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Evaluation of Malondialdehyde Levels and Total Antioxidant Capacity in Patients with Hyperthyroidism

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Abstract

***Background & Aims*:** Oxidative stress is involved in the pathogenesis of many diseases. Oxidative stress and antioxidant imbalance conditions play a major role in the incidence and development of hyperthyroidism. The aim of this study is to evaluate malondialdehyde (MDA) levels as lipid peroxidation index and total antioxidant capacity (TAC) in hyperthyroid patients compared to control group.

Materials and Methods: A case-control study was performed on 35 women with hyperthyroidism and 35 healthy women as control group. Measurement of MDA serum levels was performed by Human Malondealdhyde (MDA) ELIZA KIT from Bioassay Chinese Company by ELIZA method, and serum level measurement was performed by GmbH Zellbio from Zellbio German Company using the ELIZA method.

Results: The serum MDA concentration in patients with hyperthyroidism (20.59 ± 2.50 nmol/mL) significantly increased (P = 0.001) compared to the control group (9.94 ± 2.12 nmol/mL), while the serum TAC concentration in the hyperthyroid group (0.769 ± 0.145) significantly decreased (P = 0.001) compared to the control group (1.63 ± 0.286).

Conclusion: In this study, total antioxidant capacity in patients with hyperthyroidism was reduced compared to control group, and levels of malondialdehyde increased. These findings indicate that the thyroid gland plays an important role in the production of oxidative stress in disease conditions. Therefore, it is recommended that thyroid hormone disorders and oxidative stress be used in the therapeutic strategies of hyperthyroidism.

***Keywords*:** Hyperthyroidism, Malondialdehyde, Total antioxidant capacity, oxidative stress

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Introduction

Hyperthyroidism is a common disease in the glands and metabolism, which affects all human body systems, including growth of the organs, cardiovascular system and nutrition. The thyroid hormone regulates the metabolic rate in the body. Studies have shown that blood components are also affected by thyroid hormone (1). Several studies have shown that thyroid gland

disorders result in changes in oxidant and antioxidant systems. Pathological disorders in the thyroid gland cause functional changes in various organs of the body. The findings from in vitro and in vivo studies have shown that thyroid hormones have a strong influence on oxidative stress(2).

The incidence of hyperthyroidism is about 3 per thousand, and it is 8 times more common in women. This disease can be due to the high levels of thyroid gland activity or other conditions, such as its acute or chronic inflammation(3). Hyperthyroidism is characterized by a decrease in serum thyrotropin levels despite the presence of free thyroxine and free triiodothyronine, which results in metabolic formation and acceleration of the production of free radicals to induce changes in the activity of antioxidant enzymes (4, 5). Aerobic organisms have antioxidant defense systems that reacts with reactive oxygen species resulting from the oxidation of substances in the respiratory chain. Active oxygen species include hydroxyl radical, superoxide anions and hydrogen peroxide that are produced in response to internal and external stimuli. Low levels of ROS are required for several biological processes, including intracellular differentiation and cell growth, or prevention of apoptosis, immunity and defense against microorganisms. In contrast, high doses or the inadequate removal of reactive oxygen species causes oxidative stress, which may result in damage to biological macromolecules (6).

Free radicals and antioxidant defense system disorders have a pathogenic effect on human tissues. Hence, they are recognized as important factors in the development of various diseases. Free radicals are atoms, ions, or molecules with one or more electron pairs in their outer orbits; therefore they are very reactive. These materials are also produced in normal metabolism through biochemical reactions (7). High concentrations of thyroid hormones affect oxygen metabolism and cause the formation of free radicals in the mitochondria. Reactive oxygen species play an important role in physiological mechanisms. However, highly reactive species result in oxidative stress in molecules. Reactive oxygen species that are formed in this way, if not eliminated by essential mechanisms, are toxic to the biological membranes and lead to lipid peroxidation. Malondialdehyde (MDA) is the natural product of the peroxidation of unsaturated fatty acids with three or more dual bands(8). Thyroid hormones increase the consumption of oxygen through thermogenic effects. In hyperthyroidism, increasing basic metabolism comes along with an increase in oxygen consumption and an increase in the microsomal oxidative capacity and free radicals formation. There are contradictory results about the increase or decrease of oxidative and antioxidant activity in hyperthyroidism(9). Some studies reported that the Superoxide Dismutase (SOD) activity significantly increases as one of the antioxidant indices in individuals with hyperthyroidism (10, 11). In contrast, several studies reported that SOD activity decreases in patients with hyperthyroidism (12, 13).

A study by Fahham et al. (2015) based on the correlation between thyroid hormones and oxidative stress in infertile women showed a significant decrease in T4 levels and a significant increase in TSH levels in the infertile women group compared with the control group. The results of this study also showed that there is a significant increase in the MDA level, a significant decrease in the activity of antioxidants, such as catalase, and a significant increase in GSH levels in the infertile women group compared to the control group. The results of this study showed that there is a significant negative correlation between MDA and TSH, and there is also a positive correlation between MDA and T4. Therefore, thyroid hormone disorders and increased oxidative stress seem to be effective factors for women's infertility (14). In a study by Cetinkaya et al. (2005) held on 22 women and 8 men with hyperthyroidism at 6 months and on 21 women and 9 healthy men as the control group with the aim to investigate oxidative stress in patients with hyperthyroidism without clinical symptoms, the levels of malondialdehyde plasma as lipid peroxidation and superoxide dismutase showed a significant increase ($P < 0.01$) in the patient group compared to the control group. The results of this study

showed that oxidative stress and the antioxidant response increase in patients with hyperthyroidism(5). A study by Laithy et al. (2016) based on the correlation between oxidative stress index and antioxidant levels in thyroid disorders, which was performed on 55 men (45 ± 10 years) in three groups (20 patients with hyperthyroidism, 20 patients with hypothyroidism and 15 healthy subjects as control group), showed that there is a significant negative correlation between malondialdehyde as a lipid peroxidation index and vitamins A and C as antioxidant indices in hyperthyroid patients. Therefore, the results of this study showed that there is a correlation between thyroid dysfunction and oxidative stress, which is due to the imbalance between the formation and neutralization of active oxygen species in people with thyroid dysfunction(15).

Materials and Methods

The present study is a case-control study. In this study, 35 women with hyperthyroidism aged 25-50 years who referred to Imam Hospital of Urmia University of Medical Sciences and who had been diagnosed with hyperthyroid by endocrinologist were registered as the case group after obtaining written informed consent, and the control group was 35 healthy people who had no symptoms of thyroid gland disorders. Inclusion criteria include hyperthyroidism and suppressed TSH, and exclusion criteria are patients with hyperthyroidism due to medication, pregnant women and age below 25 or over 50.

In the present study, malondialdehyde (MDA) as one of the most important biological markers and an overall index for lipid peroxidation was measured by ELIZA technique due to its high solubility in serum. By measuring total antioxidant capacity (TAC), there is also an estimate of the combination of different potential antioxidants in the body that interact with each other. It seems better to measure this index than measuring individual indices of antioxidant status (such as antioxidant enzymes SOD, CAT, GPX), because there is a complex interaction between the oxidants and antioxidant forces in the living environment, and what is estimated by this index is the net result of these interactions.

5cc venous blood samples were taken from all patients to measure MDA activity and total antioxidant capacity, and after centrifugation and serum isolation, samples were kept at -20 °C until analysis.

Malondialdehyde (MDA) Assay:

Malondialdehyde assay was performed by HumanMalondealdhyde (MDA) ELIZA KIT from Bioassay Chinese Company by the ELIZA method using the ELIZA Reader device. The basis of the assay was redox colorimetry. To do this, at first, 20 standards were mixed and diluted with 120 µl of diluent, then 50 µl standard and HRP solution (40 µl serum and 10 µl MDA antibodies) was added to each well. After washing and adding dyes A and B to the wells, a reaction stopping solution was added. At the end, samples' optical absorption was read by the device at 450 nm wavelength and unit conversion was done. The sensitivity of this kit is 0.14 nmol/ml and the diagnostic range is (0.2 - 60 nmol/ml).

Total antioxidant capacity assay:

To measure the total antioxidant capacity, the German Zellbio measurement kit was used with the ELIZA method on the Eliza Reader device, where the basis of the assay was redox colorimetry. The kit contains 1 ready-to-use reagent, X 100 buffer, dye powder, reaction stopping solution, standard and a 96-well microplate. In this assay, the amount of the total antioxidant capacity, equivalent to an amount of antioxidant in the sample, was compared with ascorbic acid as a standard. The kit's sensitivity was equal to 0.1 mM and the diagnostic range was (0.125 - 2 mM). The final absorbance was read at 490 nm and unit conversion was performed.

Statistical analysis of data was performed using SPSS software. All quantitative variables were expressed as mean and standard deviation. T-test was used to compare the mean of quantitative variables in the two groups, and Pearson correlation coefficient was used to investigate the relationship between quantitative variables. The statistical significance level was defined as $P < 0.05$.

Results

In this study, the levels of MDA and TAC in serum samples of patients with hyperthyroidism and control group were studied. Laboratory results showed that MDA levels in patients with hyperthyroidism were different in comparison with the control group. The mean and standard deviation of MDA were 20.59 ± 2.50 nmol/ml in the group of patients with hyperthyroidism and 9.94 ± 2.12 nmol/ml in the control group. Statistical comparison showed a significant difference between the two groups in terms of MDA ($P < 0.05$). As shown in the figure, the MDA level in the patient group is significantly higher than the control group (Table 1). Furthermore, the results of the analysis of the obtained values of TAC indicate that the mean and standard deviation of the patient group is 0.769 ± 0.145 mM/L, and the mean and standard deviation of the control group is 1.63 ± 0.286 mM/L. Statistical comparison showed a significant difference between the two groups ($P < 0.05$) (Table 2). The results of the experiments indicate that there is correlation between MDA and TAC indices in the patient and control groups (Table 3).

Table 1: Descriptive statistics markers of malondialdehyde(serum malondialdehyde concentration) measurement in the control group and the group of patients with hyperthyroidism

Hyperthyroidism Status MDA	Sample number	concentration Concentration mean MDA (nmol/ml)	Concentration SD MDA maximum (nmol/ml)	Concentration median MDA (nmol/ml)	Concentration minimum MDA (nmol/ml)	(nmol/ml)
The patient group	35	20.5971	2.50722	20.1000	16.10	25.70
The control group	35	9.9429	2.12911	9.2000	6.90	15.10

MDA=Malondialdehyde, SD=Standard deviation

Table 2: Descriptive statistics markers of Total antioxidant capacity (serum Total antioxidant capacity concentration) measurement in the control group and the group of patients with hyperthyroidism

Hyperthyroidism Status	Sample number	concentration mean TAC (mM)	Concentration SD TAC (mM)	Concentration median TAC (mM)	Concentration minimum TAC (mM)	Concentration maximum TAC (mM)
The patient group	35	0.7694	0.14594	0.7400	0.49	0.99
The control group	35	1.6371	0.28603	1.7000	1.10	1.20

TAC= Total antioxidant capacity, SD=Standard deviation

Table 3: correlation between MDA and TAC indices in the patient and control groups

		MDA	TAC
MDA	Pearson Correlation	1	-.775**
	Sig. (2-tailed)		.000
	N	70	70
TAC	Pearson Correlation	-.775**	1
	Sig. (2-tailed)	.000	
	N	70	70

**. Correlation is significant at the 0.01 level (2-tailed).

Discussion and Conclusion

Hyperthyroidism or thyrotoxicosis is a clinical condition due to excessive increase in thyroid hormones, particularly triiodothyronine (T3) and thyroxine (T4). The most common cause of hyperthyroidism is toxic goiter or Graves' disease (16). Under normal circumstances, epithelial cells of the thyroid have a moderate production of reactive oxygen species (ROS) that are physiologically required for the formation of thyroid hormones. These are not necessarily toxic because they are continuously toxicized by the synthesis of the hormone or the endogenous antioxidant system(17). It is deduced that oxidative stress occurs when reactive free radicals cause oxidative damage to the macromolecular structures of the cell. The thyroid gland plays an important role in producing general oxidative stress in conditions of the disease (18).

Hypermetabolic status in hyperthyroidism is associated with oxidative tissue damage. Thyroid hormones play a role in regulating the status of basic metabolism and in oxidative metabolism. These hormones can lead to high changes in the number and activity of mitochondrial respiratory chain compounds, which may result in increased production of reactive oxygen species (ROS) (19, 20). Oxidative stress is a general term used to describe the condition of damage caused by ROS(20, 21). These materials can lead to oxidative damage in cellular macromolecules, such as lipids, proteins, and DNA(22). In fact, the cell contains various materials that are capable of destroying free radicals and protecting them from harmful effects. These materials include enzymatic antioxidants, glutathione reductase (GR), glutathione peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD), while non-enzymatic antioxidants are glutathione (GSH), vitamin E, vitamin C, beta-carotene and flavonoids(23). When ROS production exceeds the antioxidant capacity of the cell, oxidative stress occurs. Oxidative processes occur mainly in the mitochondria(24). On the other hand, the mitochondria are interesting targets of thyroid hormones. During the synthesis of thyroid hormones, a stable production is absolutely necessary(25). Experimental studies and epidemiological data have shown that hyperthyroidism is associated with an increase in the production of free radicals and lipid peroxidation levels(26). In recent years, the possible correlation between thyroid gland disorders and reactive oxygen species has been increasingly taken into account. Considering that the association of oxidative stress and thyroid hormone disorders is very less studied in Iran, and since the thyroid gland plays an important role in regulating the basic metabolism, thyroid hormone disorders and increased oxidative stress seem to be effective factors in many other diseases. To this end, this study was conducted so that its results be used in the therapeutic strategies of hyperthyroidism.

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