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ORIGINAL ARTICLE

Study of DIO2 Thr92Ala Genetic Polymorphism (rs225014) In Hypothyroid Patients Who Achieved Biochemical Euthyroidism

Shimaa Kamal El-Din Zewain[1], Heba Ashraf Ibrahim EL-Malky*[1] , Mohammed Abd El Raouf Korany[1], Sally Mohammed Ibrahim El- Hefnawy[2 3] , Moustafa Bakrey Hamed Ata[1]

[1] Department of Internal medicine, Endocrinology unit , Faculty of Medicine, Menoufia University, Menoufia, Egypt

[2] Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Menoufia University, Menoufia, Egypt.

[3] Department of Basic medical science, Faculty of Dentistry, Alryada University for Science and Technology (RST), Sadat city, Egypt

***Corresponding author:**

Heba Ashraf Ibrahim EL-Malky

Email:
elmalkyheba80@gmail.com

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ABSTRACT

Background: Many hypothyroid patients often experience persistent symptoms despite being biochemically euthyroid on levothyroxine replacement. Type 2 deiodinase activates triiodothyronine from thyroxine. DIO2 Thr92Ala genetic polymorphisms are associated with a variety of clinical conditions. This study aimed to assess the relationship between DIO2 Thr92Ala (rs225014) polymorphism and persistent hypothyroid symptoms in levothyroxine-treated hypothyroid patients and achieve biochemical euthyroidism.

Methods: This case-control study was carried out on 144 participants attending outpatient endocrinology clinics at Menoufia University hospitals from November 2022 to June 2024. Participants were categorized into three groups of 48 each: Group A: matched for age and sex with a normal thyroid profile. Group B: symptomatic hypothyroid patients on levothyroxine, achieving biochemical euthyroidism for 6 months. Group C: Asymptomatic hypothyroid patients on levothyroxine, also achieving biochemical euthyroidism for 6 months. History-taking, clinical examination, and investigations, which included the genotyping of DIO2 Thr92Ala (rs225014) using real-time PCR, were administered to all participants.

Results: There was no association among the DIO2 Thr92Ala genetic variation (rs225014) and the following: anti-thyroid peroxidase antibody titer, free triiodothyronine, thyroid stimulating hormone, free thyroxine, illness duration, or levothyroxine dosage. In group B, it correlated significantly with certain thyroid-related quality of life (THYPRO39) questionnaire subscales like tiredness, daily life, and overall score. In group C, only the emotional subscale was associated (p-value <0.05).

Conclusions: DIO2 polymorphism rs225014 may be correlated with chronic hypothyroid symptoms but not correlated with TSH level or levothyroxine dosage.

Keywords: Deiodinase type 2; Genetic polymorphisms; Hypothyroidism; Levothyroxine; ThyPRO39 questionnaire.

INTRODUCTION

Hypothyroidism, affecting up to 10% of women, is a common endocrine disorder [1]. In the context of hypothyroidism, levothyroxine (LT4) is considered the most effective treatment [2], aiming to alleviate symptoms and prevent long-term complications [3]. It is catalyzed by DIO1 and DIO2 to convert inactive thyroxine (T4) to active thyroid hormone 3, 5, 3′-triiodothyronine (T3). DIO2 is accountable for 70% circulating T3 in euthyroid humans' ultimate concentration [4].

Pituitary gland, brain, thyroid, skeletal muscle, cardiac muscle, and adipose tissue are all sites of DIO2 expression [5].

The DIO2 gene, which is situated on chromosome

14q24.3, was the subject of analysis. Two polymorphisms were the primary focus of the analyses, one in exon 2 (DIO2-Thr92Ala, rs225014) and one in exon 1 (DIO2-ORFa-Glu3Asp, rs12885300) [6].

Five to ten percent of hypothyroid patients still have hypothyroid symptoms even when their levels of thyroid stimulating hormone (TSH) and free thyroxine (fT4) are normal [7]. The catalytic efficacy of DIO2 is diminished as a result of the substitution of Thr92 with Ala, which leads to localized hypothyroidism. As a result, levothyroxine monotherapy may only provide limited benefits to hypothyroid carriers of Ala92, even if their TSH level returns to a normal range [1]. Thus, the Thr92-to-Ala substitution may suggest the necessity of a higher dosage of levothyroxine or a combination therapy with liothyronine [1, 7].

In comparison to individuals who are heterozygous Thr/Ala or homozygous Thr/Thr carriers, homozygous Ala/Ala carriers of the D2-92Ala have been hypothesized to experience impaired cognitive functions and a reduction in health-related quality of life (HRQoL) [8].

Furthermore, it is hypothesized that DIO2's Thr92Ala polymorphism (rs225014) may increase the likelihood of developing a wide range of health problems, including type II diabetes, insulin resistance, high blood pressure, abnormal lipid profiles, obesity, osteoporosis, osteoarthritis, and mental health issues [9, 10]. This indicates that a change in DIO2 activity may result in specific clinical phenotypes without influencing the serum levels of thyroid hormone [9].

This study aimed to assess the association among the DIO2 Thr92Ala (rs225014) polymorphism and persistent hypothyroid symptoms in hypothyroid patients who obtained biochemical euthyroidism after undergoing six months of levothyroxine replacement therapy.

METHODS

This case-control study evaluated 144 participants who attended outpatient endocrinology clinics in the department of internal medicine of Menoufia University hospitals from November 2022 to June 2024. The Research and Ethics Committee of the Faculty of Medicine, Menoufia University, authorized the study. Institutional Review Board (IRB) approval was obtained on 11/2022 INTM 38. Each participant provided verbal consent prior to his or her participation in the research.

The subjects under study were split into 3 equal groups, with 48 patients in each: **Group A:** apparent healthy participants matched for age and sex with normal thyroid profile. **Group B:** symptomatic hypothyroid patients on levothyroxine treatment and achieved euthyroidism for 6 months. **Group C:** Asymptomatic hypothyroid patients on levothyroxine treatment and achieved euthyroidism for 6 months. Patients who were aged 18-65 years and had hypothyroidism, either due to primary hypothyroidism or post-total thyroidectomy, were eligible for the study. Their thyroid function levels and body mass index (BMI) were used to determine the dosage of L-T4 replacement therapy, and they were able to stay in a euthyroid state for at least six months after treatment. This was completed with or without persistent hypothyroid symptoms, as determined by the Thyroid-Related Patient-Reported Outcome-39 (ThyPro39) questionnaire [11]. The PRO is the first comprehensive questionnaire measuring QoL and validated for those with benign thyroid diseases. ThyPRO-39 aggregates 39 items of the 85 items of the Thy PRO into 13 scales. On the QoL-impact scale, the total score varies from 0 to 100 [11].

Those with diabetes mellitus, hypertension, autoimmune diseases, cardiac diseases, pregnancy, malignancy, liver disorders, renal failure, and psychiatric disorders unrelated to hypothyroidism symptoms were excluded. Additionally, patients receiving medications that affect levothyroxine bioavailability or those with medical conditions impairing levothyroxine absorption and patients who were inconsistent in using a certain brand of levothyroxine tablets were also excluded from the research.

All patients were assessed for a comprehensive history, which included demographic data, etiology, duration of hypothyroidism, and medication history. A complete physical examination was conducted, measuring blood pressure, body height, weight, waist-hip ratio, and waist circumference, and BMI was calculated [12].

Blood Sampling: Ten milliliters of venous blood were collected following a 12-hour fast. A simple vial was used to transfer seven milliliters of blood, which was then centrifuged at 4000 r.p.m. for ten minutes. Two fractions were subsequently formed from the samples. We used the Fried Ewald equation [13] to find the serum lipid profile, which comprised TC, Tgs, HDL, and LDL (low-density lipoproteins) as well as total cholesterol (TC), total lipids, and total cholesterol. Further investigation involved quickly freezing the thyroid profile at -20 ºC. This comprised FT3, TSH, FT4, titer of anti-thyroid peroxidase antibody (Anti-TPO), and results from hepatic and renal function assessment.

Genotyping by real-time PCR:

Using the Gene JET Genomic DNA Purification reagent (Lithuania), DNA was isolated from whole blood by Thermo Scientific. We rinsed the isolated DNA and kept it at -200 C to make it usable in future PCR procedures. An allelic discrimination assay was conducted using a TaqMan probe (Applied Biosystems, USA) to genotype the rs225014 polymorphism within the DIO2 gene in a real-time PCR. Primers, probes, and Master Mix (40X) were provided by Thermo Scientific. The probe sequences used were TTGCCACTGTTGTCACCTCCTTCTG[C/T] ACTGGAGACATGCACCACACTGGAA. In order to develop a reaction mixture, 3.5 µl of nuclease-free water, 10 µl of Master Mix, and 1.5 µl of the primer/probe mixture were combined. Each reaction contained five microliters of extracted DNA. The following is a comprehensive summary of the cycling conditions: A 40-cycle sequence was initiated with a preliminary denaturation at 95°C for 10 minutes, followed by primer annealing at 50°C for 60 seconds, denaturation at 94°C for 15 seconds, and extension at 72°C for 2 minutes. The terminal extension was executed at 72°C for one minute to finalize the procedure. Data analysis was performed using the software that is included with the ABI7500 real-time PCR device (V.2.0.1).

Statistical analysis:

The data that was obtained was then tabulated and analyzed using SPSS software, version 26, on computers that were compatible with IBM. While numbers and percentages (No & %) were used to represent qualitative variables, standard deviation (SD), range, and mean ($\bar{x}$) were used to represent quantitative data. In order to examine the connections between qualitative characteristics, the Chi-squared test ($\chi 2$) was used. A one-way ANOVA test was implemented in conjunction with a post-hoc test when contrasting quantitative variables that were normally distributed across more than two groups. This is correct when dealing with quantitative variables that deviate from a normal distribution. In order to address this, we used a post hoc Kruskal-Walli's test. The student t-test was employed to compare two continuously distributed quantitative variables. Two variables that did not follow normal distributions were subjected to analysis using the Mann-Whitney U test. A Hardy-Weinberg equilibrium (HWE) was used for gene genotyping. We determined statistical significance with a p-value below 0.05.

RESULTS

The baseline demographics, laboratory investigations, and clinical characteristics of the groups investigated are presented in **Table 1.** The prevalence of hypothyroidism in the investigated groups was higher in females than in males (female patients constituted 90.3% of the investigated group). The mean age was 41.54±8.08 years.

As regards BMI and waist-hip ratio, a significant statistical difference was observed among the control group and hypothyroid groups (B, C).

Additionally, no statistically significant difference was observed between the hypothyroid groups (B, C) in terms of BMI, waist-hip ratio, etiology, and overall disease duration. The levothyroxine dose was significantly different among the two groups (p-value<0.001).

Hypothyroid groups (B, C) exhibited a statistically significant difference in anti-TPO titer and TSH level. Nevertheless, the two groups didn't differ statistically in terms of lipid profiles, which include TC, TG, and LDL, and FT3, FT4.

The genotyping frequency of DIO2 (rs225014) among studied groups is shown in **Table 2.** The genotyping frequency did not change significantly between the groups of study, confirming Hardy-Weinberg equilibrium (HWE) (p-value > 0.05).

Genotype frequencies and allelic distribution of DIO2 Thr92Ala (rs225014) are presented in **Table 3.** Even though group B had a CC genotype with a higher frequency (mutant variant) than group A (22.9 % vs. 16.7%) and group C (22.9 % vs. 18.8%) and had a higher C allele frequency than group A (41.7 % vs. 36.5%) and group C (41.7 % vs. 40.6%), the study groups didn't show any statistically significant difference regarding genotype frequencies and distribution of allelic.

The relation of genotyping of studied groups to clinical characteristics and laboratory findings is displayed in **Table 4**, and no significant statistical difference was found among the three genotypes (TT, CC, TC) of DIO2 Thr92Ala in the three groups regarding the duration of the disease, the dose of levothyroxine, and the laboratory investigations including TSH, FT3, FT4, anti-TPO titer, serum TC, and LDL-c (P-value > 0.05).

The relation of genotyping of hypothyroid groups to THYPRO39 questionnaire subscales is shown in **Table 5 and Table 6**. In group B, a significant association among DIO2 Thr92Ala (rs225014) and certain THYPRO39 subscales (tiredness, daily life, and total score) was noticed. Additionally, there is only an association with the emotional subscale in group C (p-value <0.05).

Table 1: Distribution of the studied groups regarding their baseline characteristics and laboratory investigations (n=144)

Variable	Group A (n=48)		Group B (n=48)		Group C (n=48)		Test of significance P value	*Post Hoc* test
	No.	%	No.	%	No.	%		
Gender Male Female	5 43	10. 89.6	4 44	8.3 91.7	5 43	10.4 89.6	$\chi 2$=0.16 P=1.000	--
Age (Years): Mean ±SD	41.50 ±8.39		41.96 ±6.91		41.17 ±8.94		F=0.12 P=0.892	--
BMI (Kg/m2): Mean ±SD	24.33 ±0.47		28.75 ±3.80		27.58 ±2.96		F=32.20 **P<0.001***	**P1<0.001*** **P2=0.001*** P3=0.126
Waist to hip ratio: Mean ±SD	0.74 ±0.03		0.92 ±0.11		0.90 ±0.09		F=69.32 **P<0.001***	**P1<0.001*** **P2=0.001*** P3=0.638
Etiology of hypothyroidism Primary hypothyroidism Post total thyroidectomy	-- --	-- --	23 25	47.9 52.1	18 30	37.5 62.5	$\chi 2$=1.06 P=0.302	--
Duration of disease (Years): Mean ±SD	-- -		6.38 ±3.02		5.83 ±2.88		U=1.04 P=0.297	--
Dose of levothyroxine (µg/ day): Mean ±SD	-- -		135.94 ±29.13		112.50 ±24.19		t=4.30 P<**0.001***	--
TSH (mU/L)	3.70 ±0.44		3.79 ±0.58		2.83 ±1.31		K=11.20 **P=0.004***	P1=0.328 **P2=0.022*** **P3=0.001***
F T3 (pg/L)	3.17 ±0.25		2.85 ±0.46		2.99 ±0.44		F=7.97 **P=0.001***	**P1<0.001*** P2=0.102 P3=0.201
F T4 (ng/L)	1.35 ±0.16		1.25 ±0.23		1.34 ±0.26		F=3.35 **P=0.038***	P1=0.062 P2=1.000 P3=0.105
Anti TPO (IU/ml)	25.27 ±4.79		100.67 ±62.33		64.42 ±34.46		K=46.59 **P <0.001***	**P1<0.001*** **P2<0.001*** **P3=0.037***
TC (mg/dl)	154.83 ±9.66		194.54 ±44.78		183.73 ±28.57		F=20.82 **P <0.001***	**P1<0.001*** **P2<0.001*** P3=0.274
TG (mg/dl)	73.39 ±19.55		161.56 ±54.84		146.06 ±42.21		K=84.41 **P <0.001***	**P1<0.001*** **P2<0.001*** P3=0.264
HDL (mg/dl)	57.94 ±12.29		46.31 ±8.93		51.48 ±6.98		F=17.49 **P <0.001***	**P1<0.001*** **P2=0.004*** **P3=0.029***
LDL (mg/dl)	82.62 ±7.36		116.01 ±40.42		103.07 ±27.71		K=36.19 **P <0.001***	**P1<0.001*** **P2<0.001*** P3=0.063

***:** Statistically significant, SD: Standard deviation, χ2: Chi-squared test, F: One Way ANOVA test, t: Student t test, U: Mann-Whitney U test, BMI: Body mass index, F T3: Free Triiodothyronine, F T4: Free thyroxine, TSH: Thyroid stimulating hormone, Anti TPO: anti thyroid peroxidase, TC: Total cholesterol, TG: Triglycerides, HDL: High density lipoprotein, LDL: Low density lipoprotein, Group A: Control group matched for age and sex with normal thyroid profile- Group B: Hypothyroid patients on levothyroxine who achieved biochemical euthyroidism for 6 months with persistent hypothyroid symptoms-
Group C: Hypothyroid patients on levothyroxine treatment and achieved biochemical euthyroidism for 6 months with no hypothyroid symptoms.
P1: P value between Group A and Group B, P2: P value between Group A and Group C, P3: P value between Group B and Group C

Table (2): Genotype profile of DIO2 Thr92Ala (rs225014) among studied participants (n=144)

SNPs	Total (n=144)		Group A (n=48)		Group B (n=48)		Group C (n=48)	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
TT	58	52.6	21	19.4	19	16.3	18	16.9
CC	28	68.9	8	6.4	11	8.3	9	7.9
TC	58	22.6	19	22.2	18	23.3	21	23.2
χ2	3.59		1.02		2.51		0.416	
P value	0.058		0.313		0.113		0.519	

If P < 0.05 - not consistent with HWE (Hardy-Weinberg equilibrium)
Group A: Control group matched for age and sex with normal thyroid profile- Group B: Hypothyroid patients on levothyroxine who achieved biochemical euthyroidism for 6 months with persistent hypothyroid symptoms-Group C: Hypothyroid patients on levothyroxine treatment and achieved biochemical euthyroidism for 6 months with no hypothyroid symptoms

Table (3): Genotypes of DIO2 Thr92Ala (rs225014) frequencies and allelic distribution among studied groups (n=144)

		Group A (n=48)	Group B (n=48)	χ2	P value	OR (95% CI)	Group A (n=48)	Group C (n=48)	χ2	P value	OR (95% CI)
Genotype	TT	21 (43.8%)	19 (39.6%)	0.60	0.741	1.0	21 (43.8%)	18 (37.5%)	0.39	0.823	1.0
	CC	8 (16.7%)	11 (22.9%)			1.52 (0.5-4.58)	8 (16.7%)	9 (18.8%)			1.31 (0.42-4.11)
	TC	19 (39.6%)	18 (37.55%)			1.05 (0.43-2.56)	19 (39.6%)	21 (43.8%)			1.29 (0.53-3.12)
Alleles	T	61 (63.5%)	56 (58.3%)	0.55	0.459	1.0	61 (63.5%)	57 (59.4%)	0.35	0.553	1.0
	C	35 (36.5%)	40 (41.7%)			1.24 (0.70-2.23)	35 (36.5%)	39 (40.6%)			1.19 (0.67-2.13)

*: Statistically significant, χ2: Chi-squared test, OR: Odds ratio, CI: Confidence interval
Group A: Control group matched for age and sex with normal thyroid profile- Group B: Hypothyroid patients on levothyroxine who achieved biochemical euthyroidism for 6 months with persistent hypothyroid symptoms-Group C: Hypothyroid patients on levothyroxine treatment and achieved biochemical euthyroidism for 6 months with no hypothyroid symptoms.

Table (4): Genotyping in relation to clinical characteristics and laboratory finding in Group A, B and C (n=48 in each)

Variable	Group A			P value	Group B			P value	Group C			P value
	TT	TC	CC		TT	TC	CC		TT	TC	CC	
	Mean ±SD	**Mean ±SD**	**Mean ±SD**		**Mean ±SD**	**Mean ±SD**	**Mean ±SD**		**Mean ±SD**	**Mean ±SD**	**Mean ±SD**	
Duration of disease (Years)	----------	----------	----------	----------	6.32 ±2.26	6.56 ±3.94	6.18 ±2.68	0.914	5.94 ±2.65	5.76 ±2.76	5.78 ±3.87	0.784
Dose of levothyroxine (µg/day)	----------	----------	----------	----------	138.16 ±34.76	134.72 ±25.92	134.09 ±25.67	0.915	115.28 ±27.30	110.71 ±23.15	111.11 ±22.05	0.832
TSH (mU/L)	3.69 ±0.39	3.70 ±0.48	3.73 ±0.48	0.977	3.73 ±0.54	3.90 ±0.60	3.70 ±0.65	0.602	3.04 ±1.42	2.57 ±1.24	3.01 ±1.25	0.465
F T3 (pg/L)	3.12 ±0.23	3.21 ±0.27	3.21 ±0.28	0.506	2.81 ±0.47	2.89 ±0.49	2.85 ±0.43	0.852	3.06 ±0.45	2.98 ±0.44	2.93 ±0.49	0.767
F T4 (ng/L)	1.33 ±0.16	1.37 ±0.15	1.37 ±0.16	0.578	1.24 ±0.23	1.28 ±0.23	1.19 ±0.25	0.608	1.32 ±0.29	1.33 ±0.26	1.41 ±0.20	0.701
Anti TPO Positive Negative	---------- ----------	---------- ----------	---------- ----------	---------- ----------	14(73.7%) 5(26.3%)	12 (66.7%) 6(33.3%)	8 (72.7%) 3 (27.3%)	0.922	13 (72.2%) 5(27.8%)	12 (57.1%) 9(42.9%)	5 (55.6%) 4 (44.4%)	0.679
Anti TPO (IU/ml)	25.14 ±4.95	25.16 ±4.59	25.88 ±5.44	0.929	105.21 ±61.56	92.11 ±64.80	106.82 ±63.95	0.579	73.72 ±37.12	58.57 ±32.50	59.44 ±33.08	0.450
TC (mg/dl)	155.19 ±10.29	153.58 ±8.99	156.88 ±10.30	0.711	194.11 ±29.39	203.33 ±61.58	180.91 ±32.84	0.433	190.28 ±29.39	184.24 ±31.09	169.44 ±14.77	0.204
TG (mg/dl)	70.39 ±19.63	75.42 ±20.96	76.41 ±16.90	0.651	174.89 ±55.99	145.39 ±45.91	165.00 ±63.94	0.282	150.72 ±40.98	142.86 ±48.41	144.22 ±31.01	0.398
HDL (mg/dl)	58.39 ±13.12	58.19 ±11.27	56.14 ±13.80	0.91	44.37 ±10.45	49.67 ±7.66	44.18 ±6.76	0.417	50.94 ±7.63	51.81 ±7.44	51.78 ±4.79	0.922
LDL (mg/dl)	83.15 ±6.41	80.74 ±7.12	85.69 ±9.70	0.259	115.20 ±26.09	124.37 ±56.77	103.73 ±26.13	0.369	109.18 ±27.21	103.94 ±31.26	88.82 ±13.41	0.305

Table (5): Genotyping THYPRO39 questionnaire among Group B (n=48)

Variable	DIO2 Thr92Ala			Test of significance P value	*Post Hoc* test
	Homozygous TT (n=19)	Homozygous CC (n=11)	Heterozygous TC (n=18)		
	Mean ±SD	Mean ±SD	Mean ±SD		
Hypothyroid subscale	5.89 ±2.18	5.36 ±1.96	5.67 ±1.68	K=1.27 P=0.531	--
Tiredness subscale	4.79 ±0.63	4.45 ±0.82	5.39 ±1.24	F=3.75 **P=0.032***	P1=1.000 P2=0.180 **P3=0.039***
Cognition subscale	7.16 ±1.54	7.27 ±1.19	7.33 ±1.91	F=0.06 P=0.946	--
Anxiety subscale	1.68 ±0.82	1.18 ±0.60	1.94 ±0.80	K=5.90 P=0.052	--
Depression subscale	3.58 ±1.02	2.73 ±1.35	3.94 ±1.59	K=5.36 P=0.069	--
Emotional subscale	3.63 ±1.30	2.64 ±0.81	3.72 ±1.49	K=5.81 P=0.055	--
Impaired social life	3.26 ±1.82	2.18 ±1.33	3.72 ±1.90	K=4.85 P=0.088	--
Daily life subscale	5.84 ±2.04	4.27 ±1.79	5.78 ±1.17	K=7.23 **P=0.027***	**P1=0.024*** P2=0.713 **P3=0.011***
Appearance subscale	4.42 ±2.06	3.64 ±2.50	5.00 ±2.20	K=1.83 P=0.401	--
Overall quality	2.74 ±0.73	2.36 ±0.51	2.72 ±0.58	F=1.44 P=0.249	--
Total score	42.95 ±8.39	36.09 ±8.53	45.22 ±8.81	F=3.98 **P=0.026***	P1=0.122 P2=1.000 **P3=0.024***

*: Statistically significant, SD: Standard deviation, K: Kruskual Wallis test, F: One Way ANOVA test
Group B: Hypothyroid patients on levothyroxine who achieved biochemical euthyroidism for 6 months with persistent hypothyroid symptoms. P1: between TT and CC .P2: between TT and TC. P3: between CC and TC

Table (6): Genotyping THYPRO39 questionnaire among Group C (n=48)

Variable	DIO2 Thr92Ala			Test of significance P value	*Post Hoc* test
	Homozygous TT (n=18)	Homozygous CC (n=9)	Heterozygous TC (n=21)		
	Mean ±SD	Mean ±SD	Mean ±SD		
Tiredness subscale	3.61 ±0.50	3.56 ±0.53	3.48 ±0.51	F=0.34 P=0.712	--
Depression subscale	3.39 ±0.50	3.44 ±0.73	3.29 ±0.64	F=0.26 P=0.773	--
Emotional subscale	3.50 ±0.62	4.00 ±0.00	3.52 ±0.51	F=3.34 **P=0.045***	P1=0.062 P2=1.000 P3=0.071
Total score	10.50 ±1.09	11.00 ±1.12	10.29 ±1.23	F=1.19 P=0.314	--

*: Statistically significant, SD: Standard deviation, F: One Way ANOVA test
-Group C: Hypothyroid patients on levothyroxine treatment and achieved biochemical euthyroidism for 6 months with no hypothyroid symptoms
P1: between TT and CC
P2: between TT and TC
P3:betweenCCandT

DISCUSSION

Levothyroxine (LT4) is a safe and effective hormone replacement therapy for hypothyroidism, aimed at normalizing serum TSH levels. The effectiveness of this therapy relies on the bioavailability of L-T4, which can be affected by various factors involving administration timing, body mass, gender, age, concurrent diseases, pregnancy, cytochrome P450 inducers, and genetic polymorphisms [14].

There is increasing concern regarding the influence of deiodinases genetic polymorphisms on the determination of levothyroxine dosage [15].

The body composition and thyroid hormones are closely related. Hypothyroidism leads to decreased thermogenesis, decreased metabolic rate, and a higher body mass index, leading to a higher prevalence of obesity. [16]

The current study demonstrated a significant statistical disparity among the groups of control and hypothyroid in relation to waist-hip ratio and BMI (group B > group C > group A).

In the present study, the levothyroxine dose means and the level of TSH in group B were greater than that of group C while both groups achieved euthyroid state, and this agreed with Rasheed et al. [15] and disagreed with Castagna et al. [17].

In the present study, no statistically significant difference was detected for FT3, FT4, and TSH among the hypothyroid groups, and this disagreed with Castagna et al. [17], and this may be explained by the small frequency of the mutant CC genotype in both groups.

There was no significant variation in genotype frequencies or allelic distribution among the groups of study (p-value > 0.05), despite the fact that, in comparison to group A and group C, group B exhibited higher CC genotype frequencies (mutant variants) and C alleles, and this was agreed with Mang et al. [18] and Zyara et al. [19], who studied the therapeutic response to LT4 in 150 hypothyroid patients under the influence of the DIO2 gene, and it was determined that the CC genotype was more prevalent among the patients being investigated.

In the current study, no statistically significant difference was noticed among genotypes (TT, CC, TC) in hypothyroid groups as regard to levothyroxine dose, and that disagrees with Rasheed et al. [15] and Torlontano et al. [20], who stated that the homozygous variants (TT and CC, respectively) necessitated higher LT4 concentrations. They ascribed this to the reduced pituitary feedback that resulted from aberrant pituitary/hypothalamic DIO2 activity.

In this study, there was no significant statistical difference between genotypes (TT, CC, TC) in hypothyroid groups as regard to TSH, free T3, and free T4, and that was agreed with by Wouters et al. [8] and Zyara et al. [19] and disagreed with by Arici et al. [21] and Castagna et al. [17], who concluded that a reduced FT3 level is linked to the Ala/Ala D2 genotype, indicating that some of these patients may not benefit from conventional LT4 therapy.

This research did not reveal any statistically significant distinctions between the lipid profiles of hypothyroid groups and genotypes (TT, CC, TC), and that agreed with Kang et al. [22].

Hypothyroidism can significantly impact HRQoL, leading to mood-related issues, sexual dysfunctions, cosmetic concerns, and neurocognitive problems. Thus, daily and social activities are frequently impaired [Watt T., 2019] [23].

In the prospective study, group B exhibited a statistically significant difference in DIO2 Thr92Ala genotypes (CC, TC) concerning their correlation with certain Thy PRO 39 subscales, including tiredness, daily life, and overall score. The discovery further corroborates the hypothesis that impaired DIO2 enzyme activity may result in the development of a hypothyroid condition in the brain in individuals with the CC genotype of rs225014. Improvements in psychological well-being were observed in hypothyroid patients who were identified with the CC genotype at rs225014 when they were administered a combination of T3 and T4 therapy [24]. This observation aligns with Panicker et al. [25], who reported an association between the CC genotype of rs225014 and poorer well-being; however, it contrasts with the results of Wouters et al. [8], who evaluated the populations under investigation using the RAND 36-Item Health Survey and detected no correlation between the DIO2 Thr92Ala polymorphism and cognitive functioning or quality of life.

The present study offers a unique strength: to our knowledge, it is the first to investigate the relationship between the DIO2 Thr92Ala (rs225014) polymorphism and persistent hypothyroid symptoms in levothyroxine-treated hypothyroid patients who achieved biochemical euthyroidism, specifically among Egyptian patients. However, the unicentric study's relatively small sample size represents a limitation and necessitates additional research with a larger sample size in order to obtain more conclusive results.

Conclusion

The current study demonstrated that hypothyroid

patients who are treated with LT4 and continue to experience hypothyroid symptoms despite achieving biochemical euthyroidism do not exhibit a correlation among the genetic polymorphism of DIO2 Thr92Ala (rs225014) and levels of thyroid hormone, anti-TPO, disease duration, or levothyroxine dosage. Moreover, there was a significant association with certain subscales of the ThyPRO39 questionnaire.

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