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Increased frequency of chromosomal aberrations and sister chromatid exchanges in peripheral lymphocytes of radiology technicians chronically exposed to low levels of ionizing radiations

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ABSTRACT

Chromosome aberrations (CAs) and sister chromatid exchanges (SCEs) frequencies were estimated in peripheral lymphocytes from 21 radiology technicians, and from 21 non-exposed control subjects. We exclusively considered individuals who neither smoke nor consume drugs or alcohol for a period of at least two years prior to the analysis. Significant differences were found between exposed and controls in terms of SCEs and CAs frequencies.

Technicians showed a significant higher number of high-frequency individuals (HFIs) with respect to the control group. Nevertheless, the mean frequency of SCEs observed among technician HFIs did not significantly differ with respect to that observed among control HFIs. Vice versa, the non-HFIs belonging to technicians group showed a statistically higher difference in the SCEs/NSM value with respect to the non-HFIs belonging to control group. Since the differences in the SCEs frequencies between the two groups are due to non-HFIs, our results seem to indicate a general genotoxic effect of the IR, not affected by HFIs.

Among technicians, the level of chromosome damage correlated neither with years of radiation exposure nor with the age of the subjects. Vice versa, in the control group, a positive correlation was found between the number of SCEs and age. In both samples the gender status did not influence the frequencies of CAs and SCEs.

Our results suggest that chronic long-term exposure to low doses of ionizing radiation could increase the CAs and SCEs frequencies. This study reinforces the relevance of the biomonitoring of hospital workers chronically exposed to ionizing radiation.

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1. Introduction

Personnel employed in radiology units represent workers chronically exposed to low doses of ionizing radiation (IR). The International Commission on Radiological Protection (ICRP) recommends an effective dose limit of 20 mSv per year, averaged over 5 years, with the further provision that the effective dose should not exceed 50 mSv in any single year (ICRP, 2001). Although in many hospital radiology units the radiation exposure remains below these levels, there is a higher risk for hospital workers handling diagnostic X-ray machines and γ-cameras due to the chronic but cumulative exposure to low doses of IR.

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One consequence of the exposure to IR is the formation of free radicals, such as peroxynitrites and hydroxyl radicals (Zhang et al., 2000; Spitz et al., 2004), able to produce some important alterations in the living cells. At DNA level, IR exerts its genotoxic effect by the induction of single- and double-strand breaks, a-basic sites and oxidized bases. Moreover, the exposure to IR has been associated with gene mutations, amplification of genetic material, chromosomal rearrangements and increased frequencies of apoptosis, chromosomal aberrations (CAs), micronuclei and sister chromatid exchanges (SCEs) (Lazutka et al., 1999; Limoli et al., 2000; Engin et al., 2005; Dias et al., 2007; Sari-Minodier et al., 2007; Sakly et al., 2012a,b). These genotoxic effects of the radiations would result in each individual in the induction of a carcinogenic process (Breimer, 1988; Overbeek et al., 1999).

Monitoring of personnel occupationally exposed to IR consists of regular film dosimetric control. Nevertheless, this physical control makes it difficult to calculate the effective doses and to determine which are the risks of whole-body radiation exposure. Moreover, personal dosimeter may underestimate the real exposure, not only because of the detection threshold of dosimeters but also because of improper wearing (Zakeri and Hirobe, 2010). In this scenario, the additional information obtained with cytogenetic studies, complement physical dosimetry data and enables better evaluation of the radiation health effects.

It is known that cancer incidence among healthy individuals of a population increases with increased levels of CAs in their circulating lymphocytes (Bonassi et al., 2000, 2004). Because an increased frequency of CAs was found to be a sensitive indicator of exposure to IR (Carrano and Natarajan, 1988), the CAs assay has been applied to monitor workers professionally exposed to low but cumulative levels of IR (Lazutka et al., 1999; Zakeri and Hirobe, 2010; Bonassi et al., 1997; Maffei et al., 2004; Ballardin et al., 2007).

Analogously to CAs assay, the SCEs analysis is widely used to assess the genomic damage, because it represents a sensitive method for identifying physical DNA-damaging agents that directly induce DNA strand breaks (Natarajan and Mullenders, 1987).

The objective of the present investigation was to evaluate the frequencies of CAs and SCEs in a sample of hospital radiology technicians chronically exposed to low doses of IR, in comparison with matched control individuals. Radiology technicians were chosen as study group because they represent one of the most IR exposed hospital workers categories. Indeed, although over the recent decades the exposures of workers in conventional radiology are generally well controlled as a result of efficient protection policy among radiologists, radiology technicians and interventional physicians, such as cardiologists, are the worker categories that receive some of the highest radiation doses in the medical sector (UNSCEAR, 2008; Ballardin et al., 2007; National Research Council BEIR V, 1990).

Effects of donor age, gender and years of exposure on CAs and SCEs frequencies were also evaluated. Finally, because cigarette smoking was found to affect both the radiosensitivity of the cells (Wang et al., 2000) and the fidelity of DNA repair systems (Au et al., 1998), in our study we recruited only non-smokers subjects.

2. Materials and methods

2.1. Study population

The exposed group comprised 21 technicians (13 males and 8 females) working in a hospital radiology unit and chronically exposed to low doses of IR (≤20 mSv/year, as established by the Italian law). Their occupational exposure to X-rays ranged from 1 to 29 years (mean ± S.D., 9.714 ± 8.730). The control group consisted of 21 unexposed healthy individuals (13 males and 8 females) belonging to the administrative staff, and working in the same hospital without any work-related exposure to hazardous agents and with no history of radiation exposure. All the subjects of both groups lived in the same urban area. The procedures followed in this work were approved by the local responsible committee on human experimentation and have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

All the participants were healthy volunteers that received information about the study and that gave their written consent. Prior to blood collection, each individual was extensively interviewed by a specialized physician who filled in a structured questionnaire specifying gender, date of birth, smoking status, dietary habits, alcohol consumption, work-related exposure to hazardous agents, previous exposure to diagnostic X-ray as a patient, and use of therapeutic drugs. In our sample we exclusively considered individuals who neither smoke nor consume drugs or alcohol, and who were not exposed to X-ray for medical treatments, for a period of at least two years prior to the analysis.

We do not have quantitative data about the exact radiation doses received by the workers. Nevertheless, when interviewed, technicians confirmed that their activity conforms to the radiation protection procedures as established by the Italian law, and that their exposure to IR was below the permitted levels (<20 mSv per year). In this sense, results of this study must be considered as the effect of their prolonged exposition to low levels of IR during their work activity.

2.2. Blood sample collection

Blood samples were obtained by venipuncture (5–10 mL) and collected into heparinised tubes, for genotoxicity testing. All blood samples were coded, cooled (4 °C), and processed within 2 h after collection. Heparinized venous blood (0.3 mL) was cultured in 25 cm^2 flasks in 6 mL RPMI-1640 (Biological Industries) supplemented with 20% fetal calf serum (FCS), 2% of the mitogenic agent phytohemagglutinin-M (PHA, Difco, 0.2 mL), L-glutamine (2 mM), antibiotics (100 IU/mL penicillin, and 100 μg/mL streptomycin). The cultures were incubated for 48 h for CAs assay and 72 h for SCEs assay, at 37 °C in an atmosphere of 5% CO_2 in the air. To arrest cells in mitosis, colchicine (Sigma, 0.25 μg/mL) was added at a concentration of 0.06 μg/mL during the last 2 h of culture. Chromosome preparation was done following standard procedures. Cells were centrifuged (≤800 rpm for 5–10 min), slowly resuspended in 10 mL of pre-warmed hypotonic solution (0.075 M KCl, pre-warmed to 37 °C), and incubated for 15 min in a 37 °C water bath. The cells were centrifuged again and fixed in cold

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