

The levels of oxidative and nitrosative stress in patients who had ^{99m}Tc -MIBI myocardial perfusion scintigraphy and ^{99m}Tc -DMSA, ^{99m}Tc -MAG-3 renal scintigraphy

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Abstract

BACKGROUND: Ionizing radiation is a strong stimulator of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These reactive species may cause oxidative and nitrosative stress. In this study, we aimed to evaluate possible effects of ^{99m}Tc -methoxyisobutylisonitrite (MIBI), ^{99m}Tc -dimercaptosuccinic acid (DMSA), ^{99m}Tc -mercaptoacetyltriglycine (MAG-3) on oxidative and nitrosative stress biomarkers in patients who were performed myocardial perfusion scintigraphy (MPS) and renal scintigraphy.

MATERIAL AND METHODS: Patients ($n = 29$) who were referred to nuclear medicine department were chosen as the patient group. They were divided into three subgroups according to the type of disease and ^{99m}Tc labelled agent. The first patient group had MPS ($n = 9$). The second patient group had ^{99m}Tc -DMSA renal scintigraphy ($n = 12$). The third patient group had ^{99m}Tc -MAG-3 renal scintigraphy ($n = 8$). The blood samples were taken from first, second and third patient groups 1 h, 3 h, 45 min after injection of the agent, respectively. The samples were taken from healthy volunteers ($n = 25$) as a control group. Alterations in catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA) levels as oxidative stress biomarkers and nitric oxide (NO) and 3-Nitrotyrosine (3-NTx) levels as nitrosative stress biomarkers in all blood samples were evaluated.

RESULTS: Results of MPS and renal scintigraphy performed patients were compared with control group separately. CAT, SOD, MDA and 3-NTx levels were higher in the first group than the control group ($p < 0.05$). Although NO levels were higher in the first group than the control group, it was not statistically significant ($p > 0.05$). CAT and SOD levels were lower in second and third groups than the control group ($p < 0.05$). However, MDA, NO, 3-NTx levels were higher in second and third groups than the control group ($p < 0.05$).

CONCLUSIONS: These results show that oxidative and nitrosative balance is impaired due to ionization radiation. These reactive species might stimulate an adaptive and protective cellular defense mechanism in irradiated cells soon after exposure to radiation. Thereby, this mechanism protect organism from the effects of low dose ionizing radiation.

KEY words: ionizing radiation; oxidative stress; nitrosative stress

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Introduction

^{99m}Tc and ^{99m}Tc -labeled radiopharmaceuticals widely are used more than 50 years in clinical nuclear medicine applications. ^{99m}Tc has both 140 keV gamma emission

and approximately 5 low energy Auger and internal conversion electrons per decay. ^{99m}Tc 's half-life ($t_{1/2}$) is six hours. ^{99m}Tc -methoxyisobutylisonitrite (MIBI) is used for myocardial perfusion scintigraphy (MPS) [1, 2]. ^{99m}Tc -dimercaptosuccinic acid (DMSA) is used for renal cortical scintigraphy and ^{99m}Tc -mercaptoacetyltriglycine (MAG-3) is used for dynamic renal scintigraphy [3]. Although the importance of ^{99m}Tc has been accepted in the worldwide, it results high ionization radiation intensity near the radionuclide's decay position due to emitted beams [2]. After intravenous (i.v.) injection of the radiopharmaceutical, it distributes into living cells and ionizing radiation is absorbed by these living cells. Cellular exposure to ionizing radiation initiates oxidizing process. This process damages cells and their structures at the atomic level via direct interactions with macromolecules such as DNA, RNA, proteins and lipids or radiolysis of water. Furthermore, this damage might spread to neighbour cells via bystander effect and persist in the progeny for many generations [4–7].

During the radiolysis of water-reactive oxygen species (ROS) including superoxide anion, hydrated electron, hydroxyl radical, hydrogen peroxide, organic hydroperoxides, alkoxy and peroxy radicals, hypochlorite, peroxyxynitrite are produced. Ionizing radiation also causes the production of reactive nitrogen species (RNS). As a result of that, large amounts of nitric oxide and peroxyxynitrite anion are produced [7, 8].

ROS/RNS initiate harmful process in cells. Different signalling cascades adaptive responses including DNA repair, antioxidant reactions and different signalling cascades are stimulated to overcome these harmful process. Ionizing radiation regulates antioxidant enzyme activity depending on dose and linear energy transfer. While a low dose of LET radiation (gamma rays) at low dose-rate stimulates antioxidant defense system, high LET radiation spreads oxidative stress both in the irradiated cells and nearby cells by means of bystander effect [7]. Antioxidants defense systems play a crucial role for preventing damage caused by ROS/RNS and eliminating ROS/RNS. The imbalance in the production and the elimination of ROS/RNS and various reducing or antioxidant systems of the body causes oxidative/nitrosative stress [7, 9, 10].

To our knowledge, this is the first study in the current literature that investigates the effects of ^{99m}Tc -MIBI, ^{99m}Tc -DMSA and ^{99m}Tc -MAG-3 oxidative/nitrosative damage in human. The present study was aimed to evaluate levels of both oxidative stress biomarkers including catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA) and nitrosative stress biomarkers including nitric oxide (NO) and 3-Nitrotyrosine (3-NTx) in patients who were clinically indicated and underwent ^{99m}Tc -MIBI MPS, ^{99m}Tc -DMSA renal cortical scintigraphy and ^{99m}Tc -MAG-3 dynamic renal scintigraphy.

Material and methods

Ethics

This study was conducted after obtaining approval from the local ethical committee of Sutcu Imam University, Medical Faculty, Kahramanmaraş, Turkey. Also, the study was conducted in accordance with the Declaration of Helsinki on medical protocols and ethics. As we used routinely generated data with no identifying information, the study carries no harm whatsoever to patients. Data were

used only for scientific purposes. All participants were informed extensively about this study and signed an informed-consent form before they were enrolled in this study.

Study population

A total of 29 patients who were referred to the nuclear medicine department and who were routine scintigraphic imaging methods were indicated were chosen as the patient group. The patients were divided into three subgroups based on the type of disease and ^{99m}Tc labelled agent. The ^{99m}Tc labelled imaging agents were administered at clinical doses.

In 9 patients (2 female, 7 male) with a mean age of 52 ± 9.7 years (range 38–67 years), MPS with ^{99m}Tc -MIBI was obtained (group 1).

In 12 patients (8 female, 4 male) with a mean age of 20 ± 21.6 years (range 1–66 years), renal cortical scintigraphy with ^{99m}Tc -DMSA was carried out (group 2).

In 8 patients (6 female, 2 male) with a mean age of 28 ± 21.4 years (range 6 and 66 years) dynamic renal scintigraphy with ^{99m}Tc -MAG-3 was performed (group 3).

The blood samples were taken from first group 1h after intravenous (i.v.) injection of ^{99m}Tc -MIBI, from second group 3h after i.v. injection of ^{99m}Tc -DMSA, from third group 45min, after i.v. injection of ^{99m}Tc -MAG-3. The samples were also taken from a total of 25 healthy volunteers with a mean age of 39 ± 13.1 years (range 18–63 years) as a control group.

Alterations in both CAT, SOD, MDA levels as oxidative stress biomarkers and NO and 3-NTx levels as nitrosative stress biomarkers in all blood samples were evaluated.

Biochemical analysis in erythrocytes

The blood samples were centrifuged at 3000 g for 10 minutes at 4°C. Plasma was separated and the buffy coat was discarded by aspiration. Erythrocytes were washed 4 times with cold physiological saline and stored at -70°C until analysis. The CAT activity in erythrocyte was measured in samples by the method applied by Beutler [11]. The decomposition of the substrate H_2O_2 was monitored spectrophotometrically at 240 nm. The activity of CAT was expressed as U/g Hb. The SOD activities in erythrocyte were estimated by the use of the method described by Fridovich [12]. SOD activity was expressed as U/g Hb. Lipid peroxidation level in the plasma samples was expressed in MDA. Measurement was based on the method of Ohkawa [13]. MDA levels were expressed as nmol/mL. NO and 3-NTx levels in plasma samples were determined with a "sandwich" enzyme-linked immunosorbent assay kits (mybio-source human elisa kits, USA) according to the manufacturers' protocol. Then, 3-NTx levels were given as nmol/L.

Statistical analysis

Data were analyzed using the statistical package SPSS for Windows version 20.0 (SPSS Inc., Chicago, IL.). Results were expressed as mean \pm SD. The conformability of the quantitative data to the normal distribution was examined by the Kolmogorov–Smirnov test. The Mann–Whitney U-test was used to compare mean values for all parameters between patients and control groups. p value < 0.05 was accepted as statistically significant.

Results

Results of MPS and renal scintigraphy performed patients were compared with control group separately.

Initially, MPS performed patients group were evaluated. The antioxidant enzyme activities of CAT and SOD were statistically significantly higher in the first patient group than the control group ($p < 0.05$) as shown in Figure 1A and 1B. CAT and SOD levels were approximately 1.9 and 1.7 fold higher in the first patient group than healthy subjects, respectively. In addition, MDA levels were also statistically significantly higher in the first patient group than the control group ($p < 0.05$) and its levels were nearly 1.6 fold higher in the first patient group as shown in Figure 1C. 3-NTx levels were statistically significantly nearly 1.8 fold higher in the first patient group than control group ($p < 0.05$) as shown in Figure 2B. NO levels were nearly 1.2 fold higher in the first patient group than control group ($p > 0.05$) as shown in Figure 2A.

Then, renal scintigraphy performed patients group were evaluated. CAT and SOD levels were statistically significantly lower in second, third and total renal scintigraphy performed patient groups than control group ($p < 0.05$). CAT and SOD levels were nearly 2 and 3 fold lower in the second patient group than control group, respectively (Fig. 3A and 3B). CAT and SOD levels were nearly 1.7 and 3 fold lower in the third patient group than control group, respectively (Fig. 5A and 5B). CAT and SOD levels were nearly 2 and 3 fold lower in total renal scintigraphy performed patients group than control group, respectively (Fig. 7A and 7B). MDA levels were statistically significantly higher in second, third and total renal scintigraphy performed patient groups than the control group ($p < 0.05$). MDA levels were nearly 2, 2 and 1.8 fold higher in second, third and total renal scintigraphy performed patient groups than the control group, respectively (Fig. 3C, 5C and 7C). NO levels were nearly 1.5, 2 and 1.6 fold higher in second, third and total renal scintigraphy performed patient groups than the control group, respectively ($p < 0.05$) (Fig. 4A, 6A and 8A). 3-NTx levels were nearly 2.3, 2.85 and 2.6 fold higher in second, third and total renal scintigraphy performed patient groups than the control group, respectively ($p < 0.05$) (Fig. 4B, 6B and 8B).

Discussion

In this study, we investigated the effect of ionizing radiation on both oxidative and nitrosative stress parameters in patients who were clinically indicated ^{99m}Tc -MIBI MPS, ^{99m}Tc -DMSA renal cortical scintigraphy, ^{99m}Tc -MAG-3 dynamic renal scintigraphy. This is the first study that evaluates levels of nitrosative stress parameters in patients with these scintigraphic imaging agents.

^{99m}Tc -MIBI is a lipophilic cationic complex, enters cells by passive diffusion. It is localized mostly in the mitochondria of myocytes due to negative mitochondrial membrane potential [1, 2, 15]. ^{99m}Tc -DMSA is a renal cortical agent that primarily bound in the proximal tubule in the renal cortex for a prolonged time after injection. ^{99m}Tc -MAG-3 is commonly used renal tubular agent in nuclear medicine practice [3]. It is known that the DNA-binding traits of ^{99m}Tc labelled agents augments radiotoxicity of these agents [1, 2, 14–16].

CAT, SOD, MDA, 3-NTx levels were found statistically significantly higher in MPS performed patients than control group ($p < 0.05$)

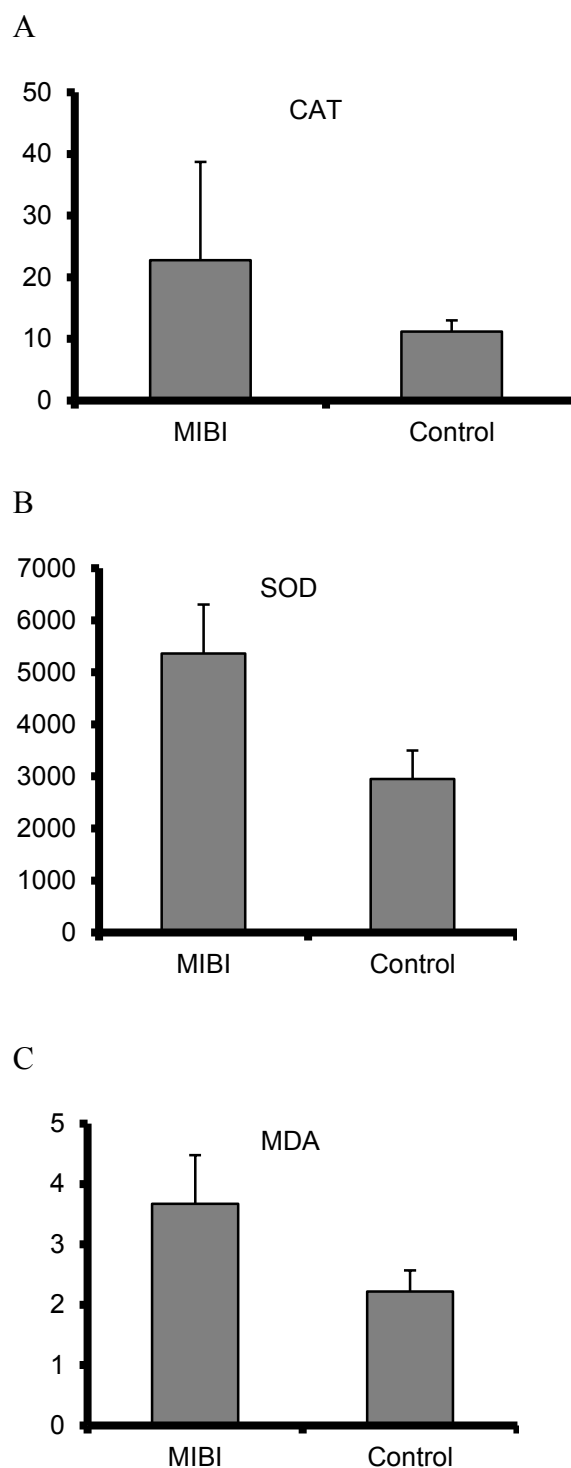


Figure 1. The activities of catalase (CAT); A. Superoxide dismutase (SOD); B. and malondialdehyde (MDA); C. Levels as oxidative stress biomarkers in first patient group and control group

and 1.9, 1.7, 1.6, 1.8 fold higher in patients, respectively. NO levels were 1.2 fold higher in patients than control group ($p > 0.05$). The results of this study showed that antioxidant enzyme activities of CAT and SOD levels were significantly higher in ^{99m}Tc -MIBI administered group than the control group ($p < 0.05$). Because shortly after exposure to ^{99m}Tc -MIBI, cellular protective pathways are triggered to overcome radiation-induced ROS. In addition, we

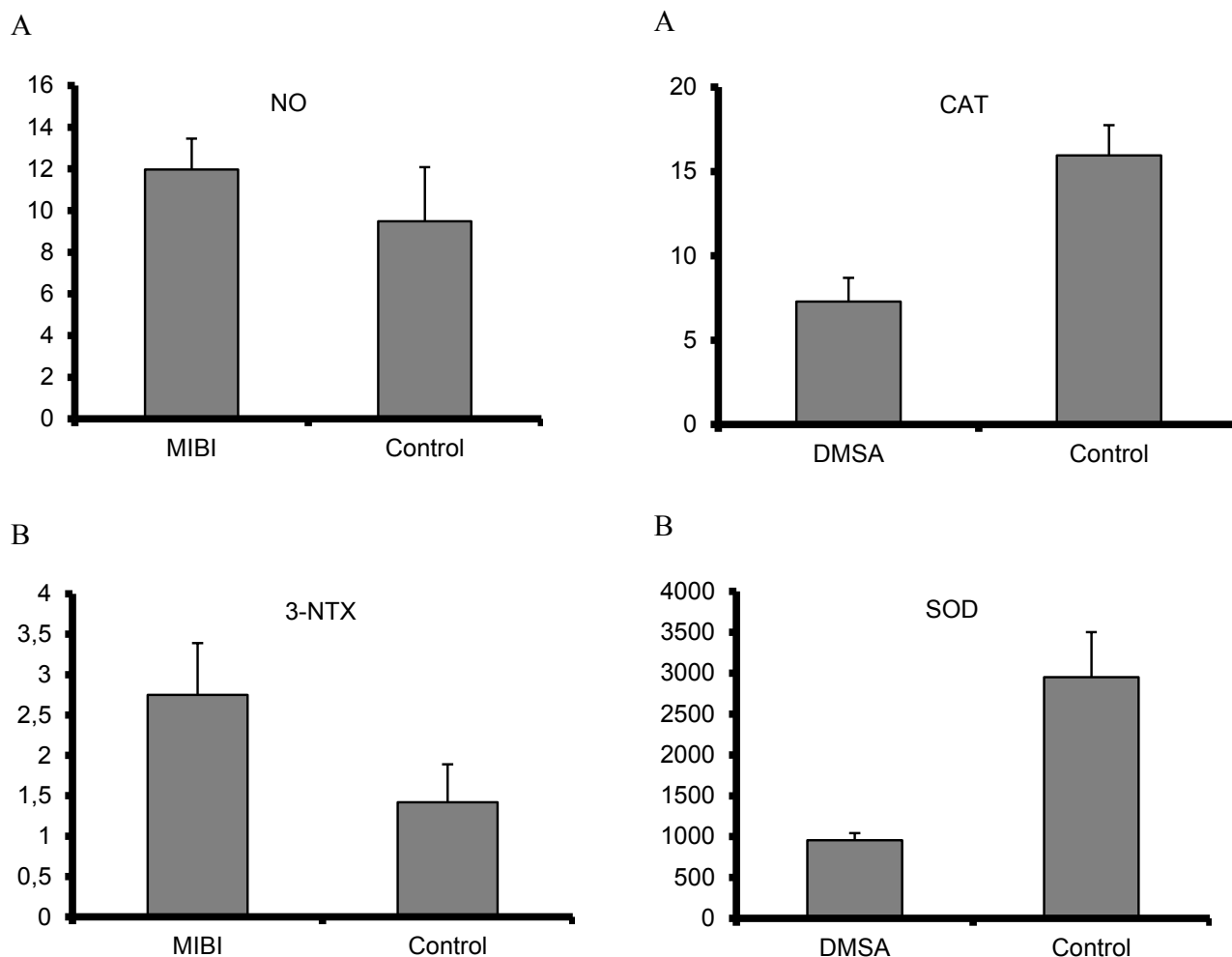


Figure 2. The activities of nitric oxide (NO); A. and nitrotyrosine (3-NTx); B. Levels as nitrosative stress biomarkers in first patient group and control group

previously reported same results in patients have thyroid and bone scintigraphy [17, 18]. However, ^{99m}Tc -MIBI caused decreased SOD levels in mice [19].

In contrast to MIBI administered group, CAT and SOD levels were significantly lower in ^{99m}Tc -DMSA, ^{99m}Tc -MAG-3 administered patients and total renal scintigraphy performed patients than control group in this study ($p < 0.05$). ^{99m}Tc -DMSA stays in the proximal tubule in the renal cortex for a prolonged time after injection. ^{99m}Tc -MAG-3 is a renal tubular agent. Renal imaging agents might induce free radical production more than MPS imaging agent. In addition, renal diseases cause oxidative stress. Therefore, antioxidant enzyme levels decreased in this group of patients. It has also been reported in the literature that exposure to radiation due to different radiopharmaceuticals including ^{99m}Tc -diethylene trimine pentaacetic acid (DTPA) [20], ^{99m}Tc -MIBI, ^{201}Tl Thallium, ^{99m}Tc -MIBI, ^{99m}Tc -methylendiphosphonate (MDP) [19, 21–23] and 2.45 GHz microwave radiation [24] caused same results in animal and human studies.

MDA levels were significantly higher in ^{99m}Tc -DMSA, ^{99m}Tc -MAG-3 administered patients and total renal scintigraphy performed patient groups than the control group ($p < 0.05$). In addition,

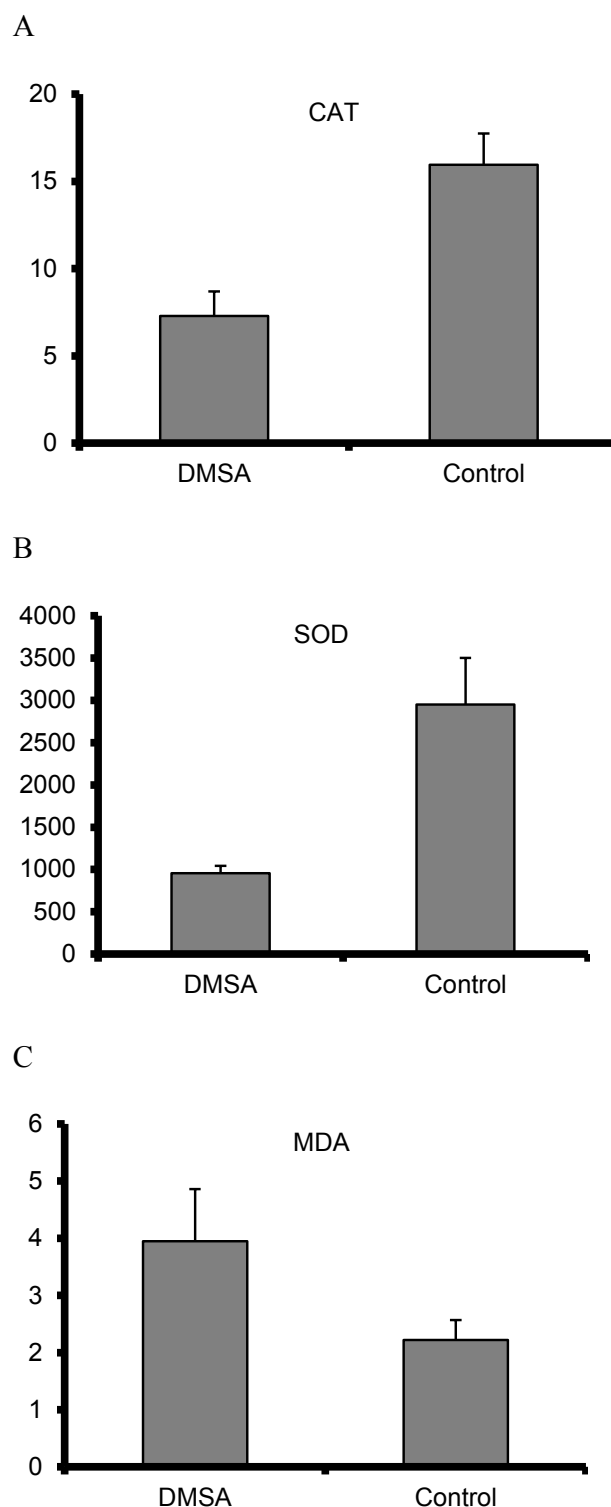


Figure 3. The activities of catalase (CAT); A. Superoxide dismutase (SOD); B. and malondialdehyde (MDA); C. Levels as oxidative stress biomarkers in second patient group and control group

researchers stated that gamma radiation at clinically used doses stimulates lipid peroxidation in human red cells [25]. ROS can initiate lipid peroxidation that is a chain reaction. Once lipid peroxidation is initiated, it results in the oxidative deterioration of polyunsaturated fatty acids and caused increased MDA levels [25].

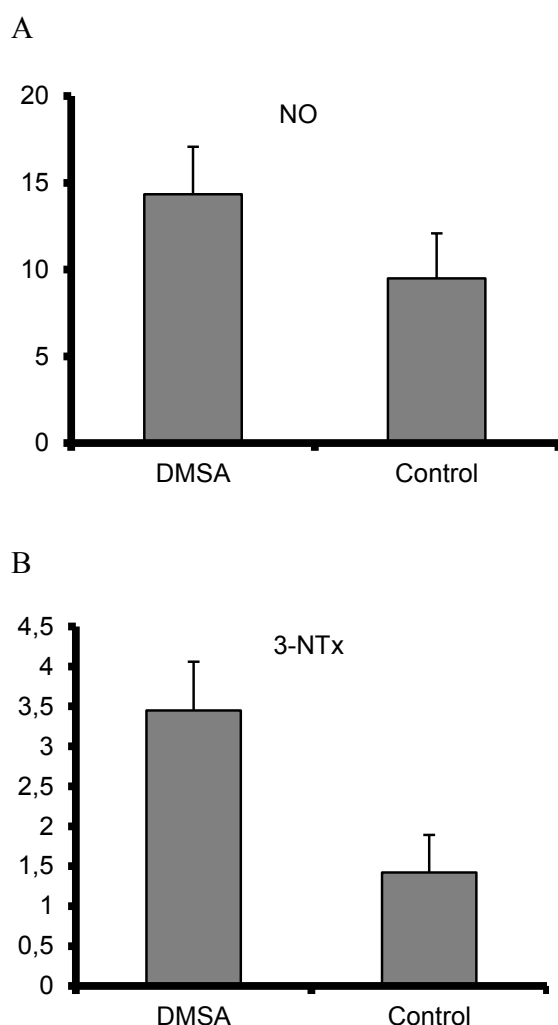


Figure 4. The activities of nitric oxide (NO); A. and nitrotyrosine (3-NTx); B. Levels as nitrosative stress biomarkers in second patient group and control group

Furthermore, these results are in agreement with previous animal and human studies that demonstrated ^{99m}Tc -DTPA [20], ^{99m}Tc -MIBI [21] and microwave radiation [24] caused increased MDA levels. However, ^{99m}Tc pertechnetate caused decreased MDA levels in patients [26].

The results acquired in this research study confirms that ionizing radiation causes different changes at antioxidant enzyme levels. However, to the best of our knowledge there is no similar clinical study reports on the effect of ionizing radiation due to ^{99m}Tc -MIBI and ^{99m}Tc -MDP on nitrosative stress parameters (NO, 3-NTx) in human. Therefore, we could not compare our results with those of others. We only could compare our findings on nitrosative stress parameters only with our previous reports that were performed in different patients groups and with different radiopharmaceutical agents including ^{99m}Tc pertechnetate and ^{99m}Tc -MDP.

NO levels were higher in the first patient group than the control group but it was not statistically significant ($p > 0.05$). 3-NTx levels were statistically significantly higher in the first patient group than the control group ($p < 0.05$).

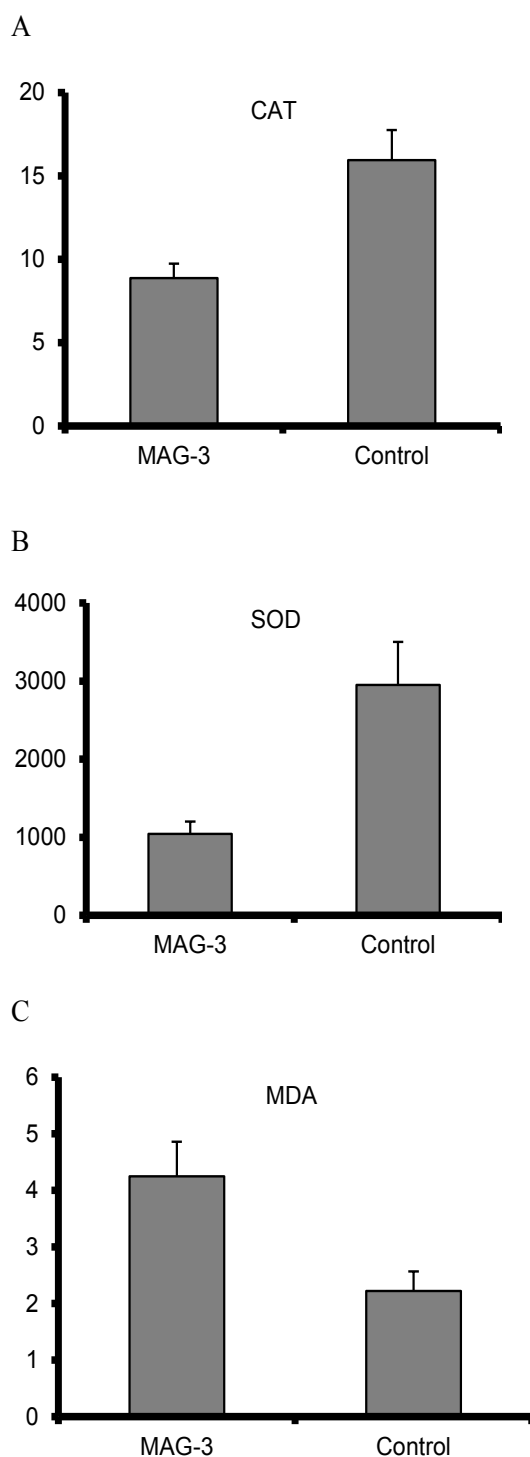


Figure 5. The activities of catalase (CAT); A. Superoxide dismutase (SOD); B. and malondialdehyde (MDA); C. Levels as oxidative stress biomarkers in third patient group and control group

NO, 3-NTx levels were higher in ^{99m}Tc -DMSA, ^{99m}Tc -MAG-3 administered patients and total renal scintigraphy performed patient groups than the control group ($p < 0.05$).

We reported that NO and 3-NTx levels were increased in patients who have performed thyroid scintigraphy with ^{99m}Tc pertechnetate and bone scintigraphy with ^{99m}Tc -MDP [17–18].

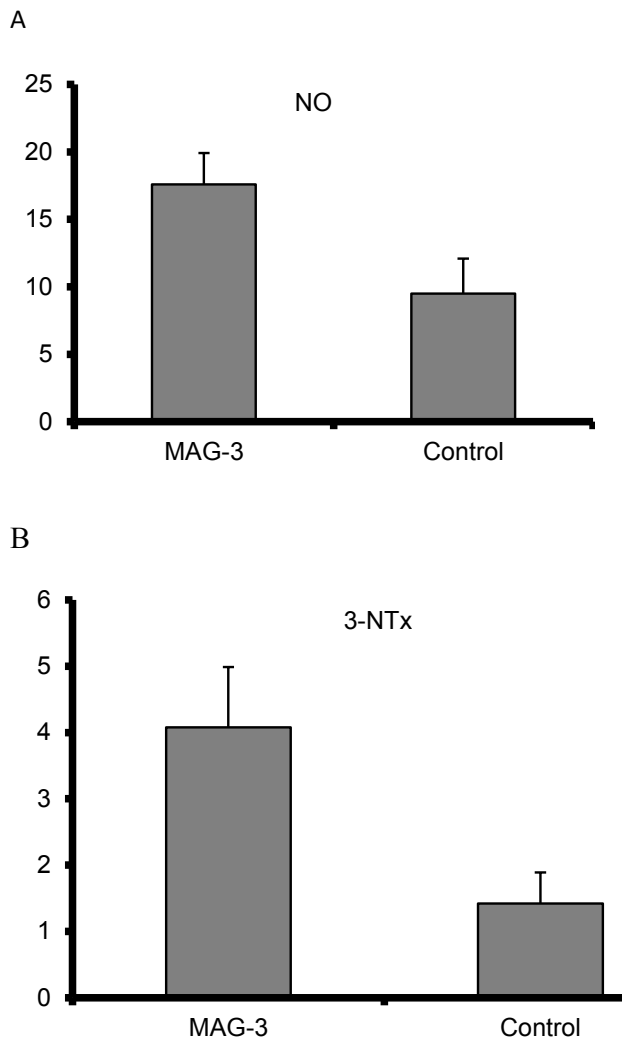


Figure 6. The activities of nitric oxide (NO); A. and nitrotyrosine (3-NTx); B. Levels as nitrosative stress biomarkers in third patient group and control group

Nitric oxide (NO) has one unpaired electron, hence it is accepted as a radical. NO is produced in biological systems via specific nitric oxide synthases (NOSs). NO plays an important role in various physiological conditions, such as neurotransmission, blood pressure regulation, defence mechanisms, smooth muscle relaxation and immune regulation. NO chemically reacts with oxygen and water, as a result of that nitrate and nitrite anions are produced. NO also reacts with superoxide anion and peroxynitrite (ONOO⁻) is produced. Reactivity of ONOO⁻ is higher than NO. ONOO⁻ reacts with proteins, thus nitrotyrosine (3-NTx) is produced. 3-NTx is a characteristic marker of nitrosative stress. It is known that NO and 3-NTx levels are increased in various diseases such as skin cancers, systemic lupus erythematosus, and atopic dermatitis [27].

NO, is produced by endothelial and endocardial tissue, has a significant effect on myocard and its vascular function. Coronary heart disease (CHD) is a chronic inflammatory process that becomes against vascular damage. Endothelial dysfunction causes CHD when endothelial cells cannot perform their functions including NO production. Furthermore, free oxygen

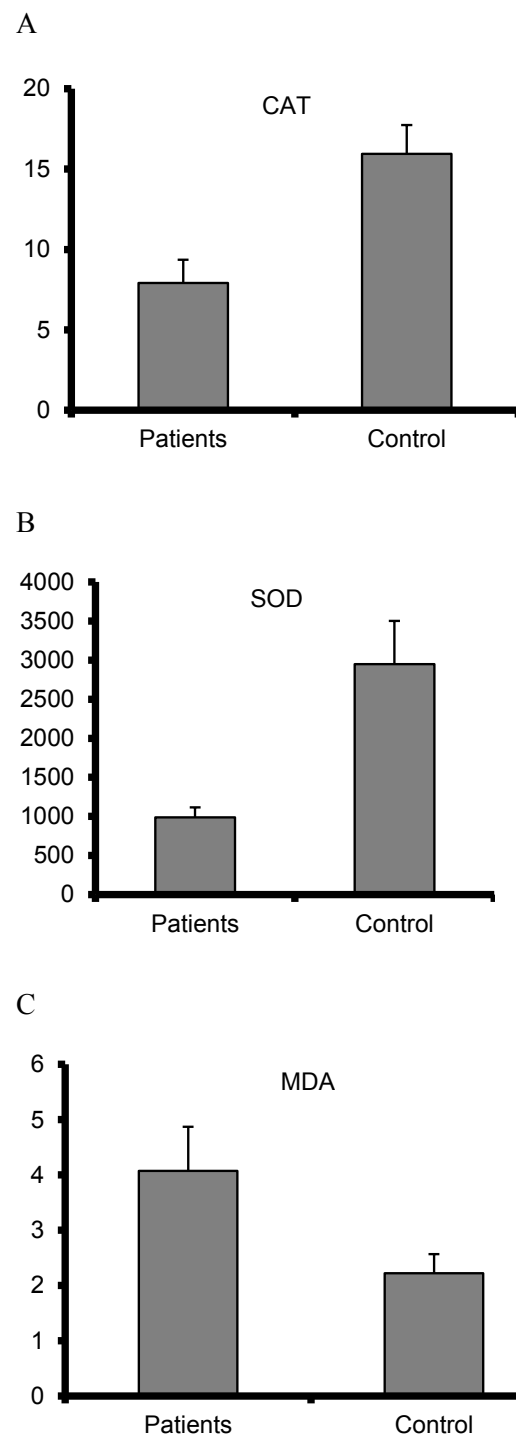


Figure 7. The activities of catalase (CAT); A. Superoxide dismutase (SOD); B. and malondialdehyde (MDA); C. Levels as oxidative stress biomarkers in total renal scintigraphy performed patient groups and control group

radicals negatively effect NO production in MPS performed patients. Because of that, NO levels might not statistically increased in ^{99m}Tc-MIBI administered group in this study [28–30].

This research, however, is subject to some limitations. There were an insufficient number of patients and number of blood samples. The samples were only taken after radiopharmaceutical

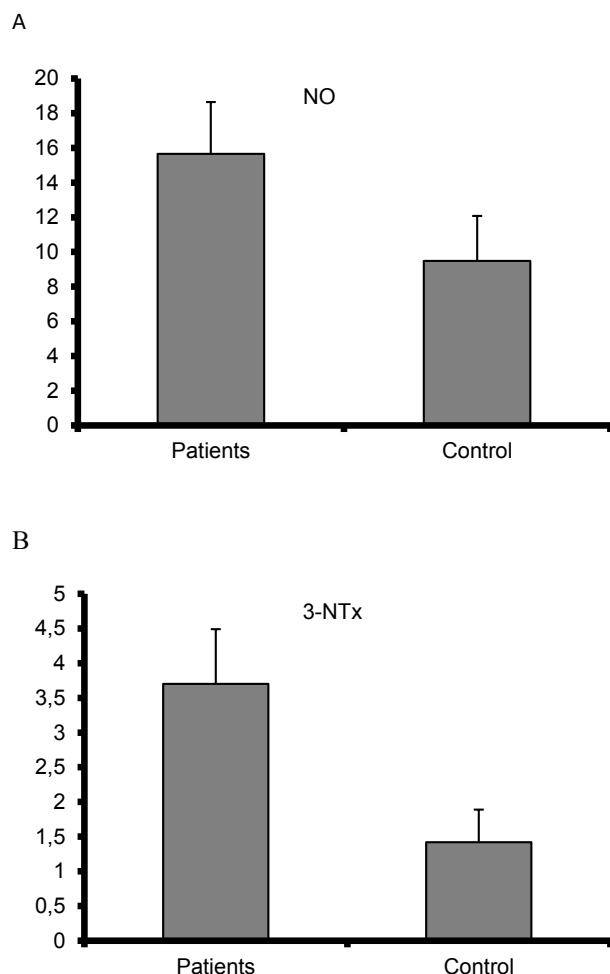


Figure 8. The activities of nitric oxide (NO); A. and nitrotyrosine (3-NTx); B. Levels as nitrosative stress biomarkers in total renal scintigraphy performed patient groups and control group

administration. If the samples were taken before agent injection, it would be more clear to understand whether oxidative/nitrosative stress parameters were changed due to ionizing radiation or cardiac and renal disease of patients.

Conclusion

Taking the results of current study into consideration, we able to show alterations in oxidative/nitrosative stress parameters due to ionizing radiation. Although, the effects of scintigraphic imaging methods on free radicals are yet to be completely elucidated, shortly after ionizing radiation exposure, cellular protective pathways are activated to overcome harmful effects of ROS/RNS production due to ionizing radiation. In the literature there are no studies evaluating nitrosative stress levels in patients who undergone MPS, dynamic renal scintigraphy and renal cortical scintigraphy. This present study demonstrated that ionizing radiation due to MPS, dynamic renal scintigraphy and renal cortical scintigraphy applications have different effects on antioxidant enzyme levels. All of these methods increased MDA levels and nitrosative stress parameters. Our study will contribute current literature understanding radiobiological

effects of scintigraphic imaging agents on especially nitrosative stress parameters. However, further studies in a larger number of the population are needed to fully understand this topic.

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