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Measurement of *SLC5A5* Gene Expression in Patients with Hypothyroidism

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P A P E R I N F O

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A B S T R A C T

This study determines the amount of gene expression level of the *SLC5A5* gene in women with hypothyroidism (HD), as it is one of the key genes influencing the development of the condition. Additionally, some risk factors were examined, including diabetes, hypertension, heart diseases, prior thyroidectomy, and family genetic history, to assess the impact of these factors on the prevalence of HD. Peripheral blood (PB) samples were obtained from 50 women with HD who were followed up by medical diagnosis and whose ages ranged from (40 to 70) years. 50 samples of healthy women who do not suffer from chronic diseases were selected as a control group. Polymerase chain reaction (qPCR-RT) technique was used to quantify expression *SLC5A5* gene levels in the samples, as well as to measure some hormonal criteria for patients with thyroid disease to demonstrate their importance in diagnosing the disease. The level of thyroid stimulating hormone (TSH) and the thyroid hormones thyroxine (T4) and triiodothyronine (T3) were measured. The results showed no statistically significant on the expression of the *SLC5A5* gene in women with hypothyroidism compared with the healthy group. In contrast, the results of thyroid hormones showed a significant decrease in both triiodothyronine (T3) and thyroxine (T4) and non-significant effect of TSH. Additionally, the findings indicated that factors such as family history, diabetes, hypertension, and heart disease contribute to the development of HD. In conclusion, risk factors such as family history, diabetes, hypertension, and heart disease were found to play a role in the development of the condition, emphasizing the multifactorial nature of HD.

1. INTRODUCTION

Hypothyroidism (HD) is a disorder characterized by an inability of the thyroid gland to produce enough thyroid hormone that performs metabolic processes [1]. Untreated HD can contribute to high blood pressure, non-equilibrium, infertility, cognitive impairment, and neuromuscular dysfunction. Its prevalence increases with age and it affects females more than males [2].

HD may result from primary thyroid failure or insufficient production stimulation of the thyroid gland by the hypothalamus or pituitary gland [3]. Primary Thyroid insufficiency can cause congenital abnormalities, autoimmune destruction (Hashimoto's disease), and iodine deficiency, HD affects growth, development, and cellular functions [4]. Also, disorders include association with transient HD that includes postpartum thyroiditis, subacute thyroiditis and silent

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thyroiditis [5]. The reasons are usually the same. HD is usually present with other manifestations of hypothalamic or pituitary dysfunction, and they stand out low or abnormal levels of TSH compared to insufficient thyroid hormone [6]. Thyroid disorders include hyperthyroidism, thyroid nodules, and thyroid cancer, but the most common disease is HD [7].

Genes play a pivotal role in determining susceptibility to hypothyroidism, as mutations or multiple genetic variations across different loci in key genes contribute to an individual's risk of developing the condition. Among the most prominent genes associated with thyroid function are *Solute Carrier Family 5 Member 5* (*SLC5A5*), *NK2 Homeobox 1* (*NKX2-1*), *Thyroid Peroxidase* (*TPO*), and *Thyroid-stimulating Hormone Receptor* (*TSHR*). These genes are linked to essential thyroid functions, including thyroid differentiation, iodide regulation, thyroglobulin synthesis, iodide transport, and iodide deiodination [8,9]. The *SLC5A5* gene is specific to the thyroid gland that plays a key role in in regulating iodide transport to thyroid follicular cells, as it encodes the sodium-iodide symporter (NIS). This transporter moves iodide from circulating blood to follicular cells, enabling the thyroid gland to synthesize hormones [10,11]. Mutations in the SLC5A5 gene disrupt iodide transport, leading to impaired thyroid hormone synthesis and resulting in hypothyroidism [12].

2. MATERIALS and MEsTHODS

2.1. Sample Collection

The study had been conducted in Karbala Governorate from November 2023 to April 2024. A total of 100 blood samples were collected and divided into two groups. The first 50 samples were obtained from the outpatient clinics at Imam Hussein (PBUH) Medical City and had included individuals diagnosed with HD, while the second 50 samples were from healthy individuals (control group).

2.2. 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 Cobas e 411. The 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.

2. 3. Determination of Serum TG Concentration

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

2. 4. Molecular Detection

2.4.1. RNA Extraction

The most important solutions used in the extraction are shown in **Table 1** aacoring to manufactures company (Promega/ USA).

TABLE 1. Extraction steps

Sequence	Reagents and materials	Volumes
1	Transzol	600 µl
2	RNA Extraction Agent	0.2ml
3	Isopropanol	0.5 ml
4	Ethanol	1ml

The process of extracting RNA from frozen samples with transsol are extracted with pre-added begins with mixing the samples using a rotary mixer, then centrifuging to separate the components. After adding 0.2 ml of RNA Extraction Agent, the samples are centrifuged again to separate the layers, and the upper aqueous layer containing RNA is transferred to a new tube. Isopropanol is added to the samples and mixed well, then centrifuged to separate the precipitate. 75% ethanol is added, then centrifuged again to form a dry layer of RNA. The RNA is dissolved in a dissolution solution and stored at -70°C, and can be stored for a long time at 55-60°C for 10 minutes.

2.4.2. 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:

$$M_1V_1 = M_2V_2$$

TABLE 2. Extraction steps

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

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

TABLE 3. Extraction steps

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

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

2.4.3. 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).

TABLE 4. Extraction steps

Primer	Direction	Sequence (5'→3')	References
Reference gene	Forward	Hs00950362_g1	Riming Liu.,et al.,2017)
SLC5A5	Reverse	Hs00166567_m1	

3. RESULTS

3.1. Change value for *SLC5A5* gene

The results of the statistical analysis in **Table 5** and Figure 1 revealed that the *SLC5A5* gene was not significant, i.e., it was unlikely to affect the HD **Table 5**.

TABLE 5. A comparison between the control group and the affected group in the fold change value for *SLC5A5* gene

Group	B actin	*SLC5A5*	ΔCt	ΔΔCt	Fold change
Control	20.287	30.109	9.822	-0.517	1.00 ±0.00
Patients	23.642	34.538	10.896	0.556	0.48 ±0.07
T-test	--	--	--	--	0.558 NS
P-value	--	--	--	--	0.1628

NS: Non-Significant.

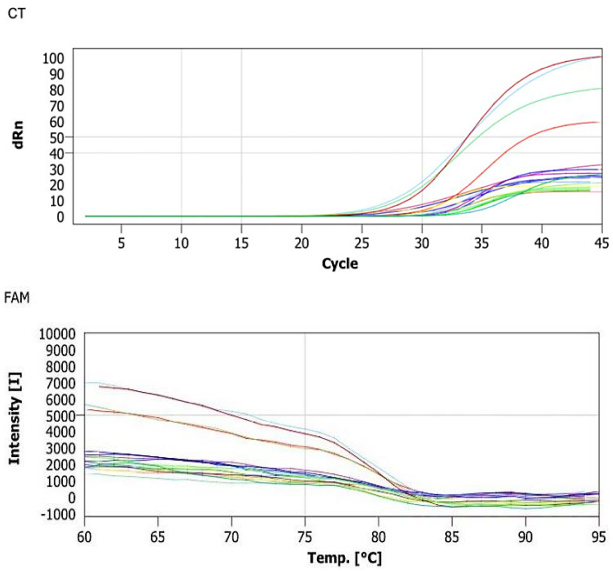

Figure 1. Exponential change curves of gene expression in the polymerase chain reaction assay for the SLC5A5 gene

3.2. Thyroid hormone tests

Table 6 shows no significant difference in TSH hormone levels between the healthy individuals and patients. However, the T3 and T4 levels were significantly lower in HD patients (P<0.01) compared to controls.

TABLE 6. Comparison between healthy group and patients in thyroid hormones.

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.**

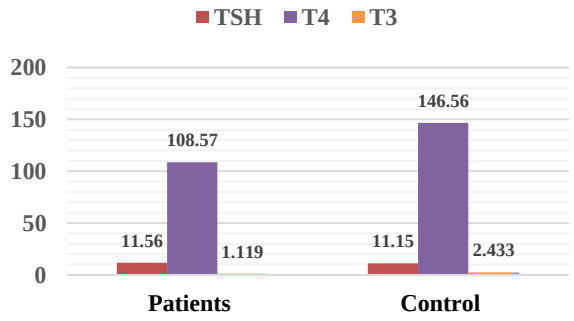

Figure 2. Comparison between healthy group and patients in thyroid hormones

3.3. Family history

The results in **Table 7** showed significant differences (P≤0.05) in the proportion of women with HD who had a family history of the condition. Additionally, the findings indicated that factors such as diabetes, hypertension, and heart disease contribute to the development of HD at a highly significant level (P≤0.01).

TABLE 7. Effect of risk factors on the development of hypothyroidism

Factors		No	Percentage (%)	P-value
Diabetes	Yes	37	74.00%	0.0007 **
	No	13	26.00%	
Pressure	Yes	36	72.00%	0.0019 **
	No	14	28.00%	
Heart disease	Yes	32	64.00%	0.0477 *
	No	18	36.00%	
Remove the gland	Yes	27	54.00%	0.571 NS
	No	23	46.00%	
Family genetic history	Yes	31	62.00%	0.0498 *
	No	19	38.00%	

* (P≤0.05), ** (P≤0.01).

4. DISCUSSION

HD is a prevalent endocrine disorder characterized by insufficient activity of thyroid hormones, which impacts a range of vital bodily functions. These functions include regulating energy metabolism, promoting growth, differentiation, and other physiological processes. Normal circulating levels of the thyroid hormones T3 and T4 are significantly reduced in HD [13].

The results of this study showed no statistically significant effect of the *SLC5A5* gene on patients with HD. These findings are consistent with the study by Geysels et al. [14], which demonstrated an absence of disease-causing variants in the coding region of the *SLC5A5* gene in three out of four patients with HD. However, our results contrast with those of Kostopoulou et al. [15], who showed that the *SLC5A5* gene is involved in thyroid dysgenesis and dyshormonogenesis. Other causes of congenital HD, such as iodothyronine transporter defects and resistance to thyroid hormones, have also been associated with genetic mutations. The reason for the lack of effect may be related to a specific sample of the population that has certain genetic patterns or environmental factors, which affects the importance of this gene in data analysis.Thus, we recommend conducting future studies on this aspect.

In contrast, HD caused a significant decrease in the levels of thyroid hormones T3 and T4 and non-affect the level of TSH. These results are consistent with Al-Farttoosi et al., [16], Hashim et al., [17], Naji [18] and Ateah et al., [14]. The decrease in hormone secretion in the results of the study is attributed to iodine deficiency or autoimmune diseases such as Hashimoto disease, that lead to produce autoantibodies, which in turn attack the thyroid cells and interfere with their ability to produce hormones [19].

Additionally, significant differences were observed in the percentage of women with HD who had a family history of the condition. In addition, the results indicated that factors such as diabetes, high blood pressure, and heart disease contribute to the development of HD, and these results are consistent with a study Hassen and Ahmed [20] which showed that family history increases the risk of hypothyroidism compared to people who do not have a family history of the disease. In terms of diabetes, the study agrees with [21] who proved that 11% of cases of HD are caused by diabetics, because diabetes cause metabolic imbalance and insulin resistance, which may affect thyroid function. Although high blood pressure and heart disease are not a direct cause of hypothyroidism, high blood pressure often coincides with other metabolic disorders and can stress the body's hormone regulation systems, indirectly affecting the thyroid gland [22].

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Arabic Abstract

هدفت هذه الدراسة إلى تحديد مقدار مستوى التعبير الجيني لجين *SLC5A5* لدى النساء المصابات بقصور الغدة الدرقية (HD)، حيث أنه أحد الجينات الرئيسية المؤثرة على تطور الحالة. بالإضافة إلى ذلك، تم فحص بعض عوامل الخطر، بما في ذلك مرض السكري وارتفاع ضغط الدم وأمراض القلب واستئصال الغدة الدرقية السابق والتاريخ الوراثي العائلي، لتقييم تأثير هذه العوامل على انتشار قصور الغدة الدرقية. بالإضافة إلى ذلك، تم فحص بعض عوامل الخطر، بما في ذلك مرض السكري وارتفاع ضغط الدم وأمراض القلب واستئصال الغدة الدرقية السابق والتاريخ الوراثي العائلي، لتقييم تأثير هذه العوامل على انتشار قصور الغدة الدرقية. تم الحصول على عينات الدم المحيطي (PB) من 50 امرأة مصابة بقصور الغدة الدرقية تم متابعتهن بالتشخيص الطبي وتراوحت أعمارهن من (40 إلى 70) عامًا. تم اختيار 50 عينة من النساء الأصحاء اللاتي لا يعانين من أمراض مزمنة كمجموعة سيطرة، تم استخدام تقنية تفاعل البوليميراز المتسلسل (qPCR-RT) لتحديد مستويات التعبير عن جين *SLC5A5* في العينات، وكذلك لقياس بعض المعايير الهرمونية لمرضى قصور الغدة الدرقية لإثبات أهميتها في تشخيص المرض وهي قياس مستوى هرمون تحفيز الغدة الدرقية (TSH) وهرمونات الغدة الدرقية الثيروكسين (T4) وثلاثي يودوثيرونين (T3). أظهرت النتائج عدم وجود فرق معنوي في مستوى التعبير لجين *SLC5A5* لدى النساء المصابات بقصور الغدة الدرقية مقارنة بالمجموعة السيطرة. بالمقابل أظهرت النتائج انخفاض كبير في هرمونات الغدة الدرقية لكل من ثلاثي يودوثيرونين (T3) والثيروكسين (T4) وعدم وجود فرق معنوي في هرمون TSH. بالإضافة إلى ذلك، أشارت النتائج إلى أن عوامل مثل التاريخ العائلي ومرض السكري وارتفاع ضغط الدم وأمراض القلب تساهم في تطور قصور الغدة الدرقية. وفي الختام، وجدا أن عوامل الخطر مثل التاريخ العائلي، ومرض السكري، وارتفاع ضغط الدم، وأمراض القلب تلعب دورًا في تطور الحالة.