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Hazards of X-Ray Radiation on the Quantitative and Phagocytic Functions of Polymorphonuclear Neutrophils in X-Ray Technicians

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Abstract: Hazards of X-Ray Radiation on the Quantitative and Phagocytic Functions of Polymorphonuclear Neutrophils in X-Ray Technicians: Sultan A MEO, *et al.* Department of Physiology, College of Medicine, King Khalid University Hospital, Saudi Arabia—Over exposure to X-ray radiation is detrimental to the living cells and may lead to development of life-threatening diseases. It is intuitive to postulate that a low level exposure may lead to functional abnormalities in human immune cells. Therefore, the objective of the present study was to study the effects of X-ray radiation on the total leukocyte count (TLC) and phagocytic activity of Polymorphonuclear neutrophils (PMN). A group of 42 apparently healthy X-ray technicians were recruited with age ranging from 25–50 years. They were matched with another group of 42 control healthy volunteer subjects in terms of age, sex and ethnic variation. The mean exposure level of X-ray radiation in X-ray technicians was 72.4 mrem per calendar quarter and 289.6 mrem per year. TLC was performed by using a Beckman Coulter counter and phagocytic activity of whole blood and PMN was determined by measuring chemiluminescence (CL) response with a chemiluminescence luminometer. The mean value of CL response was significantly decreased (p<0.0005) in X-ray technicians, even though they had low levels of exposure, compared to their controls. However, no significant difference was observed in TLC between the two groups at this low level of exposure. Exposure to X-ray radiation decreases the physiological functions of PMN as measured by decreasing chemiluminescence response even at low levels of exposure.
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Radiological investigations are one of the most frequently requested diagnostic tools. In spite of recent technological advances and other laboratory investigations, radiology remains a corner stone of, and has maintained a time-honored position in the diagnosis of different diseases[1)]. X-rays are part of the electromagnetic spectrum and generate radiation. The higher the voltage of the generator, the more penetrating will be the radiation. Penetration of ionizing radiation can destroy the living cells or make them functionally abnormal[2)]. The mechanism behind cell futility due to ionizing radiation is the generation of chemically active free radicals, which damage the molecular structure, resulting in cell dysfunction (somatic effect) or mutations (genetic damage). Workers exposed to X-ray radiations are prone to develop life-threatening diseases related to different organs of the human body including the immune system cells. Phagocytosis is one of the main defense mechanisms by which the immune system protects the body against the infection and is mediated by macrophage and or polymorphonuclear leukocytes[3)].

The mononuclear phagocyte system (MPS) and polymorphonuclear neutrophils (PMN) provide the human body with a powerful defense against various microorganisms. The ability of phagocytes to destroy target pathogens depends on their capacity to generate toxic oxygen products[4)]. The toxic oxygen product includes massive amounts of superoxide anion (O^{-2}), peroxide (H_2O_2) and hydroxyl radicals (OH^-). Superoxide and peroxide may subsequently interact to produce hydroxyl radicals (OH^-) and singlet oxygen (IO_2). In addition, H_2O_2 and Cl^- combine in the presence of myeloperoxide released from the phagosome to produce hypochlorite ($HOCL^-$). The products of oxygen reduction

(O^{-2}, H_2O_2, OH^-) constitute the key components of the oxygen-dependent anti-microbial capacity of the cells to destroy bacteria, fungi and parasites[5–7]. Failure to produce these metabolites results in decreased immunity and increased susceptibility to infection.

The phagocytic function can be impaired directly or indirectly under different conditions including quantitative and/or qualitative defects of neutrophils and monocytes, leukocyte adhesion deficiency syndrome, diabetes mellitus, chronic granulomatous disease (CGD), glucose-6-phosphate dehydrogenase (G6PD) deficiency, myeloperoxidase deficiency, Chediak-Higashi syndrome, lazy leucocyte syndrome and in Jobs syndrome (http://www.jachabacha.com/immunodeficiency.htm)[8]. Keeping in view the hazards of X-ray radiation, it is worthwhile to observe the effects of X-ray radiation on the TLC and phagocytic activity of polymorphonuclear neutrophils (PMN) stimulated with opsonised zymosan (OPZ) and measured by chemiluminescence (CL) response, in X-ray technicians and compare the results with matched controls.

Subjects and Methods

Subjects

In the present study two groups consisted of 42 subjects in each. In the first group 42 apparently healthy consenting X-ray technicians (36 males and 6 females) with ages 25–50 years were selected from different government and private sector hospitals in Riyadh, Saudi Arabia. They were matched with a second group of 42 healthy volunteer control subjects (36 males and 6 females) with the same age group. The control group was recruited from secretarial and clerical administrative staff members. X-ray technicians worked in the X-ray department 8 h a day, five days a week in different shifts. An X-ray exposure control program is run under our institutional regulations that have been devised generally in concordance with the international commission on radiological protection (ICRP) guidelines and the U.S. Department of Health, Education and Welfare Publication No. (FDA) 75-8003. Each X-ray department employee is given an X-ray radiation dose detector (TLD: thermo-luminescent dosimeter, manufactured by Harshaw/Thermo Electron, USA). These employees always keep the TLD badge with them during their duty hours and submit it monthly to the radiation safety department which calculates the X-ray exposure dose. The mean exposure level of X-ray radiation in X-ray technicians was 72.4 mrem per calendar quarter and 289.6 mrem per year. However, the mean duration of exposure to X-ray radiation was 6.55 ± 0.44 yr (mean ± S.E.M) range 2–14 yr in males and 5.33 ± 0.84 yr (mean ± S.E.M) range 3–9 yr in females. All subjects completed a questionnaire, which included anthropometric data and a consent form. Both groups met the exclusion criteria as per standard.

Exclusion criteria

Subjects with gross anemia, known history of diabetes mellitus, cardiopulmonary and autoimmune disease, malignancy, subjects with current or previous history of tobacco (smoked or chewed) addictions or who chewed betel nut were excluded from participating in the study. In addition, subjects who had been working in any industrial environment were also excluded from the study.

Methods

Collection of blood sample: Ten ml of blood was collected from each subject by venipuncture and a disposable syringe. Then 2 ml of blood was transferred to a bottle containing EDTA (ethylene diamine tetraacetic acid) at a concentration of 1.5 mg/ml to be used for TLC, and the remaining 8 ml blood was heparinized (10 IU / ml) to assess the phagocytic activity by measuring the chemiluminescence (CL) response. Each specimen bottle was labeled with a subject identification code number.

Total leukocyte count: TLC was performed on an electronic cell counter (Beckman Coulter counter, USA) that was calibrated according to our standard operating procedures.

Opsonization of zymosan: Zymosan (Sigma Chemical Co., St. Louis, MO, USA) was opsonized by suspending 50 mg in 3 ml human serum and 1 ml phosphate buffer saline (PBS). The suspension was incubated for 30 min at 37°C and then centrifuged at 300 × g for 10 min. The supernatant was then removed and the pellet was washed twice with 4 ml buffer. After the last washing, the pellet was resuspended in PBS at a concentration of 1.25 mg/ml and stored in the freezer until use. The concentration of opsonized zymosan (OPZ) used was 2 mg/ml.

Preparation of luminol: Luminol (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in DMSO to give a concentration of 10^{-2} M and this stock solution was further diluted in PBS to 10^{-4} M prior to use.

Polymorphonuclear leukocytes (PMN) separation: PMN were separated by using neutrophil isolation medium (NIM) (Cardinal Associates Inc., PO Box 5220, Santa Fe, NM 87502). Five to seven milliliters of heparinized blood were layered over 4 ml of NIM in a 15 ml tube and then centrifuged at 400 × g for 30 min at room temperature. The leukocyte-rich plasma was carefully removed with a Pasteur pipette and transferred to a 15 ml conical centrifuge tube. The tube was filled with phosphate buffered saline (PBS) and centrifuged at 350 × g for 10 min in a Heraeus centrifuge (Model GmbH, Osterode). Two milliliters of lysing buffer (E-Lyse) from the same company was added to lyse the residual erythrocytes, vortexed to resuspend the pellets and then centrifuged at 250 × g for 10 min. The supernatant was discarded and the sediment was suspended in 1 ml of 5% foetal calf serum (FCS). The cells were then counted and adjusted to the desired final concentration.

Table 1. Comparison of Age and Total Leukocyte Count between control and X-ray technicians

Parameters	Control Subjects (n=42) (mean ± SEM)	X-ray technicians (n=42) (mean ± SEM)	P value
Age (yr)	36.3 ± 1.1	37.2 ± 1.1	NS
TLC (cells/microliter)	7,280.9 ± 358.9	7,169.0 ± 384.0	NS

NS=Not Significant.

Table 2. Chemiluminescence (CL) response in whole blood stimulated with opsonised zymosan (OPZ) of X-ray technicians compared to their matched control group

Subjects	CL response (mV) (n=42)	Time to half peak (min) (n=42)	AUC (mV/ min)
Unexposed subjects (n=42)	28.3 ± 0.6	5.2 ± 2.1	512.5 ± 68.3
X-ray technicians (n=42)	19.8 ± 1.9*	9.6 ± 1.8	278.7 ± 26.1*

Values are expressed as mean ± SEM; AUC=Area under curve; PMN concentration=5 × 10^6 cells/ml; OPZ concentration=1.25 mg/ml; Luminol concentration=10^{-4}M; *p value=<0.0005.

Table 3. Chemiluminescence (CL) response of isolated polymorphonuclear neutrophils (PMN) stimulated with opsonised zymosan (OPZ) from control group of unexposed subjects and X-ray technicians

Subjects	CL response (mV) (n=42)	Time to half peak (min) (n=42)	AUC (mV /min)
Unexposed subjects (n=42)	782.5 ± 5.3	4.9 ± 0.9	21,204.5 ± 2784.6
X-ray Technicians (n=42)	510.4 ± 57.0*	5.0 ± 0.8	*11,982.4 ± 969.9

Values are expressed as mean ± SEM; AUC= Area under curve; PMN concentration=5 × 10^6 cells /ml; OPZ concentration=1.25 mg/ml; Luminol concentration=10^{-4}M; *p value=<0.0005.

Chemiluminescence assay: For luminol-enhanced chemiluminescence a Berthold luminometer (AutoLumatPlus LB 953) with a constant temperature (37°C) controller (Berthold Technologies GmbH & Co. KG, Calmbacher Strass 22, D-75323 Bad Wildbad, Germany) connected to a computer was used. The reaction mixture consisted of 100 µl of whole blood or PMN suspension and 900 µl medium containing 10^{-4} M luminol (5-amino-2,3-dihydro, 1,4-phthalazinedione; Sigma Chemical Co., St. Louis, MO, USA) and 2 ng/ml OPZ. Light emission was recorded in millivolts (mV) and the readings were recorded at 1 min intervals for 30 min. CL emission was quantified as the peak height in mV.

Statistical Analysis

The significance of statistical differences between the mean values of the two groups was evaluated by Student's paired t-test (two-tailed). Statistical difference was considered significant if the p value was less than 0.05.

Results

Table 1 summarizes the comparison of the anthropometrical parameter (age) and total leukocyte count between X-ray technicians and their matched control group. There was no significant difference between the means of age and TLC between the two groups. The mean exposure level of our exposed study subjects was 72.4 mrem per calendar quarter and 289.6 mrem per year. The mean duration of exposure of male X- ray technicians was 6.55 + 0.44 yr (mean ± SEM) range 2–14 yr, and 5.33 ± 0.84 (mean ± SEM) range 3–9 yr, in females.

Table 2 compares the phagocytic activity of PMN in X-ray technicians and their matched controls measured by the CL response in whole blood (WB) stimulated with OPZ. The mean values of CL response in whole blood were significantly decreased (p<0.0005) in X-ray technicians compared to their matched control subjects.

Table 3 shows the phagocytic activity of PMN

measured by the CL response and AUC in isolated PMN of X-ray technicians and their matched controls. The mean values of CL response in isolated PMN and AUC were significantly decreased ($p<0.0005$) in X-ray technicians compared to their matched control subjects.

Discussion

X-ray radiations have potentially detrimental effects on living tissues and can destroy the living cells or make them functionally abnormal by free radical mechanisms. Recently, the National Cancer Institute (NCI) of the USA has been considering including medical X-rays in the list of human carcinogenis[9]. The amount of scattered radiation to which an X-ray technician may expect to be exposed is less than 200 mrem (2 mSv) per month, exposures greater than this will usually indicate improper procedures on the part of the technician. Personnel dosimetry records are maintained at all good institutions. Under current guidelines each person may receive a maximum dose of 1,250 mrem (12.5 mSv) per calendar quarter; however our study group had a far lower level of exposure than this (72.4 mrem) per calendar quarter and (289.6 mrem) per year. Keeping in view the hazards of X-ray radiation and poorly reported phagocytic dysfunctions induced by X-ray irradiation, the present study was designed to determine the affects of X-ray radiation on the quantitative and phagocytic activity of PMN. The present results show impaired phagocytic function of leukocytes in X-ray technicians through a significantly decreased CL response in whole blood (Table 2) and isolated PMN (Table 3) compared to their matched controls. However, no significant difference was observed in TLC between the two groups (Table 1).

Rozgaj *et al*.[10] reported that long-term exposure to low doses of ionizing radiation may affect the cells and tissues and result in various adverse health effects. Hrycek *et al*.[11] showed that workers handling X-ray equipment in X-ray laboratories had disturbances of peripheral blood neutrophils metabolism. They also reported that phagocytic activity of neutrophils was weakened in subjects working over five years with X-ray equipment. Our results confirm the results observed by Hrycek *et al*.[11].

Maltsev *et al*.[12] conducted experiments on rats, dogs, and monkeys, irradiated with X rays or gamma rays and observed decreases in the indices of phagocytosis at 3–4 wk after irradiation. In addition, Takeuchi *et al*.[13] found that X-ray radiation had a destructive action on immune system cells and organs and depressed their functional activity. Similarly, the present results are in agreement with the results reported by Maltsev *et al*.[12] Takeuchi *et al*.[13].

In addition, Hrycek, *et al*.[14] showed that subjects operating X-ray equipment in radiology departments had a significant reduction in neutrophil adherence as well as spontaneous migration of leukocytes. Neutrophil adherence and spontaneous migration of leukocytes are components of phagocytosis, thus, our findings correlate with the results reported by Hrycek, *et al*.[14]. Similarly, Ferrer, *et al*.[15] demonstrated that X-ray-induced apoptosis promotes a short-lasting phagocytic response.

Our present study correlates with the findings of earlier studies suggesting that X-ray radiations adversely affect the phagocytic function. The point that deserves to be focused on is that, in this study we observed impaired phagocytic activity in whole blood as well as in isolated PMN in X-ray technicians compared to their matched controls, despite low levels of exposure. This is important in the sense that it not only shows the magnitude of the effect in our studied population but also demonstrates the need for more stringent strategies for prevention of exposure. It is advisable, therefore, that X-ray department workers, their employers and health officials should work together to adopt more stringent technical preventive measures including compulsory wearing of appropriate protective equipment, like lead apparel, lead goggles, and thyroid shields etc. These measures will help to minimize the hazards of X-ray radiation, which often, over time, contribute to more severe health problems. It is also suggested that X-ray technicians should undergo pre-employment and periodic medical surveillance tests including phagocytic activity of leukocytes, which will identify susceptible workers, so they can take additional preventive measures. In addition, short-term leave should be granted to X-ray technicians after every three months and in severe cases of phagocytic function impairment, as a last resort, they should be encouraged to change their profession. With stricter implementation of the governmental and institutional regulations and better awareness of occupational hazards of X-radiation in the community, it will be possible to enforce safer protective practices for X-ray technicians.

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