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Catalase and peroxidase in diabetic patients with hypothyroidism

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***Abstract*---**The production of free radicals is a major component of the oxidative stress that occurs in biological systems. This is linked to the development of various diseases, including diabetes and hypothyroidism. The presence of these free radicals can also affect the functioning of the antioxidant enzymes. Catalase is an important component of the antioxidant enzymes that protect cellular structures from the damage caused by free radicals. It also neutralizes the reactive oxygen species by preventing them from reacting with metal catalysts. The study was conducted to investigate the effects of Catalase on the activities of various antioxidants in people with diabetes and hypothyroidism. Depression of metabolism by hypothyroidism has been reported to decrease oxidant production and thus protect tissues against oxidant damage. The study included 120 subjects divided into four groups; the first group included 30 healthy control subjects, the second group includes 30 patient's hypothyroidisms with type 2 DM, the third group included 30 patient's hypothyroidisms only and the fourth group included 30 patients with type 2 DM only. The concentration of (determination the activity of serum peroxidase and determination the activity of serum catalase (antioxidant), determination the activity of serum malondialdehyde (marker for oxidative stress) and (fasting Serum glucose FSG) Triiodothyronine T3, Thyroxin T4, thyroid-stimulating hormone TSH. The results show significant differences in level of peroxidase between all studied groups that including diabetic patient hypothyroidism, hypothyroidism only, DM only and control group [(105.25±8.18), (168.25±9.18), (126.64±10.30), (301.36±15.32)]. The

results show significant differences in level of catalase between all studied groups that including diabetic patient hypothyroidism, DM only, hypothyroidism only and control group [(7.01±2.01), (8.14±2.15), (8.97±1.94), (13.19±1.82)]. The results show significant differences in level of malondialdehyde between all studied groups that including diabetic patient hypothyroidism, DM only, hypothyroidism only and control group [(1.07±0.07), (0.88±0.05), (0.75±0.07), (0.57±0.03)].

***Keywords*---**Antioxidant enzyme, Peroxidase, malondialdehyde, Catalase, diabetic, oxygen free radicals.

1. Introduction

Low levels of thyroid hormone (TH) and high levels of thyroid-stimulating hormone (TSH) in the blood indicate hypothyroidism, a metabolic disorder. TH regulates the metabolism of lipids and glucose. According to earlier research, TH boosts the synthesis of cholesterol and other lipids, and enhances the clearance of plasma cholesterol and triglycerides from the bloodstream (TG)[1]. In diabetes, insulin secretion abnormalities or peripheral tissue insulin lead to persistent hyperglycemia and impaired carbohydrate, lipid, and protein metabolism, which is a heterogeneous kind of metabolic illness [2]. Increased glucose levels in the blood are a common consequence of uncontrolled diabetes, and this can lead to long-term damage and dysfunction in a variety of organs and systems such as the eyes, kidneys, heart, and blood vessels and nerves [3].Antioxidant: Protective antioxidants, such as Catalase (CAT), Glutathione peroxide (GPx), Glutathione reductase, Superoxide Dismutase (SOD), and several minerals, including as manganese (Mn), iron (Fe), selenium, and copper, constitute the first line of defense against oxidative stress (Cu). The antioxidant vitamins E, C, flavonoids, and uric acid, which scavenge free radicals, serve as a secondary line of defense. third line of defense: de-novo enzymes and repair [4]. A decrease in the antioxidant standard can be used as an indicator of oxidative stress and as a means of determining the human body's antioxidant defense system [5]. Endogenous Antioxidants: The enzymes or cofactors that remove ROS are known as endogenous antioxidants. In the fight against free radicals, the three enzyme systems that play a significant role are GPx, SOD, and CAT. [6].Exogenous AntioxidantsPolyphenols, vitamins and their derivatives, and antioxidant minerals are all examples of exogenous antioxidants, which can be broken down into three classes. [7]. Peroxidase, an enzyme that converts reactive oxygen species (H_2O_2) to innocuous substances (H_2O) and protects red blood cell membranes, can be secreted by the serum, mammary, and mucosal glands. Peroxidases can be found in the liver, spleen, salivary glands, stomach wall, leucocytes, intestinal mucosa, and other organs and tissues. Among its many biological roles is the elimination of hydrogen peroxide, which protects cells from oxidative damage and serves as a defense mechanism for the host [8,9]. Free radicals may be neutralized by antioxidant defense mechanisms in mammalian cells, which may prevent lipid peroxidation [10]. Oxidant stress can cause cells to differ in their ability to fight back, which could be due to changes in the mix of oxidants and antioxidants in these cells. For the cells' ability to withstand diverse kinds of free radicals, the cellular radical – scavenging systems comprise chemicals, minerals, and enzymes

such as peroxidase, superoxide dismutase, and catalase [11]. One of the most essential antioxidant enzymes is catalase (EC 1.11.1.6). Heme monofunctional catalases, heme catalase-peroxidases, and manganese catalases are the three groups. In catalase enzyme, the heme active site is responsible for catalytic reactions [12].It's found in practically every aerobic organism. The molecular mass of the enzyme is around 220-240 kDa. In the structure of catalase, a channel plays a vital role in transporting *the substrate H_2O_2 to the active site [13]. Catalase converts two hydrogen peroxide molecules into one oxy*gen molecule [14].Free Radical; Free radicals are unpaired electrons in atoms, molecules, or ions that are highly unstable and active in chemical interactions with other molecules. They are made up of three elements: oxygen, nitrogen, and sulfur. The term "reactive species" has now been expanded to include reactive chlorine, bromine, and iron species [16], [17]. Oxidative Stress (OS): a pathophysiologic imbalance between oxidants and antioxidants in favor of the oxidants [18]. a disruption in redox signaling and control or a pathophysiologic imbalance between oxidants and antioxidants in favor of the oxidant's OS can be caused by a reduction in antioxidant enzymes or antioxidant levels, as well as abnormalities in the antioxidant machinery involved in the expression of antioxidant genes [17].

2. Experimental

The study included 120 subjects, divided into four groups; the first group included 30 patients with T2DM with hyperthyroidism hypo+T2DM, the second group includes 30 patient's hypothyroidisms only hypo, the third group included 30 patients onlyT2DM, and the fourth group included 30 healthy control subjects. This study was carried out with the approval of the Department of Chemistry, College of Science, and samples were collected from the national diabetes center, Al-mustansiriyah University.120 blood samples were collected, was transferred into a gel tube and allowed to clot at room temperature for 30-60 minutes. Then centrifugation at 3000rpm for 15 minutes to separate the serum. 1 ml of serum was used to determine FSG and 1.5 ml of serum used for further investigation (T3, T4 and TSH). The remained was transferred to the Eppendorf tube and stored in a deep freezer (-20°C) to be used for Determination (activity of serum peroxidase, activity of serum catalase, Malondialdehyde). The Thyroid hormone assay (TSH, T4 and T3) by using Vida's Instruments and Biomatrix kit.
Determination of the activity of serum peroxidase activity was assayed by measuring the increase in absorbance at 510nmdue to the oxidation of 4-aminoantipyrine [19]. The assay mixture contained 1.5ml of 1.7mM H_2O_2 in phosphate buffer (0.2M and pH7),1.4ml of (0.0025M) 4-aminoantipyrine 0.17M phenol, and 0.1ml of serum sample. The assay was performed at 25°C and was started by the addition of the enzyme.
The increase in the absorbance at wave length λ=510nm, was recorded for 5min.to obtain ΔA/min.

Table (1) The level of glucose in control and patient

Group	Mean± SD	SE	Min-Max	*p*-value		
				Hypo	DM	DM+Hypo
Control	87.37±6.87	1.25	72.0-98.0	0.975	0.0001*	0.0001*
Hypo	92.03±17.81	3.25	76.0-182.0	-	0.0001*	0.0001*
DM	245.23±61.43	11.22	150.0-385.0	0.0001*	-	0.043*
DM+ Hypo	215.57±57.19	10.44	139.0-356.0	0.0001*	0.043*	-

Significantat$P \leq 0.05$.

Determination the activity of serum catalase: Catalase activity was measured using a simple and accurate colorimetric approach [20] based on the reaction of hydrogen peroxide with ammonium metavanadate in an acidic environment to create a red-orange color with a maximum absorbance of 452 nm.Determination of Malondialdehyde the final product of lipid peroxidation, malondialdehyde (MDA), interacts with thiobarbituric acid to generate a color material. The most extensively used approach for measuring lipid peroxidation is the detection of MDA by thiobarbituric acid reactivity [21].

Results and Discussion**:**The result of the present study as shown in Table (1) significant the level of FSG ($p<0.001$) increasedin DM only group (245.23±61.43 mg/dl), diabetic withhypothyroidism Group (215.57±57.19 mg/dl), diagnosed hypothyroidism group only (92.03±17.81 mg/dl), as compared to control group (87.37±6.87 mg/dl).

Theresult of the present study as shown in Table (2) and (3) nonsignificant decrease in the means of (T3, T4) in patients groups compared to control group.The result of the present study as shown in Table (4). was significant increase ($p<0.001$) in the level of TSH in hypothyroidism group, diabetic with hypothyroidism group, as compared to DM only and control group.The table (5) shown the level of serum peroxidase significant increased ($p<0.001$) in control group as compared to patients' group, also The result of the present study as shown in Table (6) The level of serum catalase significantincreased ($p<0.001$) in control group as compared to patients' group.The result of the present study as shown in Table (7) Thelevelof serum MDA decreased in control group as compared to patients' group.Lower levels of total peroxidase activity were found in the current research, which agrees with the findings of a study on this enzyme activity in patients with thyroid gland cancer [22,23]. The lower peroxidase activity seen in the benign and malignant groups compared to the normal group could be due to the enzyme's product inactivating it over time.

Catalase is an important antioxidant enzyme that aids in the breakdown of hydrogen peroxide and the maintenance of cellular redox equilibrium. In

hypothyroidism and diabetic patients, malondialdehyde levels are widely used as a measure of oxidative stress and antioxidant status. Malondialdehyde (MDA) is a by-product of the peroxidation of polyunsaturated fatty acids in cells. Overproduction of free radicals is caused by an increase in free radicals.

Table (2) ThelevelofT3incontrolandpatients

Group	Mean± SD	SE	Min-Max	p-value		
				Hypo	DM	DM+Hypo
Control	1.50±0.34	0.06	1.10-2.50	0.775	0.967	0.995
Hypo	1.41±0.34	0.06	1.0-2.20	-	0.960	0.894
DM	1.46±0.40	0.07	0.90-2.50	0.960	-	0.997
DM+ Hypo	1.48±0.38	0.07	0.80-2.40	0.894	0.997	-

Significantat$P \leq 0.05$

Table (3) ThelevelofT4incontrolandpatients

Group	Mean± SD	SE	Min-Max	*p*-value		
				Hypo	DM	DM+ Hypo
Control	91.97±15.78	2.89	74.0-125.0	0.979	0.993	0.826
Hypo	93.33±14.16	2.59	64.0-122.0	-	0.910	0.967
DM	91.03±9.40	1.72	76.0-120.0	0.910	-	0.673
DM+ Hypo	94.93±13.40	2.45	73.0-124.0	0.967	0.673	-

Table (4):The level of TSH in control and patients

Group	Mean± SD	SE	Min-Max	*p*-value		
				Hypo	DM	DM+Hypo
Control	2.17±0.74	0.13	1.0-3.30	0.0001*	0.832	0.0001*
Hypo	7.94±1.38	0.25	5.80-10.0	-	0.0001*	0.957
DM	1.96±0.69	0.13	1.0-3.50	0.0001*	-	0.0001*
DM+Hypo	7.81±1.0	0.18	6.0-9.30	0.957	0.0001*	-

Table (5) The activity of peroxidase in control and patients

Group	Mean± SD	SE	Min-Max	*p*-value		
				Hypo	DM	DM+Hypo
Control	301.36±15.3	2.80	280.79-339.21	0.0001*	0.0001*	0.0001*
Hypo	126.6±10.30	1.88	94.22-141.34	-	0.0001*	0.0001*
DM	168.25±9.18	1.68	150.76-181.85	0.0001*	-	0.0001*
DM+ Hypo	105.25±8.18	1.49	94.22-122.49	0.0001*	0.0001*	-

Table (6). The activity of catalase in control and patients

Group	Mean± SD	SE	Min-Max	*p*-value		
				Hypo	DM	DM+Hypo
Control	13.19±1.82	0.33	9.90-17.62	0.0001*	0.0001*	0.0001*
Hypo	8.97±1.94	0.35	6.11-12.87	-	0.374	0.001*
DM	8.14±2.15	0.39	2.77-13.81	0.374	-	0.130
DM+ Hypo	7.01±2.01	0.37	2.84-10.65	0.001*	0.130	-

Table (7).:The level of MDA in control and patients

Group	Mean± SD	SE	Min-Max	*p*-value		
				Hypo	DM	DM+Hypo
Control	0.57±0.03	0.01	0.52-0.63	0.0001*	0.0001*	0.0001*
Hypo	0.75±0.07	0.01	0.65-0.86	-	0.0001*	0.0001*
DM	0.88±0.05	0.01	0.78-0.99	0.0001*	-	0.0001*
DM+ Hypo	1.07±0.07	0.01	0.96-1.19	0.0001*	0.0001*	-

This study also agreed with a study done by Since diabetes Meletus type two (also type one) is characterized by elevated oxidative stress, which is one of the processes that contribute to the development of chronic diabetes complications [24], However, while it is thought that diabetes is related to the increased formation of reactive oxygen species (ROS), findings on the antioxidant defense in diabetes are conflicting [25,26].

While Thyroid hormone is a major regulator of oxygen consumption and mitochondrial energy metabolism, and During oxidative metabolism in mitochondria, highly reactive oxygen species (ROS) such as hydroxyl radical, superoxide anion and hydrogen peroxide are naturally generated in small amounts as byproducts. If not disposed off quickly, they can attack biomolecules in their vicinity and cause impairment of cellular functions. However, aerobes are endowed with antioxidant defense enzymes and small antioxidant molecules to neutralize ROS generation in order to keep the cells in norm oxidant condition (27).

Conclusions

Thyroid disease and type 2 diabetes mellitus both have oxidative stress as a contributing factor (T2DM). Catalase and peroxidase are antioxidant enzymes, and their activity is required for defense against reactive oxygen species damage. MDA levels that are higher in both patient groups indicate enhanced lipid peroxidation, which may play a role in the etiology of atherosclerosis.

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