

## Apoptosis, Cytogenetic and Endothelial Progenitor Cells in the Peripheral Blood of Industrial Radiographers

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### Abstract

**Background:** Radioactive sources and fixed or mobile X-ray equipment are used for both process and quality control in the metallurgical and fertilizer industries. Workers in the nuclear industry are a suitable sector of the populace for the direct estimation of radiation effects at low doses as they are typically monitored and restricted to effective doses of 100 mSv every 5 years. A dose-related increased mortality from circulatory diseases has been observed in some studies of nuclear industry workers, but it is unclear whether this reflects a real effect of radiation exposure or a spurious one. The aim of the present study was to detect the circulating endothelial progenitor cells (EPCs) in the peripheral blood and the frequency of micronuclei (FMN) among industrial radiographers occupationally exposed to ionizing radiation at the Steamer's Welding Company and EL Nasr Company for the manufacture of Fertilizers and Chemicals in Suez and Talkha, Egypt.

**Material and Methods:** Venous blood samples were obtained from 30 industrial radiographers exposed to x-rays during industrial procedures vs. 20 persons not exposed to ionizing radiation as control subjects. Blood samples were assayed for total and differential blood counts and cell phenotype of circulating EPCs, whose surface markers were identified as CD34, CD133 and kinase domain receptor (KDR), frequency of chromosomal aberrations (FCA), apoptosis percentage in circulating lymphocytes together with plasma stromal cell derived factor-1 $\alpha$  (SDF-1 $\alpha$ ) and vascular endothelial growth factor (VEGF).

**Results:** The results of this study revealed a significant increase in FCA with respect to total number of dicentrics ( $0.09 \pm 0.03$  vs.  $0.0005 \pm 0.0001$ ) and rings ( $0.01 \pm 0.0012$  vs. 0) together with apoptosis percentage ( $7.3 \pm 2.8$  % vs.  $2.4 \pm 1.5$  %) among industrial radiographers compared to control subjects respectively, indicating radiation exposure among such workers. Also a significant increase was observed in plasma SDF-1 $\alpha$  ( $2750 \pm 370$  vs.  $2270 \pm 430$  pg/ml), VEGF ( $157.9 \pm 16.9$  vs.  $137.5 \pm 12.6$  pg/ml) among industrial radiographers compared to control subjects. Percentage of circulating mononuclear cells expressing CD34 ( $53 \pm 3.9$  vs.  $54.2 \pm 10.6/10^5$  mononuclear cells), CD133 ( $82.4 \pm 4.8$  vs.  $54.2 \pm 10.6/10^5$  mononuclear cells) and KDR ( $48.7 \pm 12.5$  vs.  $43.5 \pm 8.2/10^5$  mononuclear cells) was significantly higher among industrial radiographers compared to control subjects.

**Conclusion:** It is concluded that the industrial radiographers have increased numbers of circulating EPCs and increased levels of SDF-1 and VEGF, which denotes an increased capacity for tissue repair.

**Keywords:** Endothelial progenitor cells, apoptosis, stromal derived factor-1 $\alpha$ , Industrial Radiographers, Ionizing radiation.

### Introduction

Industrial radiography is the process of using either gamma-emitting radionuclide sources or X-ray machines to examine the safety of industrial materials. Industrial radiographers are among the radiation workers who receive the highest individual occupational radiation doses (1, 2). Those workers who are at risk for repeated radiation exposure, 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 (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), there has been emerging evidence of excess risk of cardiovascular disease at much lower radiation doses (6) that occur a long time after radiation exposure (7, 8) and in various occupationally-exposed groups (9-11), although not in all (12).

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, inflamed smooth muscle cells (SMCs), endothelial cells (ECs), leukocytes, and foam cells<sup>(13)</sup>. 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 initiation and progression of the disease<sup>(14-17)</sup>. Elevated levels of the pro-inflammatory cytokines IL-6, CRP, TNF- $\alpha$  and INF- $\gamma$  and also increased levels of the anti-inflammatory cytokine IL-10, have been observed in the Japanese atomic bomb survivors<sup>(18, 19)</sup>.

Recent studies have identified populations of multipotent progenitor cells<sup>(20)</sup> and immature hematopoietic endothelial cells from adult stem cells called endothelial progenitor cells (EPCs) that circulate in peripheral blood<sup>(21)</sup>. EPCs counteract ongoing risk factor-induced endothelial cell injury, and in response to acute hypoxia are mobilized from bone marrow to peripheral blood and participate in endothelial cell repair, regeneration and also in tissue neovascularization<sup>(22)</sup>. Experimental and human studies have shown that EPCs participate in neovascularization processes in ischemic organs<sup>(22, 23)</sup>. Increased cardiovascular risk factors and the presence of atherosclerosis are associated with dysfunction and reduced numbers of EPCs<sup>(24, 25)</sup>. Moreover, a low number of EPCs is an independent risk factor for future cardiovascular events<sup>(26, 27)</sup>. Recruitment of EPCs from remote locations such as the bone marrow into ischemic areas is promoted by the chemokine Stromal derived factor-1 $\alpha$  (SDF-1 $\alpha$ )<sup>(28, 29)</sup>, which has been shown to be up regulated in many damaged tissues as part of the injury response<sup>(30)</sup> and subsequently contributes to ischemic neovascularization *in vivo* by augmenting EPC recruitment to ischemic sites<sup>(31)</sup>.

No study has investigated EPCs nor SDF-1  $\alpha$  in the blood of radiation exposed workers. Thus, the aim of the present study was to investigate EPCs and plasma SDF-1  $\alpha$  in the peripheral blood of workers exposed to radiation, in order to determine if such cells are mobilized due to radiation exposure.

Concurrently, the frequency of occurrence of micronuclei (MNs) in dividing cells, which originate from chromosome breaks or whole chromosomes that fail to engage with the mitotic spindle when the cell divides<sup>(32)</sup> has recently been endorsed by the International Atomic Energy Agency as one of the main cytogenetic methods for assessing chromosome damage after radiation accidents and as a biological dosimeter of radiation exposure<sup>(33-36)</sup>. Since DNA aberration 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 will also assess the effects of chronic low-dose X-ray radiation exposure on the MN frequency in the subjects participating in this study.

### Subjects and Methods

Venous blood samples were obtained from 30 industrial radiographers belonging to the study group going to the medical clinic of the National centre for radiation research and technology. They included 12 technicians working at the Steamer's Welding factory who were exposed to x-ray irradiation and 18 technicians working at El Nasr Company for the production of fertilizers who were exposed to <sup>192</sup>Ir sources and to natural <sup>32</sup>P used in the preparation of these fertilizers. Exposure doses were indicated by radiation protection personnel to be within the normal permissible limits. The mean age was 44.5  $\pm$  5.2 and the period of occupational exposure was 17.32  $\pm$  5.7 years. All participants were subjected to medical examination and underwent routine hematological tests and biochemical investigations to evaluate their state of health. No deviations in the basic laboratory tests, no infections during the last three months before the study and no acute or chronic diseases were found. Their socioeconomic statuses were similar. Subjects who showed any deviation from normal blood counts and biochemical standard values were previously segregated from the study. The study population included non-smoking subjects who had not contracted an infection during the last 3 months and had no medical complaints or clinical symptoms.

The control subjects included 20 healthy non-smoking males not exposed to ionizing radiation whose mean ages were 43.8

± 4.5. Control subjects enlisted for this study also had not contracted any infection during the preceding 3 months and had no medical complaints or clinical symptoms. Their routine laboratory tests were normal.

### The Frequency of Chromosomal Aberrations (CA):

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<sub>2</sub> for 72 hours and colchicine was added (6 µg/ml) 3 hours before the termination of culture. Cells were then harvested and fixed according to the standard method used in the laboratory<sup>(37)</sup>. For each sample, 100 metaphase cells were examined using an optical microscope (magnification ×400)<sup>(38)</sup>.

### Quantification of VEGF and SDF-1α:

The plasma levels of SDF-1α and VEGF were measured using the sandwich ELISA technique according to the instructions of the manufacturer (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 circulation, peripheral mononuclear cells were first isolated from the EDTA blood samples by

Ficoll density-gradient centrifugation (Biochrom AG- Germany). The isolated cells were labeled with the R-phycoerythrin (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 circulating EPC numbers were determined by fluorescence-activated cell sorting (FACS) analysis<sup>(39-40)</sup>. Data expressed the number of EPCs per 10<sup>5</sup> mononuclear cells.

### Apoptosis assessment:

For the evaluation of apoptosis, lymphocytes were isolated using Histopaque-1077 solution, fixed with 70% ethanol for 1 hour and inspected by the fluorescent microscope after being incubated for 15 minutes at 37°C with 10-µg RNA-ase enzyme and stained with 10 µg propidium iodide and fluorescein diacetate<sup>(41)</sup>.

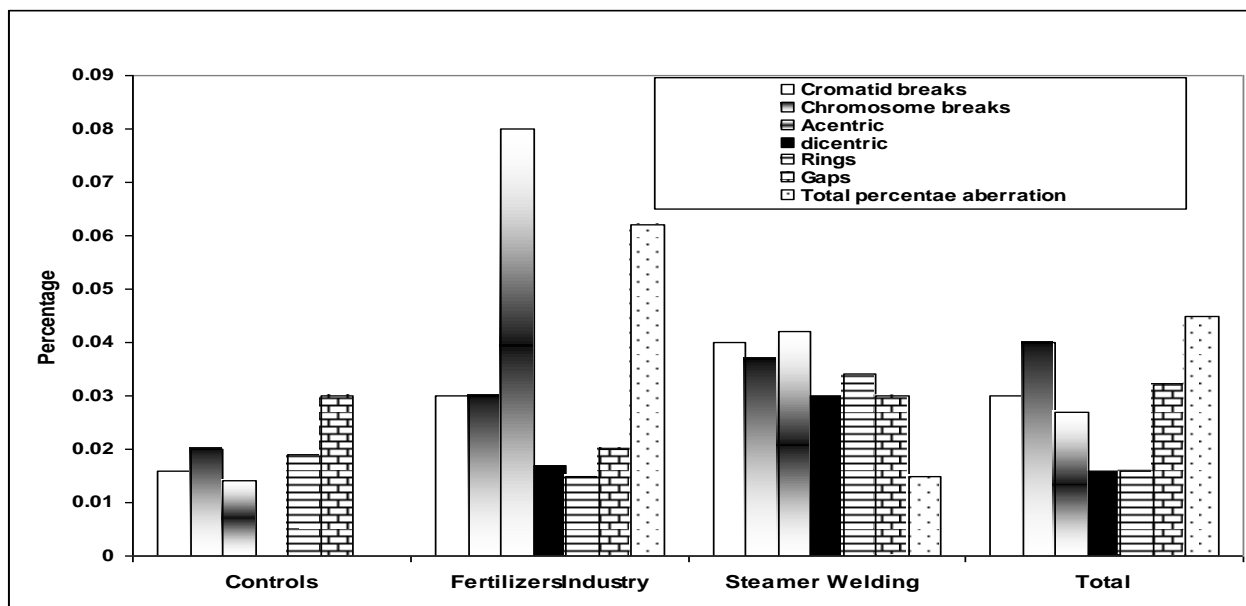
### Results

Results of this study showed an increase in the total percentage of all types and frequencies of chromosomal aberrations, including dicentrics, rings, gaps, acentrics, chromosome and chromatid breaks among both groups of industrial radiographers when compared to the control subjects (table 1 & figures 1 & 2).

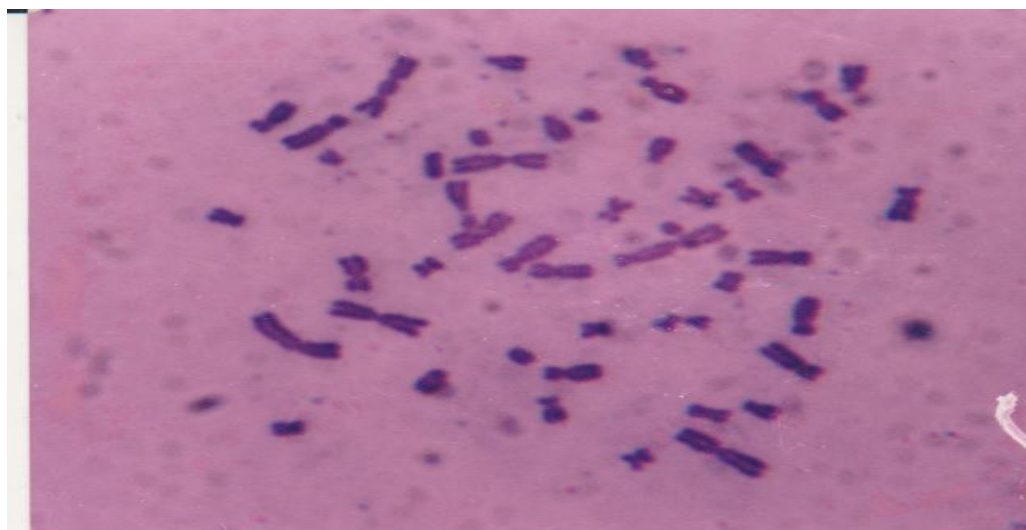
**Table 1: Cytogenetic Analysis of the Frequency of Chromosomal Aberrations.**

Subjects	Chromosome Breaks	Chromatid Breaks	Acentrics	Dicentrics	Rings	Gaps	Total Aberrations
Controls (n=20)	0.015	0.016	0.004	0.0005	0.00	0.015	0.050
Fertilizers manufacture workers (n=18)	0.033	0.038	0.078	0.077	0.0026	0.057	0.286
Steamer's welding factory workers (n=12)	0.28	0.034	0.047	0.0137	0.0075	0.065	0.195
<b>Total</b>	<b>0.328</b>	<b>0.088</b>	<b>0.129</b>	<b>0.09</b>	<b>0.01</b>	<b>0.137</b>	<b>0.531</b>

\*Each value represents the frequency of chromosomal aberrations per 100 cells examined among workers exposed to radiation and the control group.



**Fig (1):** The frequency of chromosomal aberrations (expressed as number of aberrations per 100 cells) among workers exposed to radiation and the control group.



**Fig (2):** A metaphase spread of a lymphocyte from blood of an industrial radiographer showing a dicentric chromosomal aberration.

Stromal cell derived factor-1 $\alpha$  ( $2750 \pm 370$  vs.  $2270 \pm 430$ ), VEGF ( $157.9 \pm 16.9$  vs.  $137.5 \pm 12.6$ ) and apoptosis percentage ( $14.3 \pm 4.8$  vs.  $2.4 \pm 1.5$ ) were significantly higher among industrial radiographers compared to the control subjects (table 2).

**Table 2: Plasma SDF-1 $\alpha$ , VEGF and apoptosis percentage among industrial radiographers compared to control subjects.**

	SDF-1 $\alpha$ (pg/ml)	VEGF (pg/ml)	Apoptosis %
Cases (n=30)	$2750 \pm 370$	$157.9 \pm 16.9$	$7.3 \pm 2.8$ %
Control Subjects (n=20)	$2270 \pm 430$	$137.5 \pm 12.6$	$2.4 \pm 1.5$ %
P value	<b>p&lt;0.05</b>	<b>p&lt;0.001</b>	<b>p&lt;0.0001</b>

\*Each value represents mean  $\pm$  standard deviation (SD).

A significant increase in cells expressing CD 34 ( $53 \pm 3.9$  vs.  $48 \pm 4.5$  cells/ $10^5$  mononuclear cells), CD 133 ( $82.4 \pm 4.8$  vs.  $54.2 \pm 10.6$  cells/ $10^5$  mononuclear cells) and KDR cell numbers ( $48.7 \pm 12.5$  vs.  $43.5 \pm 8.2$  cells/ $10^5$  mononuclear cells) was observed in industrial workers compared to the control subjects (table 3).

**Table 3: Endothelial progenitor cell surface markers per 10<sup>5</sup> mononuclear cells in the blood of industrial radiographers compared to the control subjects.**

	CD34	CD133	KDR
Cases (n=30)	53 ± 3.9	82.4 ± 4.8	48.7 ± 12.5
Control Subjects (n=20)	48 ± 4.5	54.2 ± 10.6	43.5 ± 8.2
P value	p<0.0001	p<0.0001	p<0.01

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

## Discussion

Workers in the nuclear industry are a suitable sector of the populace for the direct estimation of radiation effects at low doses: they form large, relatively stable populations with relatively well-measured and well-recorded external radiation doses. Large studies of combined cohorts of nuclear workers have previously reported no radiation-related excess in mortality from non-cancer diseases in these study groups<sup>(42, 43)</sup>. A dose-related increase in mortality from circulatory diseases has been observed in some studies of nuclear industry workers<sup>(43, 44)</sup> but it is unclear whether this reflects a real effect of radiation exposure or a spurious one<sup>(45)</sup>. Exposure to low dose ionizing radiation, which has been shown to induce apoptosis in macrovascular and microvascular human endothelial cells<sup>(46)</sup> and also infra-red treated peripheral blood mononuclear cells (PBMCs) interfere with endothelial cell viability and proliferative repair capacity<sup>(47)</sup>. Hence, this study was aimed at measuring the levels of some of the markers (recently implicated to be linked to atherosclerosis) among the industrial radiographers included in the study. These markers included circulating EPCs, VEGF and SDF-1  $\alpha$ , which have recently been established as specific and sensitive markers of endothelial activation and damage in a variety of vascular disorders<sup>(48, 49)</sup>.

One of the limits of this study was the unavailability of physical dosimetry. Therefore, biological dosimetry was performed with the aim of individual dose assessment<sup>(50)</sup>. Biological dosimetry is based on the analysis of chromosomal aberrations on the hypothesis that dicentric and rings are indicators of radiation exposure<sup>(51)</sup>. This study revealed that the technicians working with fertilizers exhibited the highest levels of frequency of chromosomal aberrations, where total FCA was 0.286 (range 0.02-0.29);

dicentric were 0.077 (range 0.00-0.1) and rings were 0.0026 (range 0.002- 0.005). Steamer's Welding industry workers showed lower values, where total FCA was 0.195 (range 0.03- 0.20), dicentric were 0.0137 (range 0.00- 0.014) and rings were 0.075 (range 0.00-0.1). This showed that a greater effect of hazardous radiation exposure was demonstrated in the fertilizer industry workers compared to the welding industry workers. The cytogenetic study based on the frequency of chromosomal aberrations also revealed a significant increase in FCA in each of the two groups compared to that showed by the control subjects where dicentric were 0.00051 (range 0.00- 0.001), rings were 0 and total FCA was 0.05 (range 0.00-0.06). This confirms what was previously reported by several authors<sup>(52-54)</sup>, who found a significant incidence of aberrations in workers exposed even to permissible limits of ionizing radiation. Results of the present study show a significant increase in the frequency of chromosomal aberrations among industrial radiographers compared to the control subjects. Several previous *in vivo* studies indicated that chronic low doses of ionizing radiation can lead to significant somatic DNA damage among industrial radiographers as measured using the CBMN assay<sup>(35, 56 and 57)</sup>.

A significant increase in the apoptosis percentage in circulating lymphocytes was observed among both groups of industrial radiographers compared to the control subjects. *In vitro* studies have indicated that radiation-induced apoptosis in human lymphocytes has the kinetics, sensitivity, and reproducibility to be a potential biological dosimeter<sup>(65, 66)</sup>. A study by Ilyenko *et al*<sup>(58)</sup> performed on 83 peripheral blood samples from the Chernobyl clean-up workers, found shorter telomerase lengths in their peripheral blood lymphocytes compared to healthy

controls and this was associated with a higher susceptibility to apoptosis in these workers.

Stromal cell derived factor-1 $\alpha$  in this study was significantly higher amongst industrial radiographers compared to the control subjects. SDF-1  $\alpha$  is considered as a part of host defense processes that protect stem cells from DNA-damaging agents including ionizing radiation <sup>(59)</sup>. Radiation has been shown to induce a dose-dependent increase in pro-angiogenic CXC and CXCR4 chemokines. In contrast, angiostatic chemokines and apoptosis were induced at higher (20 Gy) radiation doses <sup>(60)</sup>. SDF-1  $\alpha$  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 <sup>(60)</sup>. It is suggested that SDF-1  $\alpha$  may be secreted by hematopoietic stem/progenitor cells and be involved in autocrine/paracrine regulation of their development and survival <sup>(62)</sup>. However because a strong correlation exists between inflammation and tumor progression/metastasis, inflammation-driven expression of SDF-1  $\alpha$  may also play an important role in dissemination/metastasis of cancer stem cells <sup>(61)</sup>.

Endothelial progenitor cells in this study were significantly higher among industrial radiographers compared to control subjects. Animal studies have shown that infra-red irradiation increases stem cell active mobilization factors as it activates a novel pathway stimulating EC migration directly through the expression of SDF-1  $\alpha$  independent of HIF-1 $\alpha$  induction <sup>(62)</sup>. It is hypothesized that infra-red irradiation improves mast cell migration into ischemic tissue and that mast cells express VEGF mRNA <sup>(63)</sup>. Also overexpression of SDF1  $\alpha$  in the peripheral circulation results in the mobilization of progenitor and precursor hematopoietic cells with an increased repopulating capacity <sup>(64)</sup>. Taking into consideration the latter information and the results of the present study that show increased plasma levels of SDF-1  $\alpha$ , there is no doubt that those EPCs are subsequently significantly increased.

In conclusion, the present work has showed that occupational exposure to radiation, well within permissible levels,

leaves a genetic mark on the somatic DNA of industrial radiographers. On the other hand, exposure to ionizing radiation stimulates regenerative processes as indicated by the increase in EPCs, VEGF and SDF-1  $\alpha$ . The laborers who work as industrial radiographers should carefully follow radiation protection procedures and should minimize radiation exposure to avoid possible mutagenic effects. Routine biochemical and hematological investigations (including biological dosimetry) as well as nutritional status monitoring (that includes a high protein diet to improve tissue regeneration and antioxidants) ought to be carried out on a regular basis to detect as early as possible any adverse effects of ionizing radiation on the biological systems of the body. Workers affected by ionizing radiation should stay away from work for a specified period and should be monitored for any cardiovascular, acid base balance or nervous system reflexes disturbances. They should be given suitable treatment by the physicians following up their progress.

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