighteen patients with lumbar or thoracic compression fractures provided blood samples. Informed consent was obtained from each donor. Blood samples were collected in accordance with Bulgarian Health Law (2005/7/IV) and approved from ethical committee of National Center of Radiobiology and Radiation Protection (NCRPEC/ No. 1/09.01.2023). Patients were stationed at "St. Ivan Rilski" University Hospital (Medical

University, Sofia, Bulgaria). They were diagnosed using magnetic resonance imaging and chest or lumbar radiography. Only one patient underwent a computed tomography (CT) scan. The individuals underwent micro-invasive percutaneous vertebroplasty procedures with guidance of Philips BV Pulsera C Arm X-Ray Imaging System (Philips Healthcare). The cumulative dose at the Patient Entrance Reference Point (also known as Cumulative Air Kerma, CAK dose), Dose Area Product (DAP) and exposure time were determined automatically by the equipment. Screen images and clinical data were obtained for every subject. Patients with a history of chemotherapy or radiotherapy were excluded from the study. Two individuals had pathological fractures and were suspected to have cancer without any treatment. To eliminate the impact of previous X-ray examinations on CAs yield, blood samples were collected directly before and 15 to 30 minutes after vertebroplasty in lithium-heparin vacutainers. Thus, the baseline aberrations level before the procedures served as reference control for the consequent changes. The demographic characteristics of the patients are presented in table 1.

Table 1. Demographic characteristics of patients.

Patients and Exposure Data	
Patients, <i>n</i>	18
Male/female, %	17/83 %
Mean age (range), years	69 (54 - 90)
Body Mass Index (range), kg/m ²	25.80 (17.72 - 35.43)
Etiology, %	
Osteoporosis	83
Trauma	6
Pathological fracture	11
Anatomic location, %	
Lumbar	63
Thoracic	37
Level treated, <i>n</i> patients	
1	13
2	5
Mean fluoroscopy procedure time (range) min	1.09 (0.63 - 2.07)
Mean cumulative air kerma (CAK dose, range), mGy	24.95 (7 - 69.1)
Mean dose area product (DAP, range), Gy.cm ²	5.91 (1.90 - 16.32)

Occupational exposure to low radiation doses:

Individuals, employed in "Kozloduy" Nuclear Power Plant (Bulgaria) and protracted exposed to low LET radiation (mainly γ -rays) were included in the present study. Blood samples were collected in the period 2018-2019. Informed consent was obtained from 18 workers and 9 healthy volunteers who served as reference controls. They were interviewed, and no one reported body X-ray during the last year at the time of investigation. Only four persons reported teeth X-ray (three workers and one control). Demographic characteristics of the occupationally irradiated individuals and controls are summarized in table 2. The accumulated doses were obtained using RADOS TLD dosimeters for personal dosimetry

(Mirion Technologies, Canberra BNLS).

Table 2. Demographic characteristics of NPP workers.

Occupational exposure	Workers	Control group
Individuals, <i>n</i>	18	9
Male/female, %	100/0 %	11.1/88.9 %
Mean age (range), <i>years</i>	51 (40 - 66)	42 (29 - 65)
Mean working years (range)	28.7 (19 - 42)	12 (2 - 20)
Mean accumulated dose for entire working period (range), <i>mSv</i>	134.70 (108.9 - 177)	-
Mean accumulated dose/last 5 year (range), <i>mSv</i>	9.31 (0 - 35.49)	-
Mean accumulated dose/last 1 year (range), <i>mSv</i>	1.77 (0 - 6.91)	-

Blood processing: Whole blood samples were cultured at 37°C in 5% CO₂ for 50 hours. The cultured medium contained RPMI 1640 (Sigma-Aldrich, USA) supplemented with 10% fetal bovine serum (Sigma-Aldrich, USA) and antibiotics (50 U/ml penicillin/50 µg/ml streptomycin; Gibco, BRL). The lymphocytes were stimulated with 2% phytohemagglutinin (Gibco, BRL). After 48 h culturing, they were arrested in metaphase by adding demecolcine (0.1 µg/ml; Sigma-Aldrich, USA). The cells were harvested, treated with 75 mM KCl and routinely fixed as previously described [24]. Metaphases were spread on clean pre-chilled slides and stored at -20°C until use.

Cytogenetic analysis: Metaphase spreads were stained with 5% Giemsa (Sigma-Aldrich, USA) solution for 10 min, washed with distilled water, and air-dried. Analysis was performed manually using a Leica DM 750 microscope (Germany) equipped with an ICC50 W digital camera. Only cells with 46 centromeres were scored for structural CAs: Dicentrics (Dic) or rings (r); excess acentric fragments (i.e. not associated with Dic or r), chromatid fragments, and chromatid interchange, all reported as total CAs. Metaphases with overlapping chromosomes or undistinguishable chromosomal arms were excluded. For each experimental point, 200 - 500 metaphases were analyzed.

Statistical analysis: Statistical analysis was performed using the IBM SPSS 19 software (IBM, NY) and MS Excel. Data are presented as mean ± SE. The normality of data distribution was tested using the Shapiro-Wilk test. A paired-sample t-test was used to describe the differences between the DNA damage levels assessed in the patient group before and after the medical procedure. An independent samples t-test was used to establish whether there were statistically significant differences in DNA damage levels between NPP workers and the control group. Correlations were investigated by calculating the Pearson correlation coefficient (*r*). A 95% confidence interval was used to calculate the mean. Differences were considered significant when the *P*-value was < 0.05.

RESULTS

Patients

Over 8400 metaphases were analyzed before, and nearly as many metaphases were analyzed after the medical procedure. Our results showed that even low doses and short-term IR exposure led to DNA damage in the group as a whole. A paired-samples t-test was applied to evaluate the increase in chromosomal aberrations frequency after vertebroplasty compared to that before the procedure. A slightly higher but significant increase was observed in both acentric chromosomes and total CAs frequencies (table 3, figure 1). The values before vertebroplasty were 0.0127 ± 0.0013 per cell (acentric fragments) and 0.0143 ± 0.0013 per cell (total CAs). The mean frequencies after the medical procedures increased to 0.0188 ± 0.0015 (acentric fragments, $P < 0.001$) and 0.0213 ± 0.0016 (total aberrations, $P = 0.013$). Variability among individuals was observed with respect to the total aberrations' frequency (figure 2). On the other hand, the mean frequency of dicentrics after vertebroplasty (0.0014 ± 0.0004) was also slightly higher, but not significantly ($P > 0.05$, paired-samples t-test), compared to the baseline level (0.0009 ± 0.0003). We did not find a correlation between chromosomal aberrations yield and CAK dose, age, or BMI (Pearson's correlation test, $P > 0.05$). The coefficient values obtained from the Pearson correlation analysis were respectively $r = 0.1$ (CAK dose), $r = -0.1$ (age), and $r = 0.3$ (BMI).

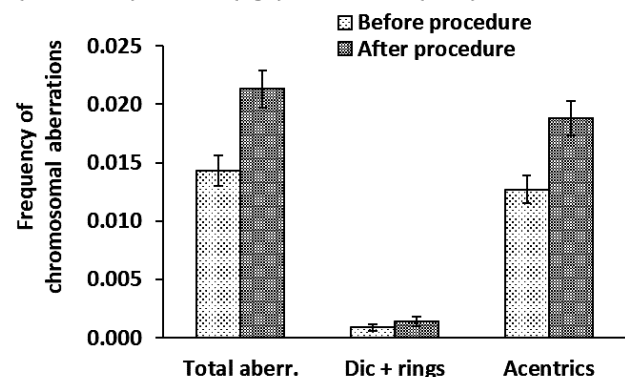


Figure 1. Mean frequency of chromosomal aberrations in patients, undergoing vertebroplasty; Total aberrations ($P = 0.013$); Acentric fragments ($P < 0.001$).

Table 3. Analysis of chromosome aberrations in patients.

Patients undergoing vertebroplasty	Before procedure	After procedure
No. of cells scored	8814	8452
Mean frequency dicentrics/rings±SE	0.0009±0.0003	0.0014±0.0004
Mean frequency excess acentrics±SE	0.0127±0.0013	0.0188±0.0015*
Mean frequency CAs±SE	0.0143±0.0013	0.0213±0.0016**

* $P < 0.001$; ** $P = 0.013$

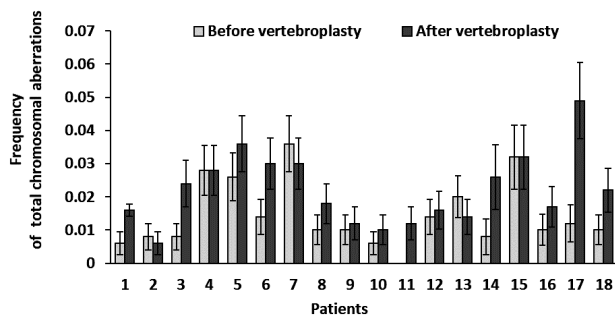


Figure 2. Individual variability in chromosomal aberrations frequency in patients, undergoing vertebroplasty.

NPP workers

In total, approximately 4060 and 1800 metaphases were analyzed for the workers and controls, respectively. Our results have shown that protracted occupational exposure to low doses of IR induces significantly higher mean frequencies of total CAs (independent samples t-test, $P=0.001$), Dic + r (independent samples t-test, $P<0.05$), and acentric chromosomes (independent samples t-test, $P<0.05$) compared to the controls (table 4, figure 3). The mean frequency of total aberrations was 0.0113 ± 0.0017 per cell, which was 4-fold higher compared to the controls. Similar results were found for excess acentric chromosomes (frequency 0.0057 ± 0.0012 per cell; ~ 3 times higher than that in controls) and Dic + r (frequency 0.0032 ± 0.0009 per cell; vs. <0.0001 for controls). At the same time, no relationship was observed between CAs yield and years of employment ($P>0.05$, Pearson's correlation test, $r=0.2$) or dose accumulated over the entire working period ($P>0.05$, Pearson's correlation test, $r=0.1$). We did not find such a correlation ($P>0.05$) in terms of cumulative dose during either recent five years ($r=0.4$) or the last one year ($r=0.4$).

Table 4. Analysis of chromosome aberrations in NPP workers.

NPP occupational exposure	Controls	Workers
No. of cells	1800	4060
Mean frequency dicentrics/rings $\pm$ SE	0	$0.0032 \pm 0.0009^*$
Mean frequency excess acentrics $\pm$ SE	0.0017 ± 0.0010	$0.0057 \pm 0.0012^*$
Mean frequency CAs $\pm$ SE	0.0028 ± 0.0012	$0.0113 \pm 0.0017^{**}$

* $P<0.05$; ** $P=0.001$

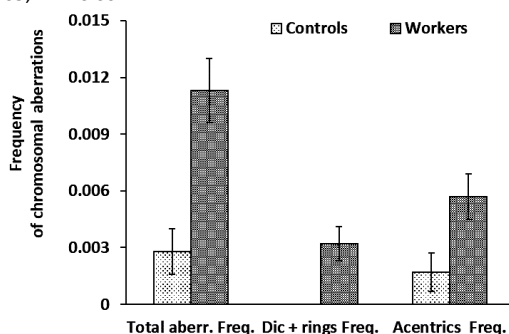


Figure 3. Mean frequency of chromosomal aberrations in NPP workers, protracted exposed to low doses radiation; Total aberrations ($P=0.001$); Dicentrics + rings ($P < 0.05$); Acentric fragments ($P < 0.05$).

DISCUSSION

The cytogenetic biomarkers are useful tool for assessment of IR-induced biological effects after environmental, occupational, accidental or medical exposure⁽²⁵⁾. In the current study, quantification of unstable chromosomal aberrations including dicentric chromosomes was used to investigate DNA damage after low-dose irradiation. The method is highly sensitive and its threshold dose limit is 50 mGy⁽²⁶⁾ or less⁽²⁷⁾. CAK doses after C-arm-guided vertebroplasty were below 100 mGy. We found that doses in the range of 7.0 to 69.1 mGy delivered to the patients for up to 2.07 min, slightly increased chromosomal aberrations yield in peripheral blood lymphocytes in a group as a whole. We observed significantly higher mean frequencies of total CAs (0.0213 ± 0.0016 per cell, $P=0.013$) and acentric chromosomes (0.0188 ± 0.0015 per cell, $P<0.001$) than baseline levels before the medical procedure. At the same time, the mean frequency of dicentrics was slightly, but not significantly higher, than that of the controls (figure 1, table 3). The present results are consistent with other studies showing that even low IR doses induce cytogenetic effects in patients after either diagnostic or therapeutic interventional radiology procedures^(7, 8, 28). However, in contrast to as, the last authors reported a stronger impact of medical irradiation on the chromosomal aberrations yield. For instance, Abe *et al.*⁽²⁸⁾ discovered a significantly higher number of dicentrics in patients after a single CT scan than before the procedure. According to them, the radiation exposure dose resulting from one CT scan is approximately 10 mSv, and it increases to approximately 50 mSv with a whole-body CT scan. However, these authors did not observe a correlation between increased dicentrics formation and effective radiation dose. Furthermore, Smith-Bindman *et al.*⁽²⁹⁾ found that different CT examinations induced variable irradiation doses depending on the anatomical body parts treated. Consequently, the resulting effects, including chromosomal aberrations, may also fluctuate. Habibi *et al.*⁽⁸⁾ also observed that the mean dicentrics frequency increased four times in patients after interventional cardiology procedures compared to the levels before the procedures. In our recent study of patients undergoing coronary angiography, the mean DAP values varied between 11 Gy.cm² and 60 Gy.cm²⁽³⁰⁾. In the current study, the range of measured DAP values vary from 1.36 Gy.cm² to 16.32 Gy.cm². Therefore, the radiation dose received by a patient during vertebroplasty is approximately 3.5 to 8 times lower compared to that received during some cardiac procedures. This may be the reason why we could not find a significant increase in dicentrics frequency in the study group. It should also be pointed out that exposure time may also affect chromosomal aberrations yield. Habibi *et al.*⁽⁸⁾ have

not provided such information, but their studies concern procedures like coronary angiography, coronary angioplasty and ablation which take more time. For instance, the fluoroscopic time for ablation can be up to 40 minutes⁽³¹⁾. During vertebroplasty, X-ray exposure time is much shorter, and in our case, it is in the range between 0.63 and 2.07 min. This may explain the observed lack of a strong effect in terms of total aberrations yield as well as a non-significant increase in dicentric frequency ($P > 0.05$) after vertebroplasty. Vertebroplasty is a minimally invasive procedure, and according to Wrangel *et al.*⁽³²⁾ the mean effective dose determined by phantom experiments for fluoroscopic-guided vertebroplasty is very low (11 mSv). Nevertheless, we found that it can induce cytogenetic effects. To the best of our knowledge, there are no data concerning the effect of fluoroscopic-guided vertebroplasty on chromosomal aberrations yield. In this line, it would be interesting to follow γ -H2AX foci yield as an early marker for radiation-induced DNA damage before repair has occurred. In contrast, the baseline CAs level in our study was slightly higher than the spontaneous frequency observed in the Bulgarian population⁽³³⁾. This could be explained by X-ray diagnostic examinations before vertebroplasty, patient age, previous non-cancer diseases, etc. We also found that CAs yield after vertebroplasty varied among patients. As we observed, it was not correlated with the cumulative dose and may be due to individual radiosensitivity (figure 2).

Regarding NPP workers, their doses accumulated mainly at the beginning of their work experience. Over the past decades, the cumulative doses after occupational exposure have decreased owing to strict safety control and low permissible dose limits. All individuals included in the present study received doses far below the ICRP (International Commission on Radiological Protection) recommended at the time of investigation when the limit was 20 mSv/year, averaged over five years (100 mSv/5 years)⁽²⁾. The mean values were respectively 1.77 mSv/last year and 9.31 mSv/last 5 years (table 2). Regardless of the low accumulated doses, we observed that chronic exposure to γ -rays (mainly) significantly increased the mean frequencies of total CAs (0.0113 ± 0.0017 , $P = 0.001$), Dic + r (0.0032 ± 0.0009 , $P < 0.05$), and acentric chromosomes (0.0057 ± 0.0012 , $P < 0.05$) compared with the control group (table 4, figure 3). These values were three to four times higher than those of the controls. Similar to the findings of Zakeri and Hirobe⁽³⁴⁾ our results show that CAs yield does not correlate with cumulative dose, years of employment, or workers' age. It could be due to the limited range of accumulated doses (100 – 200 mSv). Concerning the dicentrics and/or rings, we found their frequency to be relatively high in the studied worker group compared to our previous investigation, where increased but not significant

dicentric frequency for the same dose range was observed⁽²⁴⁾. The present results are consistent with other studies reporting that long-term exposure to IR doses close to those defined as low leads to a statistically significant increase in CAs frequency, including dicentrics, in hospital workers⁽³⁴⁻³⁶⁾. We have to keep in mind that interval between irradiation and blood sampling is very important. The half-life of dicentrics in lymphocytes is assumed to be three years⁽³⁷⁾. Chromosomal aberrations can be derived from long-life stem cells or progenitor cells that are affected by chronic IR exposure. Thus, a longer dicentric half-life has been suggested when occupational exposure of NPP workers is evaluated⁽³⁸⁾. Some factors, such as unreported recent medical IR exposure and individual radiosensitivity, could also contribute to our observations.

CONCLUSIONS

This study confirmed that doses below 100 mSv, induced cytogenetic effects after both short-term and protracted radiation exposure. It provided for the first time data that minimally invasive procedure like vertebroplasty may result in DNA damage. We found that the mean frequency of total chromosomal aberrations showed a trend to increase in patients undergoing vertebroplasty, although interindividual variability was observed. Furthermore, the workers occupationally exposed to chronic low-dose irradiation, showed 3–4 times higher levels of structural chromosomal aberrations (including dicentrics) compared to unexposed controls.

Acknowledgments: We thank all patients and volunteers for their kind involvement in our study.

Conflict of interest: None to declare.

Ethical considerations: The ethical committee of NCRP approved the study with registration number NCRPEC/No. 1/09.01.2023). All patients, workers and healthy donors provided their informed consent before participating in the study.

Authors' contribution: NK was involved in experimentation; analysed and interpreted data, drafted the manuscript. AS was involved in experimentation; analysed and interpreted data. LP was involved in experimentation; data and statistical analysis. DG; MA; II and EZ were involved in experimentation and data analysis. HH and HT performed vertebroplasty; collected blood samples and patients demographic data. RH was involved in metaphase spreads analysis; conceived the idea and finalised the manuscript.

Funding: This work was partially supported by Bulgarian National Science Fund [Contract № KII 06 H63/6/2022].

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