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Assessment of Malondialdehyde, 8-iso prostaglandin F2α, 8-hydroxy-2'-deoxyguanosine Factors and Protein Carbonyl Groups as Markers of Oxidative Stress in the Fasting Individuals in Tehran

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ABSTRACT

Background: Oxidative stress causes intracellular imbalances and various diseases. Calorie restriction can reduce the damages caused by oxidative stress.
Method: The subjects, who were healthy males and females able to fast for a month, were randomly selected from the staff of Hippocrates and Imam Khomeini hospitals. Samples of their serum and urine were collected three times, i.e. 4 days before fasting as control, on the 14th day of fasting, and on the 29th day of fasting. Plasma malondialdehyde levels were measured using HPLC technique, while the levels of protein carbonyl groups in plasma, and the levels of 8-iso prostaglandin F2α and 8-hydroxy-2'-deoxyguanosine in urine were measured by means of ELISA technique. The data was analyzed using SPSS, Ver. 20 and the level of statistical significance was considered to be $p < 0.05$.
Results: Totally, 60 subjects participated in the study, where seven participants were male (11%) and 53 subjects (89%) were female. Mean and SD of the age of the participants were 37±10 years. Concentrations of plasma malondialdehyde and 8-iso prostaglandin F2α in urine showed a significant decrease during fasting in Ramadan in all subjects compared with the control samples; moreover, their levels in the third sampling round was significantly lower than that in the second one ($p < 0.01$). Except for the concentration of 8-hydroxy-2'-deoxyguanosine, which was higher in females, no significant differences was found in the levels of other parameters between males and females.
Conclusion: The findings of this study show that fasting for a month can reduce the progression of the diseases that oxidative stress is involved in. Further studies are needed to generalize these findings.
KEYWORDS: Fasting, Malondialdehyde, 8-iso prostaglandin F2α, 8-hydroxy-2'-deoxyguanosine, Protein carbonyl.

INTRODUCTION

Oxidative stress is caused by free radicals. This can affect various organs of the organisms and may result in the failure of such organs. The harmful effects of free radicals can be found in various diseases such as diabetes, Alzheimer's, Parkinson's and heart diseases (1). One way to reduce oxidative stress is adopting a limited diet and calorie restriction.

Calorie restriction results in reduction in oxidative products due to radicals and, consequently, reduces the effects associated with oxidative damage on brain function and resulted dysfunction of neurons in animal models with Alzheimer's, Parkinson's and Huntington's diseases (2-3).

Oxygen radicals cause damage to DNA that has a role in the internal carcinogenesis, and this is a strong confirmation of the induction of oxidative DNA damage in the early phase of a metastatic tumor (4). Previous studies have shown that generally, receiving more food and calories result in increased spontaneous tumor incidences, and shorten the life (5). On the other hand, increased oxidative stress and its byproducts cause damage to DNA. The measurement indicator of DNA damage in this study has been 8-hydroxy-2'-deoxyguanosine that is much effective on tumors and carcinogens (6).

In his study on diabetic patients who were undergoing treatment regimen, Skrhga showed that the plasma malondialdehyde (MDA) levels were markedly reduced (7). MDA levels in the urine samples of those who had low-calorie diet showed a significant decrease after the diet (8, 9). The levels of oxidative DNA damage markers such as 8- hydroxy deoxyguanosine (8-OH-dGUa) also significantly reduced after the diet. Holee's studies on short-term fasting also showed that after fasting the levels of MDA and 8-iso prostaglandin F2α (8-iso PGF2α) in urine were significantly lower than before fasting (9).

High levels of protein carbonyl, which is a biomarker for oxidation of proteins, have been reported in many diseases, including Alzheimer's, Parkinson's diseases, diabetes mellitus, rheumatoid arthritis, muscular

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dystrophy and kidney tumors (10). Fasting causes a series of changes in body, all of which lead to getting rid of the wastes and repairing the damage caused in the body, and the resulted detoxification properties can prevent cell death (11).

In this study, changes MDA levels and protein carbonyl groups in plasma as well as 8-iso prostaglandin F2α and 8-hydroxy-2'-deoxyguanosine in urine during fasting were studied as indicators of oxidative stress.

METHOD

The subjects were selected by means of random sampling method from the staff of Hippocrates Laboratory and Imam Khomeini Hospital. All individuals could fast and participated in this study voluntarily with their own consent. All participants were healthy according to the clinical examinations and had no underlying disease. Serum and urine samples from subjects were collected three times: before fasting as control, on the 14th day of fasting and on the 29th day of fasting.

Plasma MDA levels were determined using HPLC method, while 8-iso PGF2α and 8-OHdG in urine and protein carbonyl groups in plasma were studied using ELISA technique. In this study, the level of 8-iso prostaglandin F2α, protein carbonyl and 8-hydroxy-2'-deoxyguanosine were measured using ELISA technique, and the samples' absorbance was measured at the wavelength of 450 nm and the concentration of each sample was calculated against the standard curve. Malondialdehyde level was evaluated by means of HPLC technique and the samples' absorbance was measured the wavelength of 532 nm, and then the concentration of each sample was calculated using the standard curve.

ELISA protein carbonyl, 8-iso PGF2α and 8-OHdG kits branded "Cell Biolabs" (made in Korea) as well as other consumables were procured from Nozhin Teb Company. The data was analyzed using SPSS, Ver. 20 and the level of statistical significance was considered to be $p < 0.05$.

RESULTS

Totally, 60 subjects participated in the study, where seven participants were male (11%) and 53 subjects (89%) were female. Mean and SD of the age of the participants were 37±10 years.

Comparison of the mean concentrations of 8-iso prostaglandin F2α and malondialdehyde in three rounds are shown in Figures 1 and 2 respectively. The results indicated that in both cases, the samples of the second and the third rounds showed a significant decrease compared with the first time (control) ($p < 0.05$). The samples of the third time also showed a significant reduction comparing that of the second time ($P < 0.05$).

Figures 3 and 4 represent the concentration of protein carbonyl and 8-hydroxy-2'-deoxyguanosine in the three times. They indicate no significant differences in the concentrations of these parameters in the different periods of fasting.

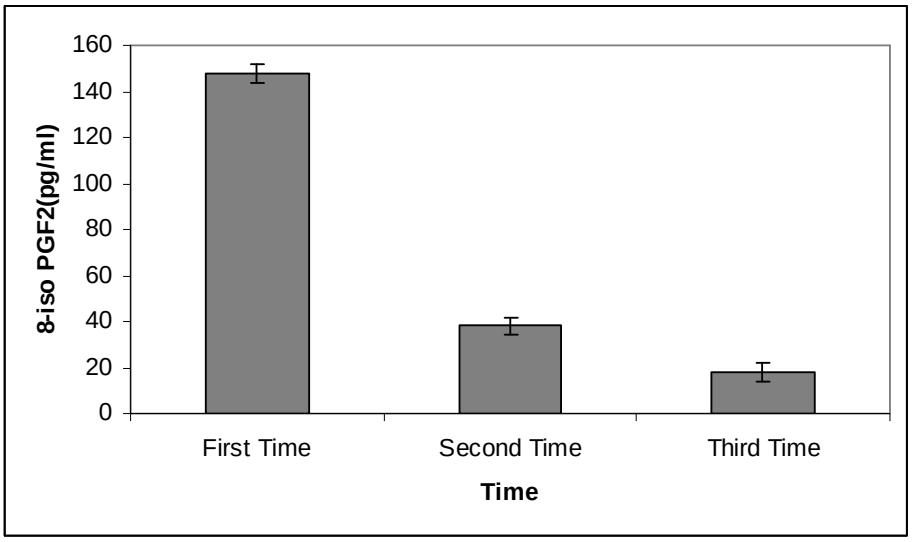

Figure 1: concentration of 8-iso prostaglandin F2α before, during and the end of Ramadan.

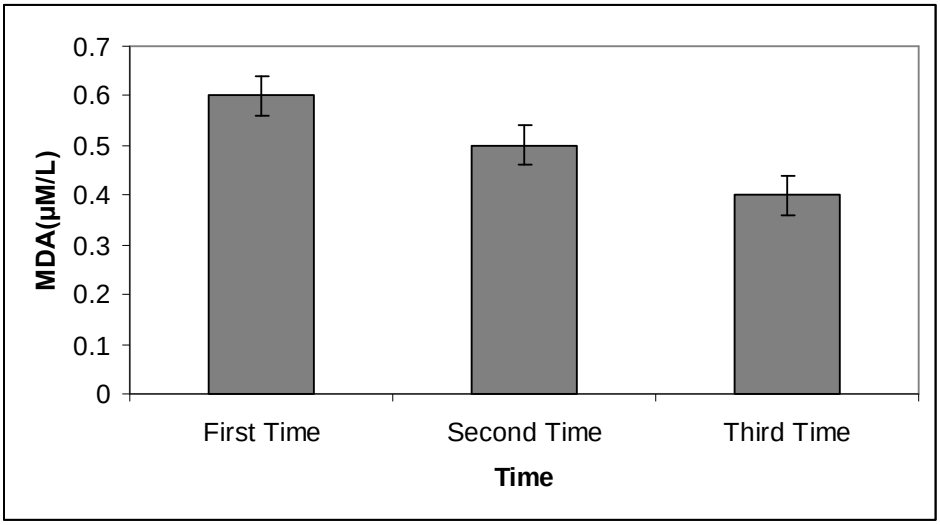

Figure 2: concentration of MDA before, during and the end of Ramadan.

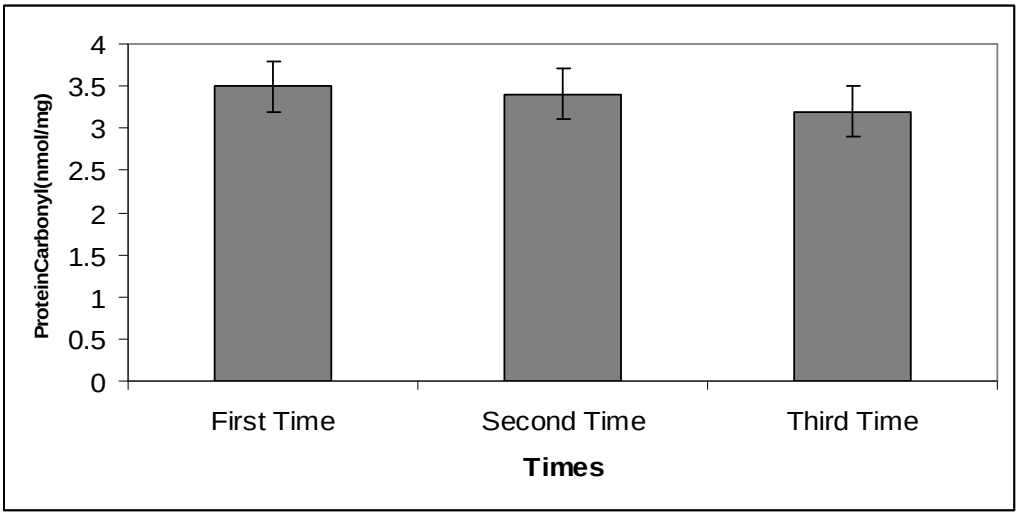

Figure 3: concentration of protein carbonyl before, during and the end of Ramadan.

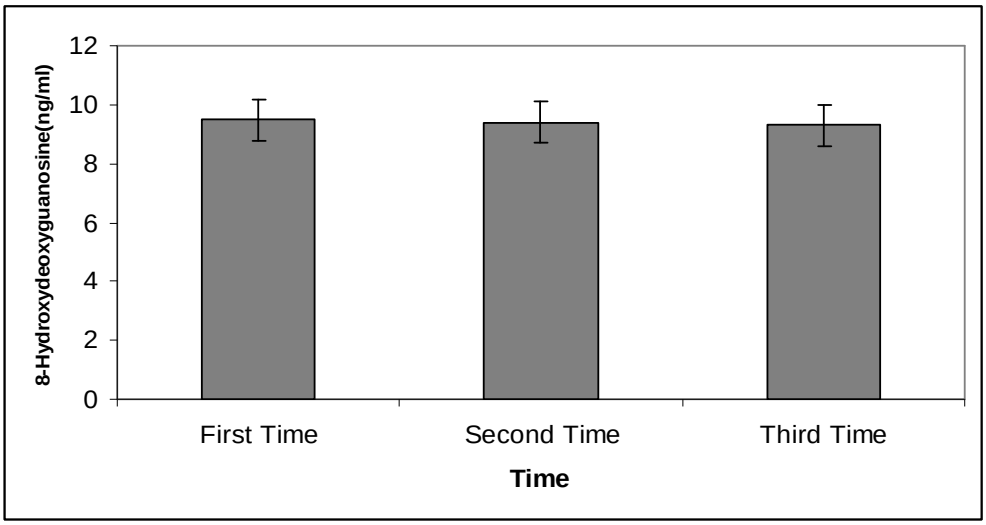

Figure 4: concentration of 8-hydroxy-2'-deoxyguanosine before, during and the end of Ramadan.

DISCUSSION

Given the widespread effects of increased oxidative stress in various diseases, as well as its effects on aging, the scientists have been concerned with oxidative stress reduction techniques for years. One of the ways to reduce oxidative stress and reactive species of oxygen and nitrogen is imposing dietary restriction and calorie restriction (CR). The study by Hook in 2006 showed that the levels of malondialdehyde and 8-iso prostaglandin F2α in urine reduced after fasting. However, such calorie restriction had no impact on the biomarkers of the oxidative damage to DNA (9). The results of the current study are consistent with these findings. The study by Ugocvhwu showed that calorie restriction will not reduce plasma MDA levels in diabetic rats, and such results are different from those of the present study, which can be due to the differences in the studies conducted on animals, rather than on human subjects (12). The study by Thompson (13) on urine samples from 28 women after 14 days of consumption of fruits and vegetables showed no change on the MDA level, and this is inconsistent with the findings of the present study. However, it measured MDA level in urine that is non-proprietary and non-sensitive and for the same reason; in this research plasma MDA levels were measured. Thompson's study showed 35% reduction in urine 8-iso prostaglandin F2α that is consistent with findings of the current study.

In the present study, the level of MDA in plasma and 8-iso PGF2α in urine during fasting showed a significant decrease compared with those levels before fasting, and during Ramadan they also decreased significantly compared to the previous periods that can be due to the reduction of oxidative stress because of decreased lipid peroxidation. Regarding the reduction of MDA, two causes can be noted: dietary restriction and reduced meal frequency decreases basal metabolic rate followed by a decrease in MDA. Here, it may be discussed that there is a difference between CR and fasting in Ramadan, since it is likely that the levels of calories remain unchanged before and after fasting. However, the studies regarding CR as "intermittent fasting" that alternates between periods of fasting, or reduced the number of meals in a day without any reduction in calories show that CR can be still useful in reduction of oxidative stress indicators (14).

In previous studies that have been performed on animals, a significant reduction in oxidative damage to DNA was observed after short-term fasting (15), while in this study, no change was found in 8-hydroxy-2'-deoxyguanosine, which may be due to the differences between animal and human studies, or other factors may be involved, e.g. differences between humans and animals regarding the ability to repair DNA. A previous study showed that the concentration of protein carbonyl levels in older people is more than that in younger people, which is caused by increased oxidative stress, proteins and lipids (16). The concentration of 8-iso PG F2α in urine and plasma protein carbonyl due to calorie restriction reduced significantly, however, the concentration of 8-hydroxy-2'-deoxyguanosine in the urine has been reported unchanged (17). The results of this research regarding the concentration of 8-iso PG F2α and concentration of 8-OHdGua in urine were consistent with our findings, while a different was observed regarding the levels of protein carbonyl groups in plasma due to calorie restriction. The period of calorie restriction in this paper was 6-month, and long-term calorie restriction had some effects on proteins and nucleic acids and reduced their oxidation, however in our study because of the short-term fasting, calorie restriction had no effect on protein carbonyl. It has been stated in previous studies that, through reduced substrate oxidation, CR reduces the respiratory chain and thus slow down the settlement of proton that ultimately reduced oxygen consumption. Moreover, CR reduced oxygen consumption as well as proton leak and consequently ROS (19).

CONCLUSION

The findings of this study show that fasting can significantly reduce oxidative stress biomarkers related to lipid peroxidation, MDA and 8-iso PG F2α; however, 8-OHdGua and protein carbonyl groups biomarkers produced in oxidative damage to DNA and proteins, respectively, showed no significant changes after fasting. Interpreting and generalizing the results regarding the slowing the path of neurodegenerative diseases or cardiovascular diseases, diabetes, kidney and liver failure, as well as the effects of CR on increased longevity require more and larger studies.

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