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Evaluation of the Serum Total Antioxidant Level and Hematological Indices in Healthy Workers Exposed to Low Radiation Doses: A Significant Increase in Platelet Indices

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Abstract

The critical role of the free radicals and the reactive oxygen species (ROS) have been well proved in the etiology of over hundred diseases. Radiation is one of the most important sources of free radicals production in human and it can destroy macromolecules, specially DNA, lipid, proteins and carbohydrate. The aim of the study was to evaluate the serum total antioxidant level (TAL) and hematological indices in healthy workers of Radiology Department of a University hospital of Mashhad, Iran. The study was performed on 70 subjects including 40 persons working with X-ray machines as the case group and 30 unexposed individuals as the controls. Radiation dose of radiology staff participating in the study was less than the maximum permissible annual level, 50 millisievert. The mean serum TALs of radiology staff (833 ± 30.4 µmol/L) was lower than control subjects (875 ± 46.8µmol/L). Nevertheless, there was no significant difference between the mean serum TALs of exposed workers and the corresponding control group ($P=0.546$). On the other hand, a significant increase in the percentage of case group PDW (platelet distribution width, 18.00 ± 0.54) and PLCR (platelet large cell ratio, 38.20 ± 1.47) was observed ($P<0.05$). This result indicates that long term low dose ionizing radiation may have side effects on thrombocytosis and coagulation function. Due to high application of fluoroscopy for interventional radiology, we suggest continuing of the research projects on radiation protection and hazards to prevent irreversible damages.

Keywords: Free radical, X-ray, total antioxidant level, PDW (platelet distribution width), PLCR (platelet large cell ratio)

Introduction

Electromagnetic radiation contains radio waves, infrared and visible light as well as high energy rays such as ultraviolet, x ray, and gamma ray. The effects of high energy rays are exerted by transferring energy to atoms and molecules through their excitation and ionization. These interactions lead to the generation of free radicals and reactive oxygen species (ROS) resulting to the oxidation of vital macromolecules or in other words oxidative stress which plays a pathogenic role in many diseases (1,2). As a result, high dose radiation can cause tissue damage and DNA breakage resulting to malignancies (3). Man has lived with natural radiation since the beginning of life, but in recent decades there has been an increase in artificial radiation, mainly from the medical uses. All ionizing radiations are harmful and thus required radiation protection. However, the human body challenges by free radicals through an integrated control of the body's antioxidant systems. This system includes enzyme and non-enzyme antioxidants. Antioxidants prevent free radical induced tissue damage by inhibiting the formation of radicals, scavenging them, or by elevating their catalysis (2). Workers operating X-ray instrument are exposed to long-term low doses of ionizing radiation which may affect their antioxidant status. In this group, some studies have demonstrated significantly reduced serum total antioxidant level in comparison to controls and associated genetic injuries (4,5). The aim of our study was to evaluate influence of low doses of ionizing radiation on serum total antioxidant levels and hematopoesis indices in a group of healthy workers operating X-ray machinery.

Materials and methods

Study design

A total of fifty subjects involving of 40 persons (24 males and 16 females) as the case group and 20 persons (15 males and 15 females) as the control group were recruited for the study. The ages of the subjects ranged from 23 to 60 years with a work experience higher than 3 years. Radiation dose of radiology staff participating in the study was less than the maximum permissible annual level, 50 millisievert.

The case group was comprised of the healthy radiology staff of Imam Reza Hospital, Mashhad, Iran. As a control group a total of 30 healthy lab staff of the same hospital, with no past history of exposure to ionizing radiation of workplace, were selected. Control subjects who had been recently exposed to medical or diagnostic radiation were excluded from the study. After obtaining the University medical research ethics committee approval, and following a through explanation of the objectives and methods of the study for the subjects, individual written informed consent was obtained.

Blood sampling

After an overnight fasting period, 7 mL of peripheral blood samples were taken from the brachial vein of the case and control subjects. Blood samples for evaluation of hematological parameters were collected into sterile tubes with anticoagulant (EDTA) and for determination of serum TAL in non anticoagulant tubes. Blood samples in non anticoagulant tubes were centrifuged at 3000 rpm for 5 min and serum samples were harvested and kept at -70 until testing.

The FRAP assay

The total antioxidant levels (TAL) in the sera from both groups were assessed using the Ferric Reducing/antioxidant power (FRAP) assay. The measurement of the serum TALs was performed by the assay based on the method of Benzie and Strain with slight modification (6). The method is based on the principle of the reduction of the ferric-tripyridyltriazine complex to the ferrous form, upon which an intense blue color develops, and the change of absorbance is measured at 593 nm. We performed the FRAP assay in a tube format, by the end-point approach. Briefly, 100 µL of sample and 3 mL of working FRAP reagent (a: acetate buffer pH 3,6; b: FeCl3 solution; c: 2,4,6,-tripyridyl-s-triazine solution; 10:1:1) were pipetted in the sterile tubes in duplicate. Then, the reaction mixture was incubated for exactly 8 min at 37 °C. The absorbance was measured by a spectrophotometer at 593 nm, against a reagent blank. The *$FeSO_4$ standard solutions* were used for the construction of the calibration curve at concentrations of *100*, 500, 1000 and 2000 µmol/L.

Determination of the hematological parameters

Complete blood cell (CBC) count was performed with a hematology cell counter (Sysmex KX 21, Japan). Routine hematological parameters including hemoglobin content (Hb), hematocrit (Hct%), red blood cell (RBC) count, white blood cell (WBC) count and platelet (Plt) count were determined on a *Sysmex KX-21N™ Automated* Hematology Analyzer. Other indices such as MCH (Mean corpuscular hemoglobin), MCHC (Mean corpuscular hemoglobin concentration), MCV (Mean corpuscular volume), MPV (Mean platelet volume), RDW (RBC distribution width), PDW (platelet distribution width), and P-LCR (platelet large cell ratio) were also reported. A blood smear was stained with Gimsa for each sample, slides were observed under light microscope. At least 100 cells were seen for differential analysis. The reticulocyte count was done using supra-vital staining (new methylene blue). An equal volume of stain was added to EDTA-anticoagulated blood, the dilution mixture incubated for 10 minutes at room temperature, and a smear was prepared. The smears were examined to measure the number of reticulocytes. An erythrocyte containing two or more particles of blue-stained material was considered as a reticulocyte.

Statistical analysis

Data were statistically analyzed using Student's *t*-test to determine significant differences in the data of two groups. Statistical tests were conducted using INSTAT software (GraphPad, Inc., San Diego, CA). P values of less than 0.05 were considered significant. The values were expressed as means ± SEM.

Results:

The calibration curve for serum TALs determination is shown in Figure 1. As can be seen, the standard curve showed excellent linearity over the 100–2000 µm/l range with an R^2 value of 0.9985. Table 1 shows the mean and SEM of the ages, work experiences, and serum TALs of the test and control subjects with no significant differences between the two groups on all parameters including serum TAL.

The hematological indices of 30 cases and 20 control subjects are described in Table 2. All parameters (except for PDW and P-LCR) did not show any significant difference between the two groups. However, a significant increase in the percentage of case group on PDW and P-LCR was observed ($P < 0.05$).

Discussion:

Ionizing radiation can produce cellular reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radical (7). ROS shows high tendency to react with cellular macromolecule components including DNA, lipids and proteins. Small amounts of these substances are necessary for daily survival (8,9). On the other hand, if ROS is over-produced, or the antioxidant content becomes low, the cells will be damaged. The intracellular redox (reduction–oxidation) state is pivotal for a correct cell function (9). In physiological conditions, there is a balance between free radical formation and anti-oxidant capacity. For example, when this balance is disrupted as a result of exposure to ionizing radiation it can lead to oxidative stress (10-12). Thus, the balance between pro-oxidant production and antioxidant defense is very important in terms of maintaining cellular homeostasis (13). One of the most vulnerable tissues to ionizing radiation is bone marrow because of the marrow's proliferative activity and lack of relative DNA repair capacity (14). Among the hematological findings of our study, the mean percentages of PDW and P-LCR of case subjects were found statistically higher than values of control group. P-LCR, a platelet large ratio, reflects changes in either the level of platelet stimulation or the rate of platelet production (15). Platelet distribution width (PDW) is a measure of heterogeneity in circulating platelet volume and reflects platelet turnover or activation (16). Regarding to the vulnerability of bone marrow to ionizing radiation, the high level of PDW and PLCR of radiology staffs may be related to adverse effects of radiation on platelet progenitor cells. However, more research is needed to prove this hypothesis.

This study showed that mean serum TALs of radiology staff was lower than control subjects, but the difference was not statistically significant. Contrary to this study, a similar study in Tehran,

Iran, showed that radiation workers have antioxidant levels significantly lower than the controls (11). The difference may be due to greater use of fluoroscopy for interventional radiological procedures. Fluoroscopy exposes a large fraction of the radiation dose delivered in diagnostic medical imaging because of continuous x-ray production and real time image output. On the other hand, in two similar studies performed in Nigeria, there was no significantly difference between the serum TALs of the control and case groups (4). Several reports indicate that very low dose ionizing radiation in professional radiation workers not only stimulates the immune system but also repairs DNA breakage, transforms free oxygen radicals, and increases longevity (17,18). However, the harmful effect of high dose ionizing radiation is well established (19,20).

In conclusion, our findings showed that serum TAL was not significantly different between healthy radiologists and unexposed controls. This may be due to well protection of the radiology workers. Nevertheless, since the PDW and P-LCR of the radiation workers were significantly higher than control group, performing a series of research studies on their platelet function and production is highly recommended. However, due to the development of the multislice Serial CT Scanners and Digital Fluoroscopy applications in our country which will lead to high radiation exposure to workers in the radiology department, performing the research projects on radiation protection and hazards should be encouraged to prevent irreversible damages.

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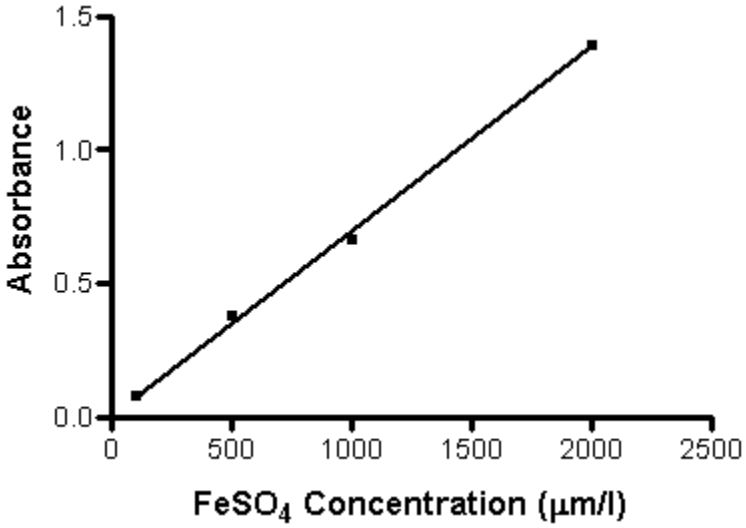

Figure 1. Calibration curve for determination of serum TAL.

Parameter	Case group	Control group	P value
Age	37.48 ± 2.1	35.55 ± 1.9	0.527
Work experience	13.68 ± 1.7	12.05 ± 1.7	0.512
Gender			
Male	24 (60%)	15 (50%)	0.751
Female	16 (40%)	15 (50%)	0.751
Serum TAL	833 ± 30.4	875 ± 46.8	0.546

Table 1. Demographic characteristics and the serum total antioxidant levels of the study groups Data shown are in terms of mean ± SE.

Parameter	Case group	Control group	P value
WBC (count $\times 10^3/\mu l$)	6.52 ± 0.29	5.88 ± 031	0.153
RBC (count $\times 10^6/\mu l$)	4.99 ± 0.11	4.83 ± 0.08	0.298
Hct (%)	41.69 ± 0.74	40.57 ± 0.60	0.280
Hb (g/dl)	14.32 ± 0.29	14.04 ± 0.24	0.497
Plt (count $\times 10^5/\mu l$)	231 ± 10	236 ± 9	0.718
MCH (pg)	28.82 ± 0.46	29.12 ± 0.39	0.650
MCHC (g/dl)	34.31 ± 0.24	34.61 ± 0.27	0.434
MCV (fl)	83.90 ± 1.01	84.10 ± 0.79	0.885
MPV (fl)	10.84 ± 0.19	10.38 ± 0.19	0.102
Neutrophil (count $\times 10^3/\mu l$)	3.70 ± 0.22	3.36 ± 0.17	0.276
Lymphocyte (count $\times 10^3/\mu l$)	2.22 ± 0.12	1.90 ± 0.12	0.070
Mix (count $\times 10^3/\mu l$)	0.60 ± 0.035	0.62 ± 0.12	0.827
RDW (%)	12.73 ± 0.15	12.81 ± 0.22	0.745
PDW (%)	18.00 ± 0.54	13.36 ± 0.34	0.028*
PLC-R (%)	38.20 ± 1.47	27.81 ± 1.41	0.0151*
Reticulocyte count (%)	0.97 ± 0.04	0.85 ± 0.04	0.119

Table 2. Comparison of hematological indices between case and control groups Data shown are in terms of mean ± SE. *$P < 0.05$ indicates significant changes compared to the control group.