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Study of arginase activity in serum of patients with hypothyroidism and type 2 diabetes mellitus

Sura jumah Abdand

University of Mustansiriyah, College of Science, Chemistry Department

Email: mustafa.sura@yahoo.com

Dr. Salma Abdul_Rudha Abbas

University of Mustansiriyah, College of Science, Chemistry Department

Email: Dr.salma_ar@yahoo.com

Dr. Baydaa Ahmed abed

University of Mustansiriyah, College of Science, Chemistry Department

Email: bayaaahmed@yahoo.com

Abstract---In L-arginine metabolizing pathways, arginase and nitric oxide synthase are regulated in a reciprocal manner. In recent years, nitric oxide has been proposed as a key player in hyperthyroidism. The importance of arginase in arginine control has not yet been studied in hypothyroidism. The goal of this study was to examine the activity of arginase in in type 2 diabetic patients with and without hypothyroidism. The two most popular endocrinopathies that exist in clinical practice are thyroid disease and diabetes mellitus DM. The thyroid hormones, on the other hand, control the metabolism of carbohydrates and the pancreas activity. The study group consisted of 120 subjects divided into four groups; the first group included 30 healthy control subjects, the second group included 30 patients with both hypothyroidism and type 2 DM, the third group included 30 patients with hypothyroidism without type 2 diabetes (T2DM) and the fourth group included 30 patients with T2DM without hypothyroidism. The concentration of fasting serum glucose (FSG), total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), very low-density lipoprotein (VLDL), triiodothyronine (T3), thyroxine (T4), thyroid-stimulating hormone (TSH) were determined. The results showed significant differences in arginase activity between all studied groups, ~~that~~ including T2DM patients with hypothyroidism, T2DM patients without hypothyroidism, and hypothyroidism patients, compared to control group [(26.70±0.32), (24.60±0.18), (18.91±0.20), (17.03±0.25)U/ml]. In

conclusion, arginase activity is considered as new predictor of type 2 diabetes mellitus with hypothyroidism.

Keywords---Arginase, hypothyroidism, type 2 DM, Thyroid Gland, Nitric oxide.

Introduction

Thyroid gland is an endocrine organ that produces and secretes thyroid hormones, and it is situated in the front, inferior part of the neck [1]. Anterior to the trachea, the thyroid can be found beneath Adam's and apple. The isthmus connects each side's two thyroid lobes in the organ's middle. The functions of thyroid hormones are critical. It oversees a wide range of metabolic activities throughout the body. The structure or function of the thyroid can be affected by a variety of thyroid diseases [2]. Infants' thyroids weigh about 1g and grow by about 1.5g per year until they are 15 years old [3]. grams per year until the Thyroid hormones are released into the bloodstream by the thyroid gland. Thyroid dysfunction can lead to a variety of health issues, including weight gain, changes in brain chemistry, depression, fatigue, and even heart disease [6]. It plays an important role in the metabolism, growth, development, and preservation of the interior environment [7]. It is vital for the formation of many tissues, such as brain gut bone skeletal muscle and the auditory system further to maintenance of tissue function throughout life and is a crucial role in the control of protein, glucose and thermogenesis [8].

Arginase Kossel and Dakin discovered Arginase (EC 3.5.3.1), the arginine hydrolytic enzyme, in the mammalian liver in 1904 [9] The hydrolysis of Larginine is catalyzed by a binuclear manganese metalloenzyme to form urea and ornithine. The enzyme consists of two isoforms, Arg I and II. Arg I is a cytosolic trimeric protein with a total size of 34,700 Da and is expressed in erythrocytes in human and higher primates. Arginase II is as well a mitochondrial trimeric protein of a total dimensions of 36,100 expressing in extrahepatic tissue such as the small intestine, brain, kidney, macrophages and monocytes [10]. Arginase II is synthesized as a preprotein and imported into mitochondria Arginase, which is mainly found in liver, kidney and erythrocytes, converts L-arginine to urea and ornithine and thus decreases substrate availability for NOS to produce NO• [11].

Both arginase isoforms are present in human plasma and are both increased in inflammatory conditions such as pulmonary hypertension and sickle cell disease [12], [13] In the early1930s, the identifying of the Krebs – Hensel urea cycle emphasized the importance of arginase; the individual hydrolytic function of arginase was previously known However, arginase interdependence and other biochemical pathways shown in the urea cycle piqued scientists' interest. Despite the fact that arginase was initially found mainly in mammalian livers [14] and to a lesser degree in kidneys [15], this enzyme also found in tissues that did not have a urea cycle [16].

1. Experimental

The study involved 120 subjects divided into four groups; the first group included 30 healthy control subjects, the second group included 30 patients with both hypothyroidism and type 2 DM, the third group included 30 patients with hypothyroidism without type 2 diabetes (T2DM) and the fourth group included 30 patients with T2DM without hypothyroidism. Blood samples were collected from the national diabetes center, Al-mustansiriyah University during the period of October 2021 to January 2022. Using disposable syringes, 5 ml of blood was drawn slowly through a vein puncture and then the blood translocated into gel tube carefully and slowly then left for 15 min at room temperature to clot. The gel tubes' samples were centrifuged for 10 minutes at 3000 rpm and four Eppendorf tubes used to store the obtained serum at -30 °C until the time of analysis. Some information were taken from patients and control, which include height, weight and age. The laboratory portion of the study was carried out at the Biochemistry Research Laboratory of Mustansiriyah University, department of chemistry science. healthy control group: Age Factor, Body Mass Index BMI and Gender Respectively:(43.43±8.41years), (25.19±1.57 kg.m⁻²) and (7male+23female).

hypothyroidism without type 2 diabetes (T2DM) Age Factor, Body Mass Index BMI and Gender Respectively:(45.97±7.10 years), (26.80±1.72 kg.m⁻²) and (5male+25female).

T2DM without hypothyroidism Age Factor, Body Mass Index BMI and Gender Respectively: (46.20±5.40 years), (26.23±1.93 kg.m⁻²) and (4male and 26 female).

both hypothyroidism and type 2 DM: Age Factor, Body Mass Index BMI and Gender Respectively:

(42.33±8.51 years), (28.13±2.14 kg.m⁻²) and (7male and 23 female).

hypothyroidism without type 2 diabetes (T2DM): Age Factor, Body Mass Index BMI and Gender Respectively:(45.97±7.10 years), (26.80±1.72 kg.m⁻²) and (5male+25female).

1.1. Serum arginase activity

Arginase activity was determined using the Roman and Ruy method [17]. In a fact, a reaction mixture of 10 mM carbonate bicarbonate buffer (pH 10), 2 mM MnCl₂, 130 mM L-arginine, and enzyme solution in a total volume of 1 ml was incubated at 37 °C for 30 minutes. By adding 10% TCA, the reaction was stopped. Centrifugation was used to extract the protein, and 0.5 ml of the supernatant was combined with 1 ml of anhydrous glacial acetic acid and ninhydrin reagent. For 20 minutes, the mixture was immersed in a boiling water bath. The produced bright red color was diluted with 2 mL of glacial acetic acid. The reagent blank was performed in parallel with the control test, and the results were compared. The optical density was calculated at 515 nm on spectrophotometer (UV 160A Shimadzu, Japan.) against reagent blank. The activity of arginase was expressed in units. A unit of an enzyme activity is defined as that amount of an enzyme, which produces one µmole of L-ornithine per min at 37°C.

1.2. Serum thyroid hormones and lipid profiles One millilitre of serum was used to determine FSG and lipid profiles, and the second 1.5 mL of serum was used for further investigation (T3, T4 and, TSH). All biochemistry (FBG, TC, HDL, LDL, and TG) measurements were done by kenza 240 TX (Biolab o) instrument

and Biolabo kits. The thyroid hormone assay (TSH, T4 and T3) was performed by using a Vida Instruments and Biomatrix kit.

Statistical analysis

Data were presented as (mean $\pm$ SD). The data were compared by SPSS version 26. Using multivariate and Pearson correlation. The differences between four groups were analyzed by multivariate, p-value equal or less than 0.05 considered significant, and non-significant when ($p > 0.05$).

Results and Discussion

Table 1 demonstrates a significant ($p < 0.0001$) increase in the arginase in hypothyroidism group, T2DM only group, diabetic with hypothyroidism compared control group. The enzyme arginase increases in hypothyroidism and diabetes, which causes a decrease in nitric oxide (NO), which causes atherosclerosis. The level of FSG was a significantly ($p < 0.001$) increase in patients compared to the control group. Thyroid disease and diabetes mellitus are the two most common endocrine diseases seen in clinical practice. Diabetes and thyroid issues have been linked for a long time, and relationships between the result of the present study as shown in Table 2 demonstrated no significant decrease in the level of (T3 and T4) in patients group compared to the control group. However, there was significant increase in the level of TSH in the hypothyroidism group, diabetic with hypothyroidism group, as compared to the T2DM only group and control group. Thyroid disease is more common in patients with diabetes mellitus, especially those with poor glycemic control (22). The result of the present study as shown in Table 3 demonstrated a significant increase in the means of total cholesterol (TC) in diabetic patients with the hypothyroidism group, and the hypothyroidism only group, T2DM only, as compared to the control group. The level of TG showed a significant increase in the diabetic with hypothyroidism group, and T2DM only group, hypothyroidism group as compared to the control group. HDL levels were significantly lower in patients as compared with the control group.

Table 1: Arginase activity and FSG level in the experimental groups

Variables	Control (n =30)	HYP0 (n =30)	DM (n =30)	HYP0+DM (n =30)
Arginase U/ml	17.03 $\pm$ 0.25	18.91 $\pm$ 0.20 ^{a,c}	24.60 $\pm$ 0.18 ^a	26.70 $\pm$ 0.32 ^a
FSG (mg/dl)	87.37 $\pm$ 6.87	92.03 $\pm$ 17.81 ^c	245.23 $\pm$ 61.43 ^a	215.57 $\pm$ 57.19 ^a

Abbreviations: HYP0, hypothyroidism; T2DM, type2 diabetes mellitus; HYP0+DM, hypothyroidism with type 2 diabetes mellitus; FSG, fasting serum glucose. Data expressed as means $\pm$ SEM. P-value < 0.05 is significant. ^a $P < 0.005$, ^b $P < 0.0001$ compared with the control group, ^c $P < 0.0001$ compared with DM group (n = 30 in each group)

Table 2: Thyroid function test

Variables	Control (n =30)	HYPO (n =30)	DM (n =30)	HYPO+DM (n =30)
T3 (nmol/l)	1.50±0.34	1.41±0.34	1.46±0.40	1.48±0.38
T4 (nmol/l)	91.97±15.78	93.33±14.16	91.03±9.40	94.93±13.40
TSH(μmol/ml)	2.17±0.74	7.94±1.38 ^{a,c}	1.96±0.69	7.81±1.0 ^{a,c}

Abbreviations: HYPO, hypothyroidism; T2DM, type2 diabetes mellitus; HYPO+DM, hypothyroidism with type 2 diabetes mellitus; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone. Data expressed as means ± SEM. P-value <0.05 is significant. ^aP < 0.005, ^bP < 0.0001 compared with the control group, ^cP < 0.0001 compared with DM group.

Table 3: Comparison of Serum Lipid Profiles

Variables	Control (n =30)	HYPO (n =30)	DM (n =30)	HYPO+DM (n =30)
Total Cholesterol (mg/dl)	124.03±19.44	191.47±34.68 ^{b,c}	188.23±32.76 ^b	192.73±37.84 ^b
Triglyceride (mg/dl)	99.70±25.64	181.20±59.14 ^b	165.50±47.89 ^b	189.57±54.05 ^b
HDL (mg/dl)	39.30±6.95	32.37±8.76 ^a	34.03±9.45	34.57±6.17
LDL (mg/dl)	64.79±20.62	122.86±34.93 ^b	121.10±29.69 ^b	120.25±41.43 ^b
VLDL (mg/dl)	19.94±5.13	36.24±11.83 ^b	33.10±9.58 ^b	37.91±10.81 ^b

Abbreviations: HYPO, hypothyroidism; T2DM, type2 diabetes mellitus; HYPO+DM, hypothyroidism with type 2 diabetes mellitus; HDL, High-Density Lipoprotein, cholesterol; LDL, Low-Density lipoprotein as means ± SEM. P-value <0.05 is significant. ^aP < 0.005, ^bP < 0.0001 compared with the control group. ^cP < 0.0001 compared with DM group (n = 30 in each group)

The level of LDL was significantly increased in the hypothyroidism group, T2DM only group, and diabetic patients with hypothyroidism, as compared to the control group. The level of VLDL was significantly increased in the hypothyroidism group, DM group, and patients with the combination of hypothyroidism and DM group. In type 2 diabetes mellitus, defective insulin secretion may lead to various abnormalities such as impaired hemostasis of lipoproteins, fatty acids, TG, and depressed the activity of lipoprotein lipase activity (23). Similar results were reported by Kumar and co-workers (24), who found that the mean total cholesterol and LDL levels were elevated in hypothyroidism patients compared to controls. Thyroid dysfunction is highly common in patients with a longer duration of diabetes, and poor glycemic control and is associated with substantially higher serum cholesterol and triglyceride levels (25). The result of correlation showed that there was a significant association between Glucose and Arginase. There is no significant correlation between T3 and T4 with Arginase, but there was a significant association with TSH. was a significant association between TC and Arginase.

In addition, correlation was performed to test whether there was association between TGs and Arginase. The result of correlation showed that there was a significant positive association between TGs and Arginase there was no significant association between HDL and Arginase. but a significant association between LDL and Arginase. At last, a significant association between VLDL and Arginase.

Conclusions

Arginase activity was significantly ($p < 0.0001$) increased in the diabetic patients with hypothyroidism group and in T2DM only group, diabetic with hypothyroidism group compared control group. There is an increase in enzyme arginase activity in patients compared to controls, therefore, monitoring arginase activity in hypothyroid patients is recommended.

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