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Measurement of TSHER gene expression in patients with hypothyroidism

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Abstract
Hypothyroidism (HD) is a common disorder of the Endocrine glands in which the thyroid gland Specifically does not produce enough thyroid hormones. In this study, we addressed Most important functional genes associated with an increased or decreased risk of hypothyroidism. To achieve this goal, we used quantitative PCR-RT based gene expression measurement. The study was conducted on 50 Woman with HD, 50 patients without any chronic diseases were used as a control group. For women with HD, clear clinical features of HD and abnormal results of routine tests of thyroid function were observed. Chemical tests showed an increased concentration of thyroid stimulating hormone (TSH) and low for the hormones T4, T3 in the serum in this group. As for measuring the percentage of lipids in serum, it was observed that lipids were increased percentage of fats (Triglycerides (TG) and cholesterol (CHOL)) in the serum of women with HD. The results of molecular analysis showed a high significance (P≤0.01) of gene expression of TSHER gene for women with HD compared to healthy women.

Keywords: *TSHER gene, Triglycerides, Thyroid stimulating hormone*

1- Introduction

HD can be defined as low of TSH in the thyroid gland. Primary hypothyroidism refers to decreased thyroid hormone secretion by the thyroid gland due to several factors affecting the thyroid gland itself (1); decreased serum thyroid hormone concentrations lead to increased TSH secretion (2). Decreased thyroid hormone secretion by the thyroid gland can also occur due to insufficient stimulation of the thyroid gland by (TSH) because of to factors that directly interfere with TSH release from the pituitary gland (secondary hypothyroidism) or indirectly by reducing TRH release from the hypothalamus (tertiary hypothyroidism) (3); it is not always possible to distinguish between secondary and tertiary hypothyroidism, and therefore they are often referred to as central hypothyroidism Which affects adults (4). Mutations in other genes involved in extrathyroidal metabolism and action of thyroid hormones in target tissues may also cause HD (5). The wide range of symptoms of HD suggest an effect on metabolism and dysfunction of multiple organ systems (6). Untreated hypothyroidism can contribute to high blood pressure, dyslipidemia, infertility, cognitive impairment, and neuromuscular dysfunction. The clinical presentation of HD is variable, but includes symptoms such as cold intolerance, fatigue, constipation, hair loss, dry skin, and other symptoms of a slow metabolism (7).

2- Materials and methods of work

Sample Collection

This study was conducted in Karbala Governorate after obtaining the approval of the Scientific and Ethical Committee. The study continued for the period from November 2023 to April 2024, where 100 blood samples were collected from the participants after obtaining written consent from them, including a detailed explanation

of the purpose of the study. The samples obtained were 50 blood samples from the reviewers who were visiting the advisory clinics in the Imam Hussein (PBUH) Medical City and who were diagnosed with hypothyroidism, while the other 50 blood samples were obtained from people who were not suffering from hypothyroidism and were treated as a control group

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Estimation of Serum Thyroid Stimulating Hormone

TSH concentrations were measured quantitatively in the laboratory by an immunoassay. The ECLIA photochemical immunoassay is designed for use with the device Cobas e 411. It was used kit is based on photoelectrochemical measurement. As with total T4, the method for measuring total T3 in serum is a competitive chemiluminescence immunoassay using the 100-sample test kit from Roche diagnostics kit

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Determination of Serum TG - CHOL Concentration

Serum TG levels were measured using a special kit linear chemicals cromatest / Spain TG - CHOL Kit.

Molecular Detection

RNA Extraction

TransZol Up Plus RNA Kit from TransGen Biotech Co., Ltd., China was used for RNA extraction according to the method of (8,9).

Estimation of RNA concentration and purity

The samples were diluted to a concentration of 20ng/µl and used in the reaction as in the equation
$M1V1^{=}M2V2$

Real-time quantitative polymerase chain reaction

The volumes of chemicals used in the reaction are shown in Table (2)

Volume	Component
10 µl	qPCR Master Mix
0,4 µl	RT Mix Buffer at concentration
0.3µl	CXR Reference Dye, 30µM
1.6µl	MgCl2 , 25mM
µl 2	Forward primer
µl 2	Reverse primer

Adjust all add-ons, transfer samples to the device, and adjust the program as in Table (3).

Cycle	Durataon	Temp.(°C)	Steps
1	10 minutes	37°C	Reversetranscription
40	15 seconds	95.0 °C	RTinactivation_Hot-start activation

40	10 Seconds 30Seconds 30 Seconds	95°C 60°C 72°C	3.StepqPCR a.denature b.Anneal-collect data c.Extend
1		60-95C°	Dissociation

Specific Primers Sequence used for PCR amplification

The specific primers shown in Table (4) were designed to determine the specific sequence of the genetic segments of the genes under study, of course, according to the standard specifications of the National Center for Biotechnology and Biotechnology (NCBI).

References	Sequence (5'→3')	Direction	Primer
Hyeon-Gun Jee.,et al.,2019)(	5′-GCTTTTGAAGGGACATGCAATGAA-3′; , 5′-AAGGGCCAGTGACACTG GTTTGAGA-3′	Forward Reverse	TSHER

3- Results

Age and weight percentage of women with and without HD

Women with HD in Karbala governorate were divided into three categories. For the first category, age (<40) was (34.00%). The second category, age (40-50), was (18.00%). Finally, the third category, age (>50), was (48.00%). The significance was high (P≤0.05). As for the weights, it was also Dividing it into three groups for patients: the first category for weights (<70) under a percentage of (22.00%), the second category (70-80) a percentage of (32.00%), and the third category for weights (>80) a percentage of (46.00%).

Biochemical tests

Measuring level of TSH and T3, T4 hormones

Table (4) showed a significant increase in TSH level (P>0.05) compared to healthy people. A significant increase was Showed in the level of T3, T4 hormones (P≤0.01).

Table 4: shows the total concentration of thyroid hormone levels in patients and control

Group	Means ±SE		
	TSH (Nmol/L)	T4 (ng/dl)	T3 (ng/dl)
Patients	11.56 ±0.62	108.57 ±1.97	1.119 ±0.03
Control	11.15 ±0.68	146.56 ±2.57	2.433 ±0.55
T-test	1.834 NS	6.424 **	1.089 **
P-value	0.659	0.0001	0.0019
** (P≤0.01), NS: Non-Significant.			

Measurement of serum lipid levels

Table (5) shows a significant increase in the level of TG and CHOL in women with HD (P≤0.01).

Table(5) : shows the levels of TG and CHOL in patients and control

Group	Means ±SE	
	CHOL (mg/dl)	TG (mg/dl)
Patients	222.36 ±3.23	191.90 ±4.38
Control	171.76 ±1.48	89.30 ±2.52
T-test	7.065 **	10.047 **
P-value	0.0001	0.0001
** (P≤0.01).		

-Molecular screening

The results of polymerase chain reaction (PCR) test shown in Table (6) showed a statistically significant increase (P≤0.01) in the level of gene expression in women with HD compared to healthy individuals.

Table (6) Gene expression level of TSHER gene in PCR

Group	B actin	TSHER	ΔCt	ΔΔ Ct	Fold change
Control	20.287	30.312	10.026	-1.024	1.00 ±0.00
Patients	23.642	31.766	8.123	-2.926	3.74 ±0.29
T-test	--	--	--	--	0.944 **
P-value	--	--	--	--	0.0052
** (P≤0.01).					

4- Discussion

Age and obesity index in women with hypothyroidism

Obesity is more common with advancing age in women with hypothyroidism. In fact, most of the factors affecting the endocrine system and metabolic disorders in women with hypothyroidism can lead to an increase in the body mass index (10). The researcher (11) mentioned that obese women have obesity associated with an increase in the concentration of CRP protein, which increases with increased gene expression of the TSHER gene. Obesity is associated with increased stress (12).

Study of hormonal changes

The results of the current study showed a significant increase in the level of TSH hormone and a clear decrease in the level of T3, T4 in the blood serum of women with HD. Thyroid hormone receptors regulate many major physiological processes for the body. Therefore, HD may lead to the emergence of a myriad of clinical signs and symptoms (13). The severity of these symptoms generally reflects the degree of thyroid dysfunction and the timeline of HD progression. Increased secretion in general of TSH hormone leads to weight gain, fatigue, poor concentration, depression, widespread muscle pain, and menstrual disorders (14). The defect in the secretion of thyroid hormones is attributed to increased gene expression of the TSHER gene. This study has reinforced the existence of a linear relationship between TSHER gene expression and HD (15).

High levels of fat in women with HD

The results of the current study showed high levels of fat (TG and CHOL) in the blood serum of women with HD compared to healthy people. Studies also confirmed an increase in fat cells in the body that secrete interleukin-6I,

which leads to fat accumulation with metabolic dysfunction and a very much increase in the secretion of TSH hormone, resulting in obesity (16).

The relative quantitative study of TSHER gene expression

The present study recorded a significant increase in TSHER gene expression in women with HD. Accordingly, the increase in body mass with age leads to an increase in gene expression, and also in fat cells of individuals suffering from diabetes and a number of diseases, blood pressure and heart diseases, in addition to fatigue and stress, are many factors that lead to an increase in expression (17).

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