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Does occupational exposure to low ionizing radiation affect endothelium health?

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Abstract. The aim of this study was to assess the effect of low-radiation exposure on detachment of the vascular endothelial cells. Circulating endothelial cells (CEC) were measured in the peripheral blood by an immunomagnetic separation (IMS) technique in 63 workers occupationally exposed to low-level ionizing radiation at the Atomic Energy Commission of Syria and in 28 controls. We found a significant difference in CEC numbers in the peripheral blood of the workers exposed to low-level ionizing radiation in comparison with those of the control samples ($p = 0.0001$) and no significant difference in the proportion of other blood elements in the peripheral circulation. Elevated number of CEC in occupational workers exposed to low doses of ionizing radiation may reflect an early affection of the endothelium. Long-term follow-up of these workers must be conducted to determine the clinical value of CEC as an early indicator of cardiovascular disease induced as a result of chronic occupational exposure to low doses of ionizing radiation.

Key words: circulating endothelial cells (CEC) • occupational exposure • low-dose ionizing radiation

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Introduction

Exposure of individuals to high doses of ionizing radiation (in such cases as the atom bomb survivors, the Chernobyl ‘liquidators’ and radiotherapy patients) is well known to contribute to both acute and chronic health effects [11, 13, 25]. In contrast, the effects of low dose are less well recognized; hence, their characteristics and early recognition are pressing problems in radiation biology. The acute damaging effects of high dose exposures of ionizing radiation on the endothelial cell system (loss of cell-cell integrity, failure of fluid barrier maintenance, cell death without replacement, new phenotype, chronic activation) are well recognized [4, 9, 10, 22]. At low doses, there is no evidence for the development of acute and/or chronic radiation pathology. However, epidemiological studies of cardiovascular morbidity seem to indicate that damage to the vasculature may indeed be a late effect of low-dose irradiation [5, 15].

The classic theory for the onset of delayed radiation damage states that the cell responsible is the endothelial cell. Two reports [7, 17] indicated that the endothelial damage is an important mechanism of early and delayed radiation injury in mammalian tissues. So, the fundamental underlying cause of late effects was dysfunction in the microcirculation. As interest in research on low-level radiation exposures expands [8] there is an increasing need for new biomarkers that can identify the effects on the individual's health with an occupational exposure to low ionizing radiation. The incorporation of cellular biomarkers into epidemiological studies has been grown exponentially during recent years in order to develop new and more effective strategies to reduce the risk, such as exposure monitoring, health surveillance and individual risk characterization.

The circulating endothelial cells (CECs), defined by membrane component CD-146, are reputedly a circulated marker of vascular damage/dysfunction [2]. Elevated CEC levels are well recognized in many affections with prognostic implications for various clinical events, such as cardiovascular disorders, infectious diseases, and various types of cancers [1, 3, 21].

The purpose of this work was to provide data on the endothelial dysfunction due to the occupational exposure to ionizing radiation by a non-invasive biomarker which is the CECs value assed by IMS technique.

Materials and methods

Subjects

The exposed group consisted of sixty-three nuclear workers (53 men – 84%, 10 women – 16%) from the Atomic Energy Commission of Syria (AECS), who had been occupationally exposed to low doses of ionizing radiation for 8 years, on average. A history of cardiovascular (Table 1) disease was reported in 4 workers.

Standardized questionnaire forms were used to collect information from all subjects who agreed to participate in this study. Each of the workers was matched by age, sex and smoking habits with an unexposed individual who lived in the same city and worked in administrative offices. The control group was composed of 28 healthy individuals – 8 women and 20 men from Damascus, who had never been occupationally exposed to radiation and was matched to the exposed subjects with respect to age and sex. The utilization of appropriate controls is essential in this type of study.

There were no reports on radiation exposure in the control group. Individuals (workers and controls) with concurrent infection, medication intake (e.g. aspirin) or medicall X-ray exposure in the last 12 months were excluded in our study. All of them gave written informed consent to participate in this study.

Exposure monitoring

The subjects in the occupational exposure group are all regularly exposed to X- and γ-rays. Their occupational exposure to ionizing radiation is routinely monitored by personal exposure measurement devices (film badges, HARSHAW, TLD 8800) that are read out every three months.

Numeration of circulating endothelial cells

Peripheral blood was drawn in an EDTA tube by venipuncture. All blood samples were treated within twenty-four hours for the isolation and quantification of CECs using a close adaptation of an established previously validated technique [27].

The first 7.5 ml were discarded to prevent contamination of the sample with endothelial cells dislodged during blood drawing. After carefully rotating of the tube, one millilitre (ml) of the blood was diluted 1:3 with isolation buffer (PBS-BSA 0.1% and NaN_3 0.1%) and incubated for ≥ 30 min with 15 μl (7 × 10^7 beads) of a preparation of anti-CD146-coupled magnetic beads (Dynal M450 IgG1, Dynal AS, Oslo, Norway) at 4°C, while gently agitating in a head-over-head mixer. The unbound cells were washed out with buffer, and bound cells were retained on the magnet, after additional wash

Table 1. Demographic and clinical characteristics of nuclear workers occupationally exposed to low-level ionizing radiation and unexposed controls

Sample characteristics	Populations	
	Exposed	Control
Number of individuals	63	28
Exposure time (years, median, range)	8 (1–24)	0
TLD (mSv, median, range)	4 (1.33–23.64)	0
Age (years, mean ± SEM)	39 ± 0.8	35.5 ± 1.5
Male (%)	84.1	71.4
BMI (mean ± SEM)	27.47 ± 0.51	26.92 ± 0.85
Smoking (%)	49.2	39.3
Medical history (number)		
Cardiovascular disease	2	2
Diabetic disease	2	0
Hypertension	6	1
Cancer		
Other	4	1
Treatment (number)		
Statin	0	0
HTA	6	1

Continuous variables are expressed as mean ± standard error of the mean (SEM) and categorical variables are represented by the number and the percentage of workers excepted for those that are expressed as median.

Abbreviations: BMI – the body mass index; HTA – antihypertensive treatment; TLD – thermoluminescent dosimeter.

cycles, the cells were incubated in 100 μl of acridine orange (5 μg/ml PBS, Sigma) for 10 min in the dark. Then, the bound cells were washed and resuspended in 100 μl of the isolation buffer to avoid the background caused by green colouring due to the use of acridine orange.

The isolated CECs were counted in a 0.500 mm. Nageotte chamber cytometer (Hecht-Assistent, Sondheim, Germany) under a fluorescent microscope (Olympus, BX51, Japan) equipped with a 500/20 nm excitation filter. The quality of images was improved by software Deltapix version 1.6 (Deltapix, Måløv, Denmark).

CEC were determined as CD-146 rosetted cells, bearing more than 5 beads with a distinctive cell morphology (diameter of 15–50 μm), and showing green fluorescence due to the presence of the specific endothelial marker, Fig. 1 [2].

This study was conducted in accordance with the declaration of Helsinki and approved by the independent local committee of ethics.

Statistical analysis

The data were categorized and processed using Statistical Package for Social Sciences (SPSS) software package, version 15 or PRISM software (Graph Pad Software Inc., San Diego, CA, USA).

The study populations was grouped according to the presence and absence of occupational exposure to ionizing radiation comparisons between groups were made using the Mann-Whitney U (continous data) and the X^2 test (nominal data). In our second round of analysis, we used Pearson's correlation coefficient to look for a correlation between the variables of CEC vs. the TLD record. Logistic regression analyses were run to identify an independent association between the occupational exposure to ionizing radiation and CEC.

Difference between the variables were considered statistically significant when the p value was < 0.05.

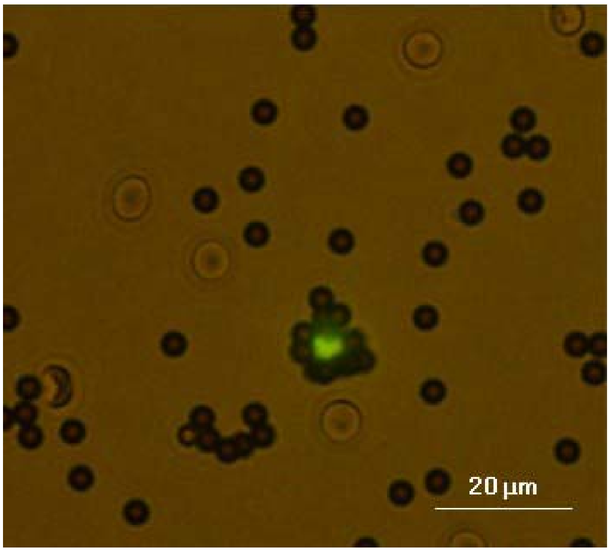

Fig. 1. Morphologic appearance of CECs isolated with immune-magnetic beads inform the blood of nuclear workers occupationally exposed to low-level ionizing radiation. CEC stained with acridine orange, photographed under fluorescent microscope, magnification ×20.

Results

Demographic data

Clinical and biological characteristic of 63 occupational exposure workers (male – 84%, female – 16%) have been illustrated in Table 1. The mean age was 39 years with a median range of (30–45) ± 4. A history of cardiovascular disease was present in 3.18% of workers.

The median TLD measured doses of ionizing radiation among the exposed workers was 4 mSv and ranged from 1.33 to 23.64 mSv; one of them had the recorded doses slightly exceeding the annual limit of 20 mSv. The mean TLD measured doses of radiation did not differ between the never smokers (6.1 ± 4.5 mSv) and current smokers (4.9 ± 3 mSv) in the exposed group ($p = 0.18$).

A detailed description of the study population that include all the informations on cardiovascular risk factors is provided. Socioeconomic status (SES) information was not available; consequently, it was not used in the present analysis.

Effect of exposure to low ionizing radiation on CEC levels

Interestingly, we found a significant elevated CEC number in the peripheral blood of the nuclear workers exposed to low-level of ionizing radiation compared to control subjects with a median of 12 (range 0–68) vs. 5 (range 0–18) respectively, ($p < 0.0001$); see Fig. 2.

However, there was no significant difference in the proportion of other blood elements (erythrocytes, leukocytes, granulocytes and platelets) in the peripheral circulation of the blood in workers exposed to low ionizing radiation compared to those of the healthy controls.

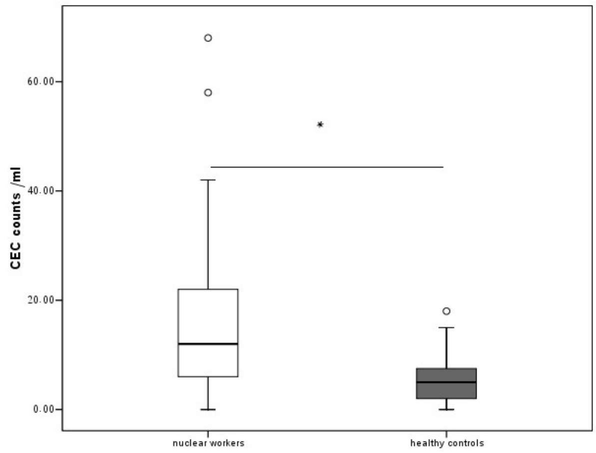

Fig. 2. The bar graphs represent the median values of CEC and range in nuclear workers occupationally exposed to low doses of ionizing radiation (open square, $n = 63$) compared to controls (closed square, $n = 28$). In both group there was an outlier with high CEC levels (circle). Numeration of CEC was performed by the immunomagnetic separation, as described in section 'Materials and methods'. Results are expressed as median and range of CEC number per mL of blood. * Significance of the difference from control in in the group of nuclear workers occupationally exposed to low-level ionizing radiation ($p < 0.0001$).

In addition, no significant correlation has been found between the CEC values and measurements ($p = 0.21$) and the other variables studied in the peripheral circulation of the workers. All the studied variables showed no significant correlation with the TLD or period of occupational exposure to ionizing radiation.

The analysis of the confounding factors that may influence CEC counts (age, sex, BMI, smoking, alcohol intake, duration of employment), did not show any influence on the CEC values in this study.

Discussion

To the best of our knowledge, this is the first study which investigates the effect of an exposure to low doses of ionizing radiation on the endothelium integrity in nuclear workers. The vascular endothelium is believed to be a target for radiation-induced injury [24]. The endothelium is the sentry against cardiovascular disease. Therefore, the risk of developing cardiovascular disease in individuals exposed to radiation from occupational radiation exposure remains a health concern.

We assessed the endothelium damage due to the occupational exposure to low ionizing radiation by measuring CEC levels. Originally, we found an increased CECs levels in the occupationally workers exposed to low ionizing radiation compared to those in healthy controls. Elevated numbers of CECs have been reported in cardiovascular diseases, including artherosclerosis [16] coronary syndrome [14, 20]. Since the number of CECs was found to correlate with other indicators of vascular damage in cardiovascular disorders [14, 16] the measurement of CECs in peripheral blood has been suggested as a potential new non-invasive method for assessing vascular damage.

In recent years, there is growing epidemiological evidence of excess risk of late occurring cardiovascular diseases after exposure to low doses of ionizing radiation [12, 15, 18]. However, the epidemiological findings are not persuasive and knowledge about the underlying biological and molecular mechanisms is needed. An examination of possible biological mechanisms indicates that the most likely causative effect of radiation exposure linked to cardiovascular damage is the damage to endothelial cells and the subsequent induction of an inflammatory response [15]. The exact primary mechanisms by which radiation-induces CEC detachment *in vivo* are not identified. Nevertheless, it seems that endothelial cell damage and/or cell loss after exposure to ionizing radiation is likely to disrupt the anti-inflammatory and antithrombotic mechanisms, thereby promoting tissue damage and fibrosis through inflammatory and thrombotic mechanisms.

In vitro, several studies have demonstrated morphologic and cytosketelal, as well as barrier function changes in endothelial cells derived primarily from animal tissues when these were exposed to ionizing radiation [6, 19, 23, 26]. In the present study, we did not found a statistical significant relationship between the radiation dose and CEC counts, but the small sample size and lack of adjustment of some factors such as diet and SES could have prevented us from finding a significant association. Additional studies are needed to confirm the present findings and demonstrate to what extent the exposure to low dose of ionizing radiation affects the state of endothelium.

Taken together, we can assume that chronic occupational exposure to low doses of ionizing radiation leads to a high number of CEC observed in the examined nuclear workers. Our assumption is supported by the absence of any effect of the factors that usually change the CEC values, but it is unclear whether this reflects a real effect of radiation exposure or is due to some confounding factors, to be identified.

Conclusions

Our report indicates that the monitoring of CEC number using IMS technique, as a non-invasive method, may be used to assess the health of occupationally workers exposed to low ionizing radiation. However, further studies with a higher number of individuals are necessary in order to obtain a more reliable correlation between the CEC levels and exposure to low doses of ionizing radiation.

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