Özgün Araştırma / *Original Article*

Bezmialem Science 2016; 3: 106-9
DOI: 10.14235/bs.2016.872

Effects of Ionizing and Non-Ionizing Radiation on Oxidative Stress and the Total Antioxidant Status in Humans Working in Radiation Environments

Hakim ÇELİK[1], İsmail KOYUNCU[2], Ali Ziya KARAKILÇIK[1], Ataman GÖNEL[3], Davud MUSA[4]

[1]Department of Physiology, Harran University School of Medicine, Şanlıurfa, Turkey
[2]Department of Medical Biochemistry, Harran University School of Medicine, Şanlıurfa, Turkey
[3]Clinic of Medical Biochemistry, Akçakale State Hospital, Şanlıurfa, Turkey
[4]Division of Biology, School of Social Sciences and Humanities, Şanlıurfa, Turkey

ABSTRACT

Objective: This study was conducted to investigate the effects of ionizing radiation (IR) and non-ionizing radiation (NIR) on oxidative stress and the total antioxidant status (TAS) in men working in radiation environments.

Methods: The serum values of total oxidant status (TOS), malondialdehyde (MDA), and protein carbonyl (PC) in men exposed to radiation and a control group were determined. In addition, the values of the total antioxidant status (TAS) were measured in serum, and the oxidative stress index (OSI) was calculated to determine the oxidative stress. Data were analyzed by SPSS 20.0 (Chicago, IL, USA).

Results: While the serum values of PC, MDA, TOS, and OSI were significantly higher in the IR group than in the control group, those of TAS were significantly lower ($p<0.001$, $p=0.003$, $p<0.001$, $p<0.001$, and $p= 0.002$, respectively). The serum values of PC, TOS, and OSI were significantly higher in the NIR group than in the control group ($p<0.001$, $p=0.021$, and $p=0.010$, respectively). In contrast, there were no significant differences in the values of TAS and MDA ($p>0.05$ and $p>0.05$, respectively) in the same groups.

Conclusion: Based on these results, we determined that it had been damaged the balance between oxidants and antioxidant status in the IR and NIR groups. This effect of oxidative stress may cause a lot of damage to cellular macromolecules including lipids, proteins, and DNA.

Keywords: Ionizing radiation, non-ionizing radiation, total oxidant status, malondialdehyde, protein carbonyl, total antioxidant status, oxidative stress index

Introduction

The number of devices emitting electromagnetic (EM) waves has been continuously increases with the development of technology. These devices are present in airports, homes, schools, and hospitals (1).

The biological effects of EM radiation (EMR) vary depending on whether they are physical and particularly ionizing radiation (IR). IR forms a high-energy photon or alpha particle, proton, and neutron current, which can be fatal (2). Non-IR (NIR), which has a lower energy than IR, does not cause lethal ionization of atoms and molecules (2, 3). X-ray devices, computed tomography devices, radio surgical instruments (Gamma Knife, Cyber Knife), some sterilizers, and ultraviolet lamps emit IR. Devices such as televisions, radio transmitters, photocopiers, mobile phone base stations, microwave ovens, computer monitors, and wireless connection devices (WiFi) emit NIR (2).

Some types of IR and NIR trigger the formation of free radicals in living organisms. These free radicals may occur directly in critical biomolecules or indirectly in water and biomolecules. The vast majority of the biological effects caused by IR are considered to occur because of free radicals in water (4). IR indirectly damages nucleic acids, proteins, and lipids by increasing the formation of free radicals via the radiolysis of water (5). The vast majority of cellular damage caused by IR occurs either with direct exposure to radiation or immediately thereafter. However, because reactive oxygen species (ROS) continuously increase owing to radiation exposure, oxidative damage can last for days or even months (6).

Address for Correspondence: Hakim ÇELİK; Harran Üniversitesi Tıp Fakültesi, Fizyoloji Anabilim Dalı, Şanlıurfa, Türkiye
E-mail: hakimcell@gmail.com

Received : 06.02.2016
Accepted : 14.03.2016

ROS include hydroxyl (OH), hydroperoxide (HO_2.), superoxide (O_2.–) radicals, hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$). The OH. radical that results from Fenton reactions is the most reactive among ROS. HO_2. and O_2.– can be transformed into the reactive OH. radical via Haber-Weiss reactions. H_2O_2 easily passes through the nuclear membrane and transforms into the OH. radical via the Fenton reaction in the nucleus. The OH. radical causes damage in the deoxyribose and phosphodiester bonds in DNA. Moreover, the OH. radical also causes single and double chain fractures in DNA (2). The OH. radical leads to the fragmentation and aggregation of proteins by affecting the amino acid residues and prosthetic groups in enzymes. In addition, ROS disrupt the physicochemical properties of the cell membrane, causing lipid peroxidation (7, 8).

Oxidative stress is the disruption of the balance between free radical formation and specific antioxidants that neutralize the free radicals. The increase in ROS in cells occurs within a period ranging from a few minutes to a few hours after radiation exposure. The antioxidant production induced in the body after the increase in ROS attempts to reduce the harmful effects of ROS and restore the oxidative balance again (4).

As can be understood from the abovementioned data, IR and NIR cause protein, lipid, and DNA damage both directly and indirectly by increasing ROS. Using new and reliable methods, we aimed to investigate the oxidative damage caused by IR and NIR and investigate whether the antioxidants that are supposed to prevent this damage are in an equilibrium condition.

Methods

Our study was conducted with three groups comprising eight people each. Our study groups were arranged as: the control group, a group that does not work in IR and NIR environments and is least exposed to radiation; the IR group, X-ray technicians working in an environment exposed to IR; and the NIR group, photocopy workers working in a NIR environment for at least eight hours a day. After 8-12 h of overnight fasting, 5 mL of venous blood was taken and centrifuged at 3000 rpm. Serums were then placed in Eppendorf tubes and stored at –80°C until the time of operation. Written informed consent was received from each individual participating in the study. The ethics committee approval for the study was received from the ethics committee of Harran University School of Medicine.

Total Oxidant Level (TOL)
Serum TOL levels were studied by means of existing diagnostic kits on the market (Rel Assay, Gaziantep, Turkey). According to this method, the oxidants in the serum convert the ferrous ion to ferric ion. Ferric ions form a colored complex with xylenol orange in acidic medium. Glycerol accelerates this reaction in the medium. The intensity of the color associated with the amount of oxidants present in the serum was measured spectrophotometrically. H_2O_2 was used as a standard, and the results were calculated as μmol H_2O_2 equivalent/L (9).

Total Antioxidant Level (TAL)
Serum TALs were studied by means of available diagnostic kits on the market (Rel Assay, Gaziantep, Turkey). According to this method; the Fe2 + -o-dianisidine complex forms the OH radical by providing a Fenton-type reaction with hydrogen peroxide. This strongly ROS reacts with the colorless o-dianisidine molecule at low pH to form yellow-brown dianisidyl radicals. The O-dianisidyl radicals increase the color formation by involving the advanced oxidation reactions. However, the antioxidants in the samples stop the color formation by suppressing these oxidation reactions. After the samples were read spectrophotometrically, the results were calculated as mmol trolox eqv./L (10).

Oxidative Stress Index (OSI)
For the calculation of OSI, which is an indicator of oxidative stress, TOLs and TALs were first calculated in μmol. Then, the OSI was calculated according to the formula of OSI (AU)=[TOL μmol/L)/(TAL μmol/L)]×100 (11). In short, it was obtained by dividing TOL by TAL.

Malondialdehyde (MDA)
The serum MDA levels were measured spectrophotometrically using thiobarbituric acid reactive substances developed by Hedge et al. in order to determine the serum lipid peroxidation (12). We used 1,1,3,3-tetraethoxypropane as the standard and calculated the results as nmol/mL.

Protein Carbonyl (PC)
The protein oxidation in the serum was studied with Cayman's protein carbonyl measurement kit. The measurement method is based on the principle that the serum PC groups react with 2,4-dinitrophenyl hydrazine to form 2,4-dinitrophenylhydrazone.

Statistical analysis
A Windows-compatible Statistical Package for the Social Sciences 20.0 (SPSS Inc.; Chicago, IL, USA) package program was used for the statistical analysis. The data was evaluated with Kruskal-Wallis H. The Mann-Whitney U test was used for comparisons within the group. All data were calculated as mean±standard deviation. A p value of <0.05 was considered significant.

Results

The demographic data of the control, IR, and NIR groups are shown in Table 1. There was no significant difference among the groups in terms of age, gender, and body mass index ($p>0.05$). The oxidative stress parameters and antioxidant results are given in Table 2. The serum PC, MDA, TOL, and OSI levels were significantly higher in the IR group in comparison to the control group ($p<0.001$, $p=0.003$, $p<0.001$, and $p<0.001$, respectively). Serum TALs were significantly lower in the IR group than the control group ($p=0.002$). As

seen in Table 2, the serum PC, TOL, and OSI levels were significantly increased in the NIR group compared to the control group (p<0.001, p=0.021, and p=0.010, respectively), but the increase in the MDA level was not significant (p>0.05). Serum TALs were lower in the NIR group than in the control group, like the IR group, but it was found that this decrease was not significant (p=0.083). There was no significant increase or decrease in the serum PC level, MDA level, TOL, OSI value, and TAL between the IR and NIR groups.

Discussion

In tissues exposed to radiation, the oxidant/antioxidant balance is distorted, and macromolecules such as protein, DNA, and lipids, which are the basic building blocks of cells, undergo oxidation (14). Although radiation-based technologies are currently relatively safe and efficient and radiation sources are tightly controlled, people are exposed to IR during many routine activities in modern life (15). IR causes cell damage either directly by affecting the target molecules or indirectly by increasing the formation of the OH•, HO2• and O2•– radicals that cause dysfunction and death of cells (16).

To determine whether IR and NIR cause protein oxidation and lipid peroxidation by increasing ROS, OSI values were calculated by determining the PC level, MDA level, TOLs, and TALs in blood plasma. While serum TALs were significantly lower in the IR group than in the control group (p=0.002), a significant increase was observed in the PC level, MDA level, TOL, and OSI value compared with the control group (p<0.001, p=0.003, p<0.001, and p<0.001, respectively, Table 2). These findings indicate that free oxygen radicals increase and antioxidant levels decrease in the IR group and that the oxidative balance is impaired. These observations are consistent with those of previous studies.

We are constantly exposed to EM NIR via wireless internet, mobile phones, computers, some medical devices, and many other electronic devices. The difference between NIR and IR is that NIR does not have enough photon energy to break off electrons from atoms and molecules and break chemical bonds. There are several mechanisms that explain the biological effects of EMR in various tissues. However, the molecular mechanisms remain to be completely explained (17). One of the most appropriate hypotheses is the impairment of the normal balance between the antioxidant defense capacity and oxidative stress. Oxidative stress refers to the production of free radicals in the cell and the imbalance among the cell defense mechanisms. ROS are radicals that cause oxidative damage to molecules such as lipids, proteins, nucleic acids, and other cellular structures (18). As seen in Table 2, while no significant difference was found between the NIR and control groups in terms of serum TALs (p=0.083), PC levels, TOLs, and OSI values significantly increased in the NIR group (p<0.001, p=0.021, and p=0.010, respectively).

Conclusion

As a result, it was determined that the antioxidant status values decreased and ROS values increased in the IR and NIR groups, especially in the IR group. For this reason, it has been thought that the increased oxidative stress may cause damage in cellular proteins, lipids, and DNA by disrupting the oxidative/antioxidant balance in people exposed to radiation. Although the number of our cases is low, it has been concluded that it may be advisable to consume antioxidant-rich foods or drugs prescribed by specialist physicians in this area to reduce increased oxidative damage and the harmful effects of free radicals when exposed to IR and NIR. In addition, it may be helpful for relevant organizations to give radiation safety training at regular intervals to the people who work in areas exposed to radiation. Taking the necessary precautions is important and can be done by taking radiation measurements at regular intervals in the fields they work.

Table 1. Kontrol, İR ve NİR gruplarının demografik verileri

	Control (n=8)	IR (n=8)	NIR (n=8)	p values
Age (year)	25.1±3.9	30.1±5.0	26.2±5.3	ns
BMI (weight/height^2)	24.0±2.9	24.5±3.6	24.9±3.5	ns

BMI: body mass index; Ns: not significant; IR: ionizing radiation; NIR: non-ionizing radiation

Table 2. TALs and OSI values of the control, IR, and NIR groups

	Control (n=8)	IR (n=8)	NIR (n=8)	p values
PC (nmol/mg protein)	1.00±0.45	4.79±1.68¥	3.29±1.07 Ɛ	<0.001
MDA (nmol/mL)	13.51±3.27	20.37±3.44¥	16.24±3.52	0.011
TOL (µmol H_2O_2 eqv./L)	7.37±1.71	11,65±1.22¥	9.84±3.31 Ɛ	0.004
TAL (mmol trolox eqv./L)	1.28±0.14	0.87±0.27¥	1.01±0.38	0.026
OSI (arbitrary unit)	0.58±0.16	1.49±0.58¥	1.14±0.58 Ɛ	0.002

IR: ionizing radiation; NIR: non-ionizing radiation; PC: protein carbonyl; MDA: malondialdehyde; TOL: total oxidant level; TAL: total antioxidant level; OSI: oxidative stress index

¥: There is a significant difference between the IR and control groups.

Ɛ: There is a significant difference between the NIR and control groups.

Ethics Committee Approval: Ethics committee approval was received for this study.

Informed Consent: Informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - İ.K., D.M., A.G.; Design - İ.K., A.G., D.M.; Supervision - İ.K., A.G., D.M.; Funding - - İ.K., A.G., D.M.; Materials - H.Ç., İ.K.; Data Collection and/or Processing - H.Ç., İ.K., A.Z.K.; Analysis and/or Interpretation - H.Ç., İ.K.; Literature Review - H.Ç., A.Z.K.; Writing - H.Ç., A.Z.K., İ.K.; Critical Review - H.Ç., İ.K., A.Z.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Furtado-Filho OV, Borba JB, Dallegrave A, Pizzolato TM, Henriques JAP, Moreira JCF ve ark. Effect of 950 MHz UHF electromagnetic radiation on biomarkers of oxidative damage, metabolism of UFA and antioxidants in the livers of young rats of different ages. Int. J. Radiat. Biol 2014; 90: 159-68. [CrossRef]
2. Sowa P, Rutkowska-Talipska J, Sulkowska U, Rutkowski K, Rutkowski R. Ionizing and non-ionizing electromagnetic radiation in modern medicine. Polish Ann Med 2012; 19: 134-8. [CrossRef]
3. Schmid E, Schrader T. Different biological effectiveness of ionising and non-ionising radiations in mammalian cells. Adv Radio Sci 2007; 5: 1-4. [CrossRef]
4. Freitinger Skalická Z, Zölzer F, Beránek L, Racek J. Indicators of oxidative stress after ionizing and/or non-ionizing radiation: superoxid dismutase and malondialdehyde. J Photochem Photobiol B 2012; 117: 111-4. [CrossRef]
5. Azzam EI, Jay-Gerin JP, Pain D. Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. Cancer Lett 2012; 327: 48-60. [CrossRef]
6. Petkau A. Role of superoxide dismutase in modification of radiation injury. Br J Cancer Suppl 1987; 8: 87-95.
7. Karihtala P, Soini Y. Reactive oxygen species and antioxidant mechanisms in human tissues and their relation to malignancies. Apmis 2007; 115: 81-103. [CrossRef]
8. Dizdaroglu M. Oxidatively induced DNA damage: Mechanisms, repair and disease. Cancer Lett 2012; 327: 26-47. [CrossRef]
9. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005; 38: 1103-11. [CrossRef]
10. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004; 37: 112-9. [CrossRef]
11. Selek S, Aslan M, Horoz M, Gur M, Erel O. Oxidative status and serum PON1 activity in beta-thalassemia minor. Clin Biochem 2007; 40: 287-91. [CrossRef]
12. Hegde KR, Henein MG, Varma SD. Establishment of mouse as an animal model for study of diabetic cataracts: Biochemical studies. Diabetes Obes Metab 2003; 5: 113-9. [CrossRef]
13. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG ve ark. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990; 186: 464-78. [CrossRef]
14. Cooke MS, Evans MD, Dizdaroglu M, Lunec J. Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J Off Publ Fed Am Soc Exp Biol 2003; 17: 1195-214. [CrossRef]
15. Liu Y, Liu F, Yang Y, Li D, Lv J, Ou Y ve ark. Astragalus polysaccharide ameliorates ionizing radiation-induced oxidative stress in mice. Int J Biol Macromol 2014; 68: 209-14. [CrossRef]
16. Spitz DR, Azzam EI, Li JJ, Gius D. Metabolic oxidation/reduction reactions and cellular responses to ionizing radiation: A unifying concept in stress response biology. Cancer Metastasis Rev 2004; 23: 311-22. [CrossRef]
17. Kovacic P, Somanathan R. Electromagnetic fields: mechanism, cell signaling, other bioprocesses, toxicity, radicals, antioxidants and beneficial effects. J. Recept. Signal Transduct. Res 2010; 30: 214-26. [CrossRef]
18. Ceyhan AM, Akkaya VB, Güleçol ŞC, Ceyhan BM, Özgüner F, Chen W. Protective effects of β-glucan against oxidative injury induced by 2.45-GHz electromagnetic radiation in the skin tissue of rats. Arch Dermatol Res 2012; 1-7. [CrossRef]