Molecular changes in population occupationally exposed to low-dose ionizing radiation: interventional radiology unit teams

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ABSTRACT

Interventional radiology team uses specific medical approach where procedures are usually done under lead aprons and in the vicinity of radiation beams. These are the main reasons for many occupationally related orthopedic conditions, as well as unwanted radiation-induced effects. Although the technological improvements decreased doses applied in such procedures, the number of them increased several fold. Strategic Research Agenda of the Multidisciplinary European Low Dose Initiative (MELODI) set priority research of cancer risks in occupational settings (particularly for doses ≤100 mSv) and individual radiation sensitivity. Therefore, the aim of the study was to assess cytogenetic changes in interventional radiology team and to relate them with occupational effective doses. Exposed population (N=24) comprised of 18 male and 6 female volunteers, at the age of 41±10, BMI of 24.65±3.26 kg/m^2, and 29% of were smokers. They were recruited in three hospitals in Zagreb, Croatia. The control group (N=24) was matched for sex, age, BMI, and smoking status to reduce the possible confounders.
Personal dosimetry using thermoluminiscent dosimeters showed average annual effective dose of 1.82±3.60 mSv (range 0 – 13.87 mSv). The baseline induction of primary DNA strand breaks was higher ($p<0.05$) in exposed population using the comet assay's tail length (Fig 1a). As for the abnormalities in chromosomal structure, the only significant increase observed was the frequency of nuclear buds (NBs) (Fig 1b), while other micronucleus test parameters did not differ among groups.
Based on our results, we managed to detect changes at the molecular level for the population occupationally exposed to low dose ionizing radiation. We also detected high inter-individual variability of the results. As for the personal dosimetry, more accurate protocols are needed in order to assess doses more precisely. Further studies are needed in order to improve occupational safety and to acknowledge ALARA principles.

KEYWORDS: Human biomonitoring, Comet assay, Micronucleus test

1 INTRODUCTION

Ionizing radiation is known human carcinogen [1] that induces DNA damage and production of reactive species. As a result it leads to molecular changes to irradiated cells, but also to surrounding cells by the bystander effect [2,3].
The use of ionizing radiation is quite usual in medicine where the development of devices and protocols led to strong decrease in doses absorbed by medical stuff performing procedures. However the need for such procedures increased remarkably [3,4].
Interventional radiology unit team is a multidisciplinary group that operates in the proximity of ionizing radiation that leads to occupational radiation exposure. Some estimates suggest that during a professional career a trained interventionalist receives a cumulative dose between 50 and 200 mSv that increases risk of development of malignant disease [5-7].
The Strategic Research Agenda by Multidisciplinary European Low Dose Initiative (MELODI) encourages to perform the studies resolving basic mechanisms of ionizing radiation at doses <100 mSv and relating them to health and risk evaluations [8].
Since the DNA is one of the targets of direct and indirect effects of ionizing radiation, we aimed to evaluate the baseline DNA damage levels in intervention radiology unit teams occupationally exposed to X-ray radiation and to compare them with matched unexposed control population. DNA damage was assessed using the comet and micronucleus tests that are widely used in genotoxicity biomonitoring. Moreover, the thermoluminiscent dosimeters (TLD-100) were used to assess personal effective dose.

2 SUBJECTS AND METHODS

2.1 Study population

Occupationally exposed physicians from intervention radiology units have voluntarily joined the study that has been approved by the Ethics committee and was performed in accordance with good medical practice and standards set by the Declaration of Helsinki. The volunteers (N=24, 18 male and 6 female) from three hospitals located in the same city were on average 41±9 years old, 1.78±0.08 m high, had 85.96±17.67 kg, while 7 of them were active smokers. Exposed volunteers had been working at position using low-dose ionizing radiation (X-ray) in range from 1 to 35 years (average 11 years). Using TLD based on LiF: Mg:Ti detectors that were worn bellow the lead apron at the left side of the chest we were able to assess their annual effective doses. While none of the volunteers reached annual dose limits, detected effective doses ranged from 0 to 13.87 mSv (1.82±3.60 mSv on average).
Volunteers donated 10 mL of blood that was placed into heparinized tubes. Based on the characteristics of exposed population, we recruited control group from the same city matched for as many parameters as possible. The control population volunteers were 41±10 years old, 1.76±0.11 m high, had 73.33±14.35 kg, with equal amount of smokers.

2.2 Comet test

Comet test was done according to Singh et al. [9] with modifications described in Gerić et al. [10]. The blood cells were embedded in the middle, 0.5% low melting point agarose gel, making a three-layer "sandwich". The slides with gels were then placed into the lysis solution (at +4°C and pH 10) and kept overnight. Following step included 20 min of DNA denaturation in electrophoresis solution (at +4°C, pH 13) and subsequently 20 min of electrophoresis took place at 1 V/cm. After the slides were washed with Tris-HCl buffer (pH 7.5), the slides were stained using 10 μg/mL ethidium bromide for 10 min. For the analysis, 200 nuclei on duplicate slides were scored using the Comet assay II image analysis software. The data was presented as tail length (TL) a useful comet descriptor for low DNA damage [11].

2.3 Micronucleus test

Micronucleus test was done according to Fenech and Morley [12] with modifications described in Gerić et al. [10]. Briefly, the 0.5 mL of whole blood was used to set up 72 h culture of peripheral blood lymphocytes in RPMI 1640 medium supplemented with 20% foetal bovine serum and 1% of phytohaemagglutinine as well as antibiotics. The culture was incubated at 37°C and 5% CO_2 atmosphere and after 44 h, 6 μg/mL cytochalasine-B was added to stop cytokinesis. Upon the 72 h incubation period, lymphocytes were isolated and fixed using series of centrifugation and fixative adding (methanol/acetic acid) steps. After the slides were dried, the cells were stained using 5% Giemsa solution for 10 min. For the analysis, 1000 binucleated lymphocytes were examined to determine micronuclei (MNi), nuclear buds (NBs), and nucleoplasmic bridges (NPBs) frequency. Furthermore, we expressed cytokinesis-block proliferation index (CBPI) on 500 cells [13].

2.4 Statistical analysis

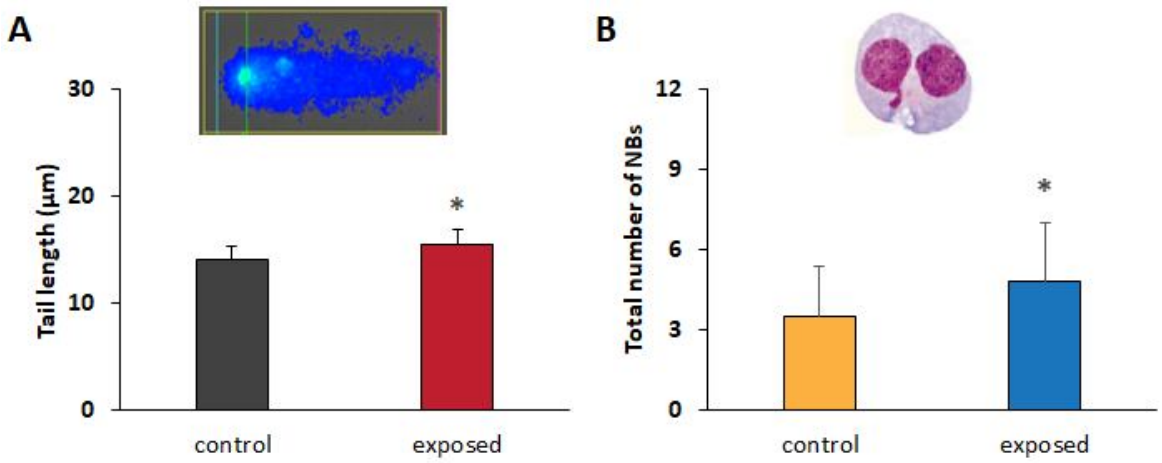
Statistical analysis was done using STATISTICA 13.2 (Dell Inc., USA). Compared groups (exposed, N=24 and control, N=24) were tested using non-parametrical Mann-Whitney U-test for parameters of the comet and micronucleus tests.

3 RESULTS

The results showed that occupationally exposed intervention radiology unit staff had 10% longer TL what was actually significant difference ($p<0.05$) compared to matched non-exposed group (Fig 1a). This results indicates more primary DNA damage induced by DNA strand breaks, DNA-DNA or DNA-protein cross linkage, and alkali-labile sites, i.e. AP (apurinic/apyrimidinic) sites or baseless sugars [14] in exposed group.

As for the micronucleus test results, we did not observe significant differences in MN and NPB frequency, nor the CBPI. However, exposed group had 38% higher frequency of NBs when compared to matched non-exposed group ($p<0.05$) (Fig 1b). NBs arise from elimination of amplified DNA and/or DNA repair complexes [15], so we can speculate that some of the DNA repair mechanisms were involved in molecular response to ionizing radiation.

Figure 1. Comparison of human biomonitoring biomarkers in interventional radiology unit workers (exposed) compared to control group. (A) the comet assay's tail length – the distance between green and magenta line; (B) total number of nuclear buds (NBs) – extruded nuclear material. Differences at $p < 0.05$ are considered significant (*).



3 CONCLUSION

The results of the study with limited number of volunteers suggest that molecular changes in occupationally exposed populations can be monitored. We have to stress out that there is a need of constant dosimetry monitoring of medical workers, however the application of molecular biology tools can improve assessment of the effects induced by ionizing radiation in working environment. Further studies with larger number of volunteers will enable insight in more reliable evaluation of radiation-induced effects and the development of biomonitoring protocols.

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Full paper available [16].

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