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Endothelial Progenitor Cells in Peripheral Blood of Cardiac Catheterization Personnel

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Abstract

Background: The aim of the present study was to evaluate the rejuvenation capacity among cardiac catheterization technicians occupationally exposed to ionizing radiation.

Subjects and methods: The individual annual collective dose information was measured by thermoluminscent personal dosimeters (TLD) for those technicians and found to be ranging between 2.16 and 8.44 mSv/y. Venous blood samples were obtained from 30 cardiac catheterization technicians exposed to X-ray during fluoroscopy procedures at the National Heart Institute in Embaba. The control group involved 25 persons not exposed to ionizing radiation and not working in hospitals in addition to 20 persons not exposed to ionizing radiation and working in hospitals. Blood samples were assayed for total and differential blood counts, micronucleus formation (FMN) plasma stromal derived growth factor-1α (SDF-1 α) and cell phenotype of circulating endothelial progenitor cells (EPCs), whose surface markers were identified as the CD34, CD133 and kinase domain receptors (KDR).

Results: SDF-1α (2650± 270 *vs.* 2170 ± 430 pg/ml) and FMN (19.9 ± 5.5 *vs.* 2.8 ± 1.4/1000 cells) were significantly higher among cardiac catheterization staff compared to those of the controls respectively. Similarly, EPCs: CD34 (53 ± 3.9 *vs.* 48 ± 8.5/10^5 mononuclear cells), CD133 (62.4 ± 4.8 *vs.* 54.2 ± 10.6 /10^5 mononuclear cells) KDR (52.7 ± 10.6 *vs.*43.5± 8.2 /10^5 mononuclear cells) were also significantly higher among cardiac catheterization staff compared to the values of controls respectively. Smoking seemed to have a positive effect on the FMN and SDF-1 but had a negative effect on EPCs. It was found that among cardiac catheterization staff, the numbers of circulating progenitor cells had increased and accordingly there was an increased capacity for tissue repair.

Conclusion: In conclusion, the present work shows that occupational exposure to radiation, well within permissible levels, leaves a genetic mark on the somatic DNA of the cardiac catheterization technician. On the other hand, exposure of workers to ionizing radiation stimulates regenerative processes as indicated by the increase in EPCs numbers and SDF-1 levels. This regenerative process is decreased by smoking as evidenced by increased levels of SDF-1 and decreased numbers of EPCs. The technicians who work in cardiac catheterization laboratories should therefore carefully follow radiation protection procedures and should minimize radiation exposure to avoid possible genotoxic effects.

Key words: Cardiac catheterization personnel, Ionizing radiation, Smoking, Endothelial progenitor cells, Stromal Derived Factor-1 α.

Introduction

The advent of complex and prolonged coronary interventional procedures has increased levels of radiation exposure among cardiac catheterization working staff (1, 2), who due to close contact with patients, have the highest potential risk of receiving a long-term exposure to low levels of ionizing radiation that greatly increase the risk of health hazards (3, 4). Although a causative link has long been established between exposure to ionizing radiation and the risk of mortality from many forms of cancer (5), recently there is emerging evidence of excess risk of cardiovascular disease at much lower radiation doses (6) and occurring a long time after radiation exposure (7, 8) and in various occupationally-exposed groups (9-13).

Atherosclerosis is the most common pathological process that leads to coronary heart disease and stroke. It is a disease of large and medium-sized arteries that is characterized by the formation of atherosclerotic plaques consisting of necrotic cores, calcified regions, accumulated modified lipids, and inflamed smooth muscle cells (SMCs), endothelial cells (ECs), leukocytes, and foam cells (14). Though previously initiation of atherosclerosis was attributed mainly to lipid accumulation within the arterial walls, it is now widely accepted that inflammation plays a vital role in the

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initiation and progression of the disease [15-17]. Elevated levels of the pro-inflammatory cytokines IL-6, CRP, TNF-α and INF-γ and also increased levels of the anti-inflammatory cytokine IL-10 have been observed in the Japanese atomic bomb survivors who had been exposed to high levels of ionizing radiation [18-20]. Recent studies have identified populations of multipotent progenitor cells [21], immature hematopoietic endothelial cells and adult stem cells called endothelial progenitor cells (EPCs) that circulate in peripheral blood [22]. EPCs counteract ongoing risk factor-induced endothelial cell injury, and in response to acute hypoxia are mobilized from bone marrow (BM) to the peripheral blood and participate in endothelial cell repair and regeneration and also in tissue neovascularization [23]. Experimental and human studies have shown that EPCs participate in the neovascularization processes in ischemic organs [24, 25]. Increased cardiovascular risk factors and the presence of atherosclerosis are associated with dysfunction and reduced numbers of EPCs [25, 26]. Moreover, a low number of EPCs is an independent risk factor for future cardiovascular events [27, 28]. Recruitment of EPCs from remote locations such as the bone marrow into ischemic areas is promoted by the chemokine SDF-1 α [29, 30], which has been shown to be up regulated in many damaged tissues as part of the injury response [31] and subsequently contributes to ischemic neovascularization *in vivo* by augmenting EPCs recruitment to ischemic sites [32].

Until now, no study has investigated EPCs numbers or SDF-1 levels in the blood of cardiac catheterization technicians exposed to radiation. Thus the aim of the present study is to investigate EPCs and plasma SDF-1 in the peripheral blood of those technicians and find out if such cells are mobilized due to radiation exposure. Simultaneously, the frequency of occurrence of micronuclei (MNs) in dividing cells was also estimated in this study. These micronuclei which originate from chromosome breaks or whole chromosomes that fail to engage with the mitotic spindle when the cell divides [33], have recently been endorsed by the International Atomic Energy Agency as one of the main cytogenetic methods of assessing chromosome damage after radiation accidents and as a biological dosimeter of radiation exposure [33-37]. Since DNA is considered to be the main initiating event by which radiation damage to cells results in development of cancer and hereditary disease, the present study also assessed the effects of chronic low-dose X-ray radiation exposure on the MN frequency in interventional cardiac catheterization technicians working in two high-volume cardiac catheterization laboratories.

Subjects and Methods:

Radiation technicians:

Venous blood samples were obtained from 30 cardiac catheterization male physicians and technicians exposed to X-rays during fluoroscopy procedures at the National Heart Institute in Embaba. The mean age was 42.8 ± 5.2 and the period of occupational exposure was 16.32 ± 5.7 years. All participants were subjected to medical examination and underwent routine haematological tests and biochemical investigations to evaluate their state of health. The study cases were free from any clinical symptoms during the preceding three months and had no medical complaints.

No deviations in the basic laboratory tests, no infections were observed during the last three months before the study and no acute or chronic diseases were diagnosed in the participating subjects. Their economic and social statuses were nearly similar. The groups of professionals were exposed to X-rays emitted from catheterization apparatuses during operations.

The control group:

Controls included 25 males (mean of age 42 ±. 4.8) not exposed to ionizing radiation and not working at hospitals in addition to 20 males (mean of age 41.2 ±. 6.9) not exposed to ionizing radiation and working at hospitals. They were non-smokers with no past history of exposure to ionizing radiations or chemicals. They had no negative experience of recent allergic responses or drug administration during the last three months and they were in a good state of health.

Description of the X-ray Systems in use:

All studies were performed in cardiac catheterization units using Integris BH5000 biplane X-ray system (Philips) consisting of a frontal Poly Diagnost C2 and a lateral L-arc 2 U. Tube settings such as peak voltage and anode current were controlled by the automatic brightness control. Pulsed fluoroscopy (12.5 frames per second) and cineangiography (25 frames per second) were used. For fluoroscopy, 2 X-ray beam filtrations

were available. The standard setting consisted of a filtration of 1.5 mm Al, combined with 0.2 mm Cu. The low-dose fluoroscopy setting had an extra filtration of 0.2 mm Cu. The half-value layers of the X-ray tubes for both fluoroscopy settings were measured at 80 kVp with a NE2571 Farmer ionization chamber (Thermo Electron, UK). For both tubes, values of 6.2 and 7.3 mm Al were obtained for the standard and low-dose fluoroscopy settings, respectively.

Radiation dose measurements:

The Entrance Surface Dose (ESD) for technicians undergoing diagnostic heart catheterization and angiocardiography were measured using thermo-luminescence dosometer (Harshow TLD-100 LiF:Mg, Ti, Harshow chemical, Solon, USA). The TLD card containing two TLD-100 chips (lithium fluoride) were mounted between Teflon sheets on aluminum substrates. The TLD system was calibrated to determine the reader calibration factor that is used in evaluating the effective dose. The TLD cards were treated at preheat temperature of 150ºC for 5s at a heating rate of 15ºC/s up to a maximum temperature of 300ºC within reading time of 13.3s.

Cytokinesis block MN assay:

Two separate cultures from each sample were set up by mixing 0.3 ml of whole blood with 4.7 ml of RPMI 1640 medium; cultures were incubated at 37°C and 5 % CO_2 for 72 h. Cytochalasin B (6 µg/ml) was added 44 h after culture initiation. Cells were then harvested and fixed according to the standard methods in use in the laboratory [(38)]. For each sample, 1000 binucleated cells were scored by use of an optical microscope (final magnification: x 400) for MN analysis, the criteria for MN acceptance being followed [(39)]. The micronucleated - binucleated cell frequency was quantified as the number of micronucleated cells per 1000 cells.

Quantification of Vasoendothelial Growth Factor (VEGF) and SDF-1α by ELISA:

The plasma levels of SDF-1α and VEGF were measured using a sandwich ELISA according to the manufacturer's instructions (R&D Systems). Absorbance at 450 nm was determined by an automated ELISA reader (Dynatech MR5000). The results were expressed in pg/ml.

Flow cytometry for Circulating Progenitor cells:

To quantify EPCs in the circulation, peripheral mononuclear cells were first isolated from the EDTA blood samples by Ficoll density-gradient centrifugation (Biochrom AG - Germany). The isolated cells were collected and labeled with the R-phycoenythrin (PE)-conjugated CD133 antibody (MACS Milteny Biotech), Fluorescein isothiocyanate (FITC)-conjugated CD34 (MACS Milteny Biotech), and Allophycocyanin (APC)-conjugated KDR (R & D Systems). The stained cells were washed with PBS/BSA and then EPCs number was determined by fluorescence-activated cell sorting (FACS) analysis (40). Data was expressed number of cells per 10^5 mononuclear cells.

Statistical Analysis:

Statistical analysis of all experimental data was performed and expressed as mean ± SD. Comparisons were made by Student's *t*-test using Statistica v.10 software program for Windows.

Results

Annual Radiation Dose:

Radiation exposure doses were measured using TLD worn under the lead apron on the chest of cardiac catheterization staff and they ranged between 2.16 and 8.44 mSv/year.

Biochemical assays:

SDF-1α (2650± 270 *vs.* 2170 ± 430 pg/ml) and FMN (19.9 ± 5.5 *vs.* 2.8 ± 1.4 per 1000 cells) were significantly higher among cardiac catheterization staff compared to controls respectively (table 2 & figure1). A significant increase in cells expressing CD 34 (53 ±3.9 *vs.* 48 ± 4.5cells/10^5 mononuclear cell), CD 133 (62.4 ± 4.8 *vs.* 52.7 ± 10.6 cells/10^5 mononuclear cell) and KDR cell numbers (48.7 ± 12.5*vs.* 43.5 ± 8.2 cells/10^5 mononuclear cell) was observed in cardiac catheterization staff compared to those of the controls (table 3 & figure 1). SDF-1α (2850 ± 250 *vs.* 2465 ± 290 pg/ml) and FMN (24.8 ± 4.5 *vs.* 13.4 ± 6.4 per 1000 cells) were significantly higher among cardiac catheterization staff who smoked compared to the non-smoking staff (table 4 & figure 2). A significant decrease in cells expressing CD 34 (37.8 ± 1.2 *vs.* 68.2 ± 6.4 cells/10^5 mononuclear cell), CD 133 (49.7 ± 3.8 *vs.* 74.5 ± 5.7 cells/10^5 mononuclear cell) and KDR cell numbers (41.5 ± 8.8 *vs.* 63.4 ± 12.4 cells/10^5 mononuclear cell) was observed in cardiac catheterization staff who smoked compared to those of the non-smoking staff (table 4 & figure 2).

Table 1: Characteristics of the study population:

	CC Staff	Controls
Mean Age	42.8 ± 5.2	42 ±. 4.8
Duration of occupational exposure (years)	15.8 ± 6.5	Nil
Smoking habits Smokers Non-smokers	 12 18	 Nil
Dose Range	2.16 – 8.44 mSv/y	Nil

Table 2: Plasma SDF-1α and frequency of micronuclei in circulating lymphocytes among cardiac catheterization staff compared to controls

	SDF-1 pg/ml	FMN/1000 cells
CC Staff (n=30)	2650± 270	19.9 ± 5.5
Control subjects (n=45)	2170 ± 430	2.8 ± 1.4
P value	p<0.05	p<0.001

*Each value represents mean ± standard deviation (SD).

Fig. 1: Plasma SDF-1α, frequency of micronuclei per 1000 cells and endothelial progenitor cell surface markers per 10^5 mononuclear cells in the circulating lymphocytes among cardiac catheterization staff compared to controls.

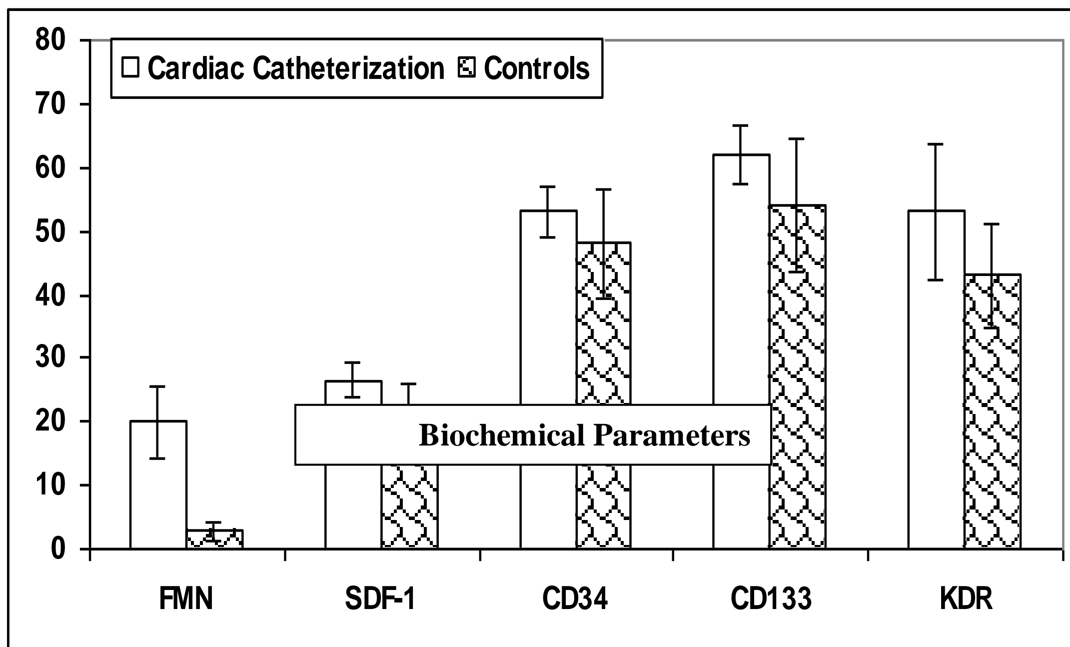


Table 3: Endothelial progenitor cell surface markers per 10^5 mononuclear cells in the blood of cardiac catheterization staff compared to controls

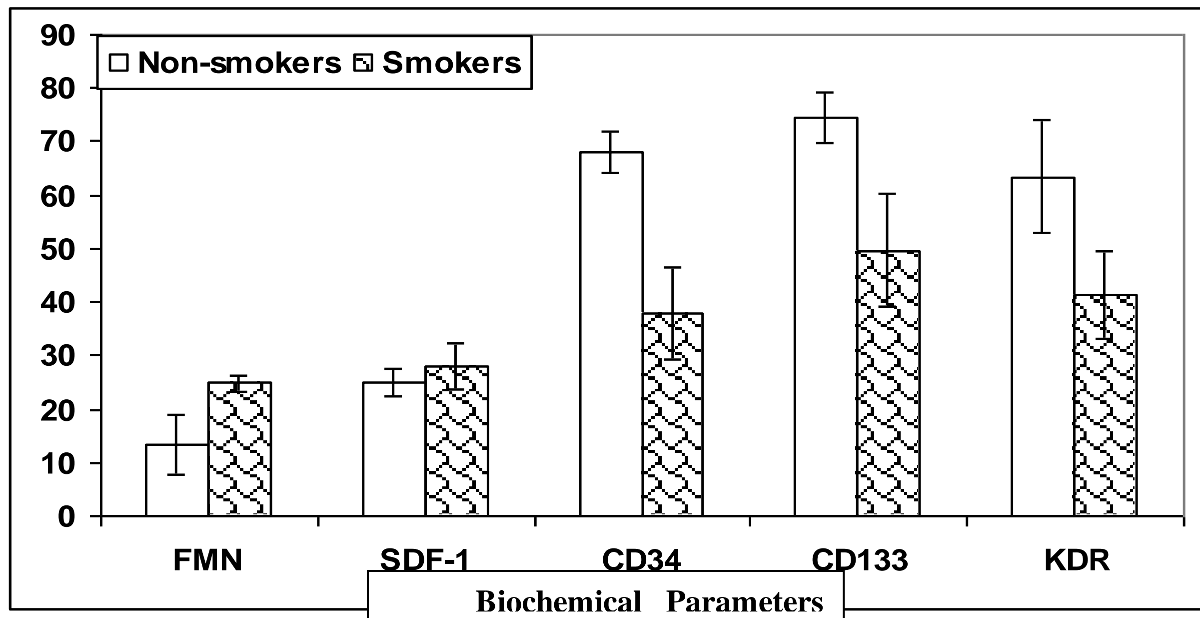
	CD34	CD133	KDR
CC Staff (n=30)	53 ± 3.9	62.4 ± 4.8	52.7 ± 10.6
Control (n=45)	48 ± 8.5	54.2 ± 10.6	43.5 ± 8.2
P value	p<0.001	p<0.001	p<0.001

*Each value represents mean ± standard deviation (SD).

Table 4: Smoking Effect on SDF-1α, frequency of micronuclei in circulating lymphocytes and endothelial progenitor cell surface markers per 10^5 mononuclear cells in the blood of cardiac catheterization staff compared to controls
***Each value represents mean ± standard deviation (SD).**

	SDF-1 pg/ml	FMN/1000 cells	CD34	CD133	KDR
Smoking CC Staff (n=12)	2850 ± 250	24.8 ± 4.5	37.8 ± 1.2	49.7 ± 3.8	41.5 ± 8.8
Non-Smoking CC Staff (n=18)	2465 ± 290	13.4 ± 6.4	68.2 ± 6.4	74.5 ± 5.7	63.4 ± 12.4
	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001

Figure 2: Smoking effect on SDF-1α, frequency of micronuclei in circulating lymphocytes and endothelial progenitor cell surface markers per 10^5 mononuclear cells in the blood of cardiac catheterization staff who smoked compared to non-smoking staff.



Discussion

The intention of the present study was to find out whether, in spite of the required protection measures being observed during cardiac catheterization procedures, there is an alteration in endothelial progenitor mononuclear cells and the frequency of cytogenetic damage in the cardiac catheterization staff occupationally exposed to ionizing radiation at Embaba Heart Institute in Cairo. The physical exposure dose was estimated to be 2.16 – 8.44 mSv/year. Those technicians' measurements were within the range of accepted occupational dose limits of exposure to ionizing radiation which are typically monitored and restricted to effective doses of 100 mSv every 5 years (i.e., 20 mSv per year), with a maximum of 50 mSv allowed in any given year [1, 2]. However, these measures are limited due to the fact that the TLD was placed beneath the lead apron on the chest of cardiac catheterization technicians with a high workload. Therefore, the true total exposure level may lead to a significant cumulative dose and radiation risk. Additionally, cardiac catheterization staffs are exposed to scattered radiation which results in non-uniform exposure doses [41]. Cardiac catheterization staffs receive high doses to the head and extremities that may be unshielded, which may increase the cumulative risk [42]. Published reports on the radiation risk of electrophysiological studies focus only on the ablation procedure or the assessment of effective radiation doses and the associated detrimental risks [3, 41, & 42]. Non-shielded parts are exposed such as the head and extremities, which may result in spurious measurements [43,

44)]. Thus, the estimated dosage from each single dosimetry badge for all the enrolled physicians may have some limitations, because some of them may occasionally forget to wear their badges. The dosimeter, when worn correctly under the lead apron, usually yields a reasonable yet non conclusive estimate of total effective dose used. A dosimetry evaluation with multiple badges would be more accurate but certainly less practical [(43)].

Biological dosimetry or biodosimetry, is mainly performed in addition to physical dosimetry, with the aim of individual dose assessment [(45)]. Biological dosimetry, based on the analysis of micronuclei (MN) in the cytokinesis-block micronucleus (CBMN) assay can be used as an alternative method for scoring dicentric chromosomes in the field of radiation protection. [(46)]. Results of the present study showed a significant increase in the frequency of micronuclei (MN) among cardiac catheterization technicians compared to the controls. Several previous *in vivo* studies indicated that chronic low doses of ionizing radiation can lead to significant somatic DNA damage in professionally exposed technicians. Studies carried out on intervention cardiac catheterization technicians in Italy showed significantly increased levels of MN compared to controls [(47, 48)]. These studies related multiple risk alleles of DNA repair genes to inter-individual differences in radiation sensitivity and genetic susceptibility [(48)]. Similar results were indicated by Iranian, Korean, Egyptian and Japanese studies [(49-53)]. A biological dosimeter that measures true cellular injury resulting from radiation could be a more accurate indicator of cancer risk than a physical dosimeter [(6)]. MN frequency has been reported in other major illnesses, including atherosclerosis and neurological diseases [(54, 55)].

Exposure to low dose ionizing radiation, which has been shown to induce apoptosis in macrovascular and microvascular human endothelial cells [(56)] and also to IR-treated peripheral blood mononuclear cells (PBMCs), interferes with endothelial cell viability and proliferative repair capacity [(57)]. Accordingly, the present study was aimed at measuring circulating EPCs numbers and SDF-1 levels, which have recently been established as a specific and sensitive marker of endothelial activation and damage in a variety of vascular disorders [(58, 59)]. SDF-1 in the present study was significantly higher among cardiac catheterization technicians compared to controls. SDF-1 is considered as a part of host defense processes that protect stem cells from DNA-damaging agents including ionizing radiation [(60)]. Radiation has been shown to induce a dose-dependent rise in pro-angiogenic C-X-C chemokine receptor type 4 (CXCR-4). In contrast, angiostatic chemokines and apoptosis are induced at higher (20 Gy) radiation doses [(61)]. SDF-1α

has been shown to be secreted by stromal and endothelial cells of many organs, suggesting that it is a pivotal regulator of trafficking of various types of stem cells in the body necessary for organ/tissue regeneration [(62)]. It is suggested that SDF-1α may be secreted by hematopoietic stem/progenitor cells and be involved in autocrine/paracrine regulation of their development and survival [(63)]. However, because a strong correlation exists between inflammation and tumor progression/metastasis, inflammation-driven expression of SDF-1α may also play an important role in dissemination/metastasis of cancer stem cells [(62)].

The level of EPCs in the present study was significantly higher among cardiac catheterization technicians compared to controls. Animal studies have shown that infra-red (IR) irradiation increases stem cell–active mobilization factors as it activates a novel pathway stimulating EPCs migration directly through the expression of SDF-1α that is independent of hypoxia-inducible factor 1-alpha (HIF-1α) induction [(64)]. It is hypothesized that IR irradiation improves mast cell migration into ischemic tissue and that mast cells express VEGF mRNA [(63)]. Also, overexpression of SDF1α in the peripheral circulation results in the mobilization of subpopulations of hematopoietic cells with repopulating capacity such as progenitor cells and precursor cells [(64)]. Taking into consideration the previous information and the results of the present study that show increased plasma levels of SDF-1α in the examined subjects, it could be concluded that those EPCs are therefore significantly increased.

Smoking significantly decreased levels of circulating EPCs and significantly

increased FMN among cardiac catheterization technicians who smoked compared to non-smokers. *In vitro* [65] and *in vivo* studies [66] support the finding that cigarette smoke is genotoxic. These results are in agreement with previous results presented by Hogsted *et al.* [67] and Stenstrand *et al.* [68] that there is a positive correlation between smoking and increased FMN in human lymphocytes. However, it contradicts Sorsa et al. [69], Migliore *et al.* [70] and Ban *et al.* [71], who reported that smoking does not affect the background MN frequency and X-ray sensitivity as determined with cytokinesis-blocking micronucleus assay. The mechanisms by which cigarette smoke induces genetic toxicity are multiple and well documented. Cigarette smoke is rich in multiple chemicals that directly form adducts with DNA and induce genetic toxicity. Of this coal tar, aldehydes, pyrolizidine products and benzopyrene derivatives present in the smoke induce the production of irritation [65, 66]. These compounds have been shown to directly induce apoptosis *in vitro* experiments [72]. Cigarette smoke, also, directly causes oxidative damage to the lung endothelial cells by inducing an inflammatory response in the tissues and promoting the recruitment of inflammatory-immune cells such as neutrophils [73]. Also, the number and function of peripheral blood lymphocytes are decreased due to cigarette smoke as the latter induces the expression of FAS mechanism [66]. Cigarette smoke also induces the production of oxygen free radicals whose increase in concentration leads to an induction of programmed cell death to the surrounding cells [74, 75].

The results of the present study which show that EPCs populations were significantly decreased among cardiac catheterization who smoked is contradictory to those found by Kondo et al. [76], who observed that in male individuals a sustained increase in the number of EPCs was observed, which was dependent on a cessation of smoking. A further study indicated that the mobilization rate of EPCs was not influenced by smoking [77]. Another study by Hill et al [78], examined the correlation between blood levels of EPC and smoking status, but found no correlation between smoking and EPCs. However, in the present study there is a synergistic effect of ionizing radiation and smoking. Ionizing radiation induces its effects by increasing both oxidative and nitrosative stress [79, 80] and cigarette smoking also causes a similar increase in both nitosative [81] and oxidative stress among smokers [82].

Data concerning levels of SDF-1α and smoking are scarce. The significant increase in SDF-1α among cardiac catheterization staff who smoked indicates that exposure to both smoking and ionizing radiation induces damage, where SDF-1α is increased. However; this damage is not repaired due to a decrease in circulating EPCs.

In conclusion, the present work shows that exposure to radiation, well within permissible levels, leaves a genetic mark on the somatic DNA of cardiac catheterization technicians. However, exposure to ionizing radiation stimulates regenerative processes as indicated by the increase in EPCs numbers and SDF-1α levels. This regenerative process is decreased by smoking as obvious by the increased levels of SDF-1α and decreased levels of EPCs. The personnel who work at cardiac catheterization laboratories should carefully adopt radiation protection procedures and should minimize overexposure to ionizing radiation to avoid possible genotoxic effects. Routine biochemical and hematological investigations should be carried for cardiac catheterization personnel for the early detection of any possible adverse effects of chronic exposure to ionizing radiation.

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