

Assessment of nitrosative stress and lipid peroxidation activity in asymptomatic exposures to medical radiation: The bystander effect of ionizing radiation

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ABSTRACT

Background: Adaptive response and bystander effect are two important phenomena involved in biological responses to ionizing radiation. **Aims:** To determine the bystander effect of ionizing radiation in medical exposures by measuring the serum nitric oxide (NO[•]), peroxynitrite (ONOO[•]), and malondialdehyde (MDA) levels. **Materials and Methods:** Twenty-five medical staff working in the Unit of Radiology and 15 medical staff working in other departments at the Al-Yarmouk teaching hospital in Baghdad, Iraq, were enrolled in the study. Venous blood was obtained from each subject for determination of NO[•], ONOO[•], and MDA levels. **Results:** Significantly higher serum NO[•], ONOO[•], and MDA levels were observed in participants working in the radiology unit as compared with serum levels in those working elsewhere. There was no correlation between the lipid peroxidation activity and ONOO[•]/NO[•] ratio. The serum NO[•] level in subjects working in the x-ray services was significantly higher than that in subjects working in the CT and MRI services. **Conclusions:** The bystander effect of radiation could be observed in asymptomatic individuals working in the radiology unit and it was particularly well observed in people working in the X-ray services as opposed to CT and MRI services. Determination of serum nitrogen species could be a useful laboratory investigation for assessment of the bystander effect of radiation.

Key words: Ionizing radiation, malondialdehyde, nitrogen species

INTRODUCTION

There is no doubt that the staff who work in radiology departments or those medically exposed to ionizing radiation need to extend their knowledge about safe radiation doses and the risks of radiation.^[1] Doctors of all grades still ignore the basic rules regarding radiation exposure even with the most common investigations, and there is even worse appreciation of the radiation involved in computerized tomography (CT) scanning.^[2]

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Adaptive response and bystander effect are two important phenomena involved in biological responses to low doses of ionizing radiation. Low doses of high-linear energy transfer (LET) radiation can induce an adaptive response characterized by lower mutation frequencies in human lymphoblastoid cells.^[3] The bystander effects of ionizing radiation are mediated by soluble factors that are released by the irradiated cells. Recently, Martin *et al.* reported the presence of a distant bystander DNA damage response mediated by inflammatory macrophages that are activated by soluble cytokines.^[4] The release of nitric oxide (NO[•]) and its metabolite from irradiated cells is the bystander effect of ionizing radiation.^[5] Some authors believe that NO[•] functions as an initiator of radiation-induced bystander and adaptive responses and there may be a correlation between the radioadaptive and bystander responses.^[6] There is experimental evidence that the total nitrate/nitrite (NO_x) and malondialdehyde (MDA) levels in both lung and liver tissues of rats are increased after a single dose of total-body irradiation.^[7] Therefore we felt that it would be worthwhile to assess the nitrosative stress and lipid peroxidation process as an indicator of the bystander effect of ionizing

radiation in medical staff working at the radiology unit at the Al-Yarmouk teaching hospital in Baghdad, Iraq.

MATERIALS AND METHODS

This study was conducted in the Department of Pharmacology in cooperation with Department of Physiology/Medical Physics, College of Medicine, Al-Mustansiriya University and the Al-Yarmouk teaching hospital in Baghdad, Iraq, from March to May 2011. This study was approved by the scientific committee of the college and verbal consent was obtained from each participant prior to admission into the study. A total of 25 patients (11 males and 14 females) who were working as medical staff in the diagnostic X-ray and computerized tomography (CT) scan and magnetic resonance imaging (MRI) units were enrolled in the study. Another 15 medical participants (9 males and 6 females) not working in the radiology unit served as control group. Information related to radiation exposure (i.e., number of exposures per day, working days per week, radiation dose per exposure, methods of protection, and accidental exposure to radiation) and medical history (i.e., past medical history, chronic diseases, and social history) was obtained from each participant. Venous blood samples were obtained from participants and the sera was separated and kept at -20°C for further chemical analysis.

Serum peroxynitrite (ONOO^{\cdot})–mediated nitration of phenol was measured as has been described earlier.^[8,9] Briefly, 50 μl of serum was added to 5 mM phenol in 50 mM sodium phosphate buffer (pH 7.4) to get a final volume of 3 ml. After incubation for 2 hours at 37°C , 50 μl of 0.1M NaOH was added and the absorbance at 412 nm of the samples was immediately recorded. The yield of nitrophenol was calculated from $\epsilon = 4400 \text{ M}^{-1}\cdot\text{cm}^{-1}$.

Nitric oxide–donating activity was determined as described by Newaz and coworkers.^[10] Briefly, 500 μl of serum was added to 50 μl HCl (6.5M) and 50 μl sulfonic acid (37.5 mM). After incubation for 10 minutes, 50 μl naphthylethylenediamine dihydrochloride (12.5 mM) was added and this was incubated for a further 30 minutes and then centrifuged for 10 minutes at 1000 g. The absorbance at 540 nm was immediately recorded. The concentration of NO as nitrate/nitrite was calculated from the standard curve of lithium nitrate.

Lipid peroxidation activity was assessed by determination of the serum level of MDA. Briefly, two volumes of cold trichloroacetic acid (10% w/v) was added to one volume of serum and the mixture was centrifuged for 10 minutes to precipitate protein. Equal volumes of supernatant and thiobarbituric acid (0.67%) were mixed and incubated in boiling water for 30 minutes. The absorbance was recorded at 532

nm using UV-visible spectrophotometer and the concentration of MDA was calculated using the extinction coefficient $1.56 \times 10^5 \text{ M}^{-1}\cdot\text{cm}^{-1}$.

Statistical analysis

The results are expressed as number (n), percentage, median, and mean \pm SD. The data was analyzed using the unpaired two-tailed Student's t -test and simple correlation test. $P \leq 0.05$ was assumed to indicate statistical significance.

RESULTS

A total of 25 subjects working in the radiological services were enrolled in the study. All the participants were qualified for practice in the unit of radiology and were aware of the risks of radiation. Seven of the 25 subjects had a history of repeated annual chest infections, 4 out of the 14 female subjects had history of abortion, and 2 out of the 25 subjects had history of diabetes mellitus [Table 1]. Active smoking was reported by three participants but none of the 25 participants gave history of alcohol intake. The radiology unit where the subjects worked handled up to 100 cases of x-ray, 20 cases of CT, and 15 cases of MRI every day. The participants had 2–3 free days per week.

There is no specific guideline or schedule for calculating the radiation dose for each patient exposure. The radiation dose of each exposure is roughly and individually estimated and sometimes up to 500 Kev is used in X-ray radiation per exposure. All the participants underwent periodic physical examinations, with laboratory investigations done every 6 months. All of them were provided with personal protective equipment. History of accidental exposure to radiation was reported by 20 of the 25 participants [Table 2]. Table 3 shows the significantly higher serum levels of NO^{\cdot} , ONOO^{\cdot} , and MDA levels in the study subjects as compared to the levels in subjects working in other departments. The $\text{ONOO}^{\cdot}/\text{NO}^{\cdot}$ ratio was 0.005 in participants working in the radiology unit as compared to 0.017 in subjects working elsewhere in the hospital. There was statistically nonsignificant association between the serum MDA level and the $\text{ONOO}^{\cdot}/\text{NO}^{\cdot}$ ratio ($r=0.054$). The differences in serum NO^{\cdot} , ONOO^{\cdot} , and MDA between participants did not reach the level of significance regarding active smoking, history of chest infection, and accidental exposure. The serum level of NO^{\cdot} in the subjects working in the X-ray unit was significantly higher than the levels in those working in the CT or MRI services [Table 4].

DISCUSSION

The results of this study show that the levels of nitrogen species and the markers of lipid peroxidation are significantly

higher in asymptomatic subjects working in the field of ionizing radiation. In addition, the levels of these biomarkers in subjects working in the X-ray unit are significantly higher than that in

Table 1: Characteristics of the study population

Gender	
Male	11
Female	14
Age in years (mean \pm SD) median age (years)	36.9 \pm 10.5 (36)
Educational status	
Primary school	1
Secondary school	1
University	22
Higher education	1
Marital status	
Single	4
Married	21
Smoking	
Current (active)	3
Passive (second-hand smokers)	12
Medical history	
Abortion	5
Repeated chest infections	7
Malignancies	0
Cataract	0
Hypertension	3
Diabetes mellitus	2

Table 2: Characteristics of exposure to radiation

Duration	2 weeks to 32 years
Working days/week	4-5 days
Number of exposures per day	2-100
Subjects had their radiation exposure measured in some way	23
Subjects undergoing check of complete blood picture every 6 months	25
Accidental exposure to radiation	20

those working in the CT and MRI services. Recently, in an *in vitro* study using human culture cells, the bystander effect of irradiation was observed in terms of decreased activity of antioxidant enzymes, activation of lipid peroxidation, and altered translation of proteins encoded by mitochondrial DNA.^[11] Formation of oxidation markers and upregulation of stress-response genes induced by the direct effect of radiation emphasize the role of oxidative stress in promoting bystander effects, i.e., in nontargeted bystander cells, and this explains the significantly higher levels of nitrogen stress species detected in this study.^[12] Previous studies have indicated that NO[•] plays an important role in mediating cell proliferation and induces double-strand break of DNA in the bystander cell population, with increased probability of mutation.^[13] *In vitro*, the bystander effect of radiation on traversed human melanoma cells was observed as a slight increase of MDA concentration, comparable decrease of glutathione peroxidase activity, and some fluctuation of mitochondrial and cytoplasmic isoenzymes of superoxide dismutase.^[14] This is the first report which shows the significant elevation of serum levels of nitrogen species and MDA in asymptomatic workers in a radiology unit. The significantly higher serum NO[•] in workers dealing with X-ray radiation is probably related to differences in the methods of protection. The high serum level of NO[•] in asymptomatic subjects working in the radiation field is cause for alarm as this is a marker that predicts future undesirable events.

The limitations of this study include (a) failure to measure real-time NO[•] using a specific NO[•] sensor and (b) failure to

Table 3: Assessment of serum nitrogen species and lipid peroxidation in subjects working in the radiology unit

	ONOO [•] /NO [•] ratio		Serum malondialdehyde (nMol)		Serum peroxynitrite (μ Mol)		Serum nitric oxide (mMol)	
	Medical exposures	Non medical exposures	Medical exposures	Medical non exposures	Medical exposures	Medical non exposures	Medical exposures	Medical non exposures
Male	0.0067	0.0165	282.1 \pm 189.46* (n=11)	7.404 \pm 3.887 (n=9)	19.731 \pm 13.188* (n=11)	2.367 \pm 0.777 (n=9)	2.943 \pm 2.009* (n=11)	0.143 \pm 2.009 (n=9)
Female	0.0042	0.0198	141.02 \pm 84.39* (n=14)	7.266 \pm 3.003 (n=6)	15.129 \pm 5.607* (n=14)	2.641 \pm 1.056 (n=6)	3.606 \pm 1.978* (n=14)	0.133 \pm 0.063 (n=6)
Total	0.0051	0.0174	214.89 \pm 162.36* (n=25)	7.349 \pm 3.444 (n=15)	17.154 \pm 9.743* (n=25)	2.482 \pm 0.886 (n=15)	3.330 \pm 1.976* (n=25)	0.142 \pm 0.031 (n=15)

The results are expressed as mean \pm SD. *P<.01

Table 4: Assessment of serum nitrogen species and lipid peroxidation in subjects working in the radiology unit

	Serum nitric oxide (mMol)	Serum peroxynitrite (μ Mol)	Serum malondialdehyde (nMol)
Active smokers vs non-smokers	3.458 \pm 1.508 vs 3.311 \pm 2.064	14.05 \pm 4.57 vs 17.58 \pm 10.24	286 \pm 133 vs 202.99 \pm 166.94
Subjects with history of chest infections vs those without history of chest infections	3.493 \pm 1.826 vs 3.262 \pm 2.084	16.0 \pm 3.46 vs 17.6 \pm 11.35	205.12 \pm 30.06 vs 217.94 \pm 186.72
Workers in X-ray services vs those in CT and MRI services	1.023 \pm 0.723 vs 3.791 \pm 1.816*	15.31 \pm 4.12 vs 17.06 \pm 10.36	173 \pm 66 vs 224.7 \pm 177
Workers with history of accidental exposure vs those without history of accidental exposure	3.071 \pm 2.118 vs 3.144 \pm 2.167	15.66 \pm 4.96 vs 22.09 \pm 20.37	221. \pm 171.9 vs 189.1 \pm 130

The results are expressed as mean \pm SD. *P<.01

determine the levels of antioxidants and that of the scavengers of reactive oxygen species.

We conclude that the determination of serum nitrogen species could be a useful laboratory investigation for assessment of the bystander effect of radiation.

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