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Entrance surface dose and induced DNA damage in blood lymphocytes of patients exposed to low-dose and low-dose-rate X-irradiation during diagnostic and therapeutic interventional radiology procedures

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ABSTRACT

The ionizing radiation received by patients and health workers due to radiological imaging may increase the risks of radiation effects, such as cancer and cataracts. We have investigated the dose received by specific areas around the head and related this to DNA damage in the blood lymphocytes of subjects exposed to interventional imaging. The entrance surface doses (ESD) to the forehead, neck, and shoulder were measured with a thermoluminescence dosimeter ($CaSO_4$ disc or polycrystalline powder of lithium tetraborate doped with Mn) and compared with that of dose area product (DAP). DNA damage was measured by γ-H2AX, $p53^{ser15}$, chromosomal aberration (CA), and micronucleus (MN) assays in lymphocytes of patients (n = 75), before and 2 and 24 h after exposure. The measured ESD values were 230.5 ± 4.9, 189.5 ± 3.55 and 90.7 ± 3.4 mGy for the forehead, neck, and shoulder, respectively. The DAP varied from 1.8 to 2047 Gy*cm^2, showing a correlation with fluoroscopy time (r = 0.417). Received doses did not increase early markers of DNA damage (γ-H2AX and $p53^{ser15}$ assays), but residual damage (CA and MN frequencies) showed a significant ($p < 0.001$) increase at 2 and 24 h post-exposure compared to pre-imaging, despite poor correlation with DAP (r = 0.1). Our results show that interventional imaging procedures deliver significant radiation doses and induce measurable DNA damage in lymphocytes of subjects, highlighting the need for rigorous patient safety protocols.

1. Introduction

Interventional radiology is an important imaging modality for early detection and treatment of complex diseases, with advantages such as non-invasiveness, timeliness, reliability, and minimum pain to the patient [1]. However, these procedures result in significant radiation doses to the individuals undergoing imaging [2,3] as well as to the general population [4]. For interventionists, these procedures present a risk of occupational health hazards, including cataract formation [5]. There has been concern over recent proposals to minimize doses to the lens of the eye during interventional radiology and cardiology, as this will require modifications of work practices and, in some cases, reductions in the numbers of procedures that an individual can perform [6–10]. Although occupational exposures to healthcare workers are regulated and monitored to ensure their safety, it is not routine practice to monitor exposures of patients, since the benefits of diagnosis and treatment presumably outweigh any associated risks [11]. However, the dose delivered to the patient is unavoidable, and its magnitude depends on many radiographic factors, such as inadequate beam filtration, increased image intensifier formats, use of continuous fluoroscopy, current, frames per second used, tube voltage, repeated views for accurate diagnosis, experience, the skill of the doctor, and confounding factors, such as the region of the body being exposed, radiosensitivity of the organ, and the age, height, and weight of the patient [12].

Mettler et al. emphasized that, apart from effective dose, absorbed organ doses are a vital quantity for measurement, since they consider high doses or sensitive tissues in the field of the primary beam [4].

Abbreviations: CA, chromosome aberrations; ESD, entrance surface dose; DAP, dose area product; DAPI, 4', 6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; ICRP, International Commission for Radiation Protection; MFI, mean fluorescence intensity; MN, micronucleus; PBL, peripheral blood lymphocytes

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Consistent with the literature [13–15], the authors of this paper have previously demonstrated that doses received during interventional radiology imaging induce significant DNA damage in the blood of patients [16] and healthcare professionals [17]. However, biological monitoring of patients undergoing interventional radiology and understanding of the relationship between DNA damage and effective dose are inadequate [18]. In the present study, we measured the entrance surface dose (ESD) around the regions of forehead, neck, and shoulder during diagnostic (angiography) and therapeutic (embolization and stenting) interventional procedures and relate this value to the registered dose area product (DAP) and to early and late DNA damage, measured in the blood lymphocytes of patients exposed to low dose of x-rays.

2. Materials and methods

2.1. Study population

The study population consisted of subjects (n = 75) who underwent interventional radiology imaging in the Department of Neuro-interventional Radiology, Sri Ramachandra University. The study protocol was approved by the Institutional Ethics Committee (IEC-NI/09/DEC/13/42). Pregnant women, subjects with family history of known genetic or DNA repair syndromes/disorders, as well as those with previous exposure to medical/occupational radiation were excluded. With informed consent, blood samples (3 ml) were collected from each study subject in sterile heparinized vacutainers, at three time points, before and after imaging: first, before the imaging procedure; second and third, 2–3 h and 24–26 h after the procedure. All blood samples were processed for γ-H2AX foci formation, expression of $p53^{ser15}$, chromosome aberrations (CA) and micronuclei (MN).

2.2. Dose area product (DAP) and measured ESD

The study subjects DAP values were derived from the built-in DAP meter present in the imaging system [Philips Allura x-per FD 20-20 biplane system auto exposure]. The system was calibrated as per the manufacturer's protocols. CaSO4: Dy disc or polycrystalline powder ($Li_2B_4O_7$: Mn, 50 mg) was measured and sealed into 1 × 1 cm packets, labeled, and placed on the forehead, neck, and shoulder regions of the subjects (Fig. 1) who underwent interventional procedures, to quantify the individual ESD to those regions [19]. After the procedure, the thermoluminescence dosimeters (TLD) were carefully collected and the dose was derived using a RISO TL/OSL reader model DA-20, as detailed elsewhere [20]. For each subject, the TL material was read three times, and the mean was taken as the final dose. According to the standardized protocol, the published calibration factor for the TL material was calculated by exposing the TL powder using 662 keV gamma rays emitted by a ^{137}Cs source.

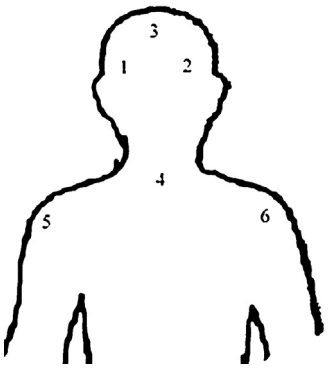

Fig. 1. Placement of TLD on patients during interventional radiological procedure.

2.3. Early DNA damage markers: γ-H2AX and $p53^{ser15}$

Blood (approximately 2 ml) was used to isolate lymphocytes using Ficoll Histopaque 1077 density gradient separation; lymphocytes were washed twice with phosphate-buffered saline and processed for γ-H2AX and $p53^{ser15}$ measurements, as described earlier, with slight modifications [21–23]. Briefly, cells were fixed with 2% para-formaldehyde, permeabilized using 0.25% Triton X-100, and blocked with 2% bovine serum albumin. The lymphocytes were divided into two aliquots; one was stained with primary antibody (H2AX, Millipore; USA, Cat-05-636) and the second with antibody to $p53^{ser15}$ (Abcam; UK, Cat-ab1431) for 2 h, at room temperature. After incubation, excess antibody was washed away with PBS, and the cells were stained with secondary antibody for γ-H2AX (Millipore; USA, Cat-AP124F-2 mg) and $p53^{ser15}$ (Abcam; UK, Cat-ab6717, conjugated with FITC) at room temperature for 1 h. The cell pellets were re-suspended in Roswell Park Memorial Institute medium (RPMI 1640, 1 ml) and analyzed for γ-H2AX and $p53^{ser15}$ using FACS caliber (Becton Dickinson, USA). Debris and cell aggregates were excluded by gating in forward scatter and side scatter plot. A minimum of 10,000 stained cells were acquired and the γ-H2AX and $p53^{ser15}$ fluorescence intensity was displayed as an FL1 histogram and analyzed as mean fluorescence intensity (MFI), using Cell Quest software. The average pre-exposure MFI was compared to that obtained post-exposure. γ-H2AX foci frequency was also compared between samples obtained before and after exposure. For this purpose, a fraction of the cells stained with γ-H2AX were placed on glass slide, counterstained with DAPI, and sealed with a coverslip. Then the slides were used to calculate the foci frequency by counting (~100 cells) using epi-fluorescence microscopy (Olympus, Japan, BX60) with UV and green filters [24].

2.4. CA and MN assays

Blood (approximately 1 ml) was cultured in duplicates with 8 ml RPMI 1640 medium and 2 ml fetal bovine serum supplemented with 400 µl phytoheamagglutinin (1 mg/ml) at 37 °C in 5% CO_2 atmosphere. In one set of cultures, the cells were arrested at metaphase stage by adding colchicine (20 ng/ml) at 24 h and harvested at 48 h, using hypotonic solution (75 mM KCl), and fixed and washed twice with Carnoy's fixative. The cells were placed on a pre-chilled glass slide, air dried, stained with Giemsa and scored (500 metaphases per sample) for CA (both chromatid and chromosome types, including dicentrics) [25].

To another set of cultures, cytochalasin B (6 µg/ml) was added at 44 h, harvested at 72 h, and fixed and stained, similarly to metaphase chromosome preparation. The slides were coded and stained; binucleated cells (1000 cells per sample) were scored for MN using a microscope at 100 X magnification [25]. The mean aberration frequency (with standard error) was calculated.

2.5. Statistical analysis

The mean aberration frequency was calculated from the ratio of number of aberrations (CA and MN) to total cells scored, and mean fluorescence intensity (MFI) of γ-H2AX and $p53^{ser15}$ from the samples obtained before and after exposure, for each donor. Student's *t*-test was performed to assess significance of differences pre-vs. post-exposure; ANOVA was used to compare the aberration frequencies obtained among the three time points. Pearson's correlation analysis was performed between fluoroscopic time and effective dose, ESD, as well as for CA and MN.

3. Results

3.1. ESD and DAP of individual subjects

The demographic details of the study subjects are summarized in