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Original Article *Özgün Araştırma*

THE EFFECT OF COENZYME Q10 ADMINISTRATION ON THE EXPRESSION OF *Gdnf, Plzf, Sox3, Thy1* GENES IN RATS WITH HYPOTHYROIDISM

Hipotiroidili Sıçanlarda Koenzim Q10 Uygulamasının Gdnf, Plzf, Sox3, Thy1 Genlerinin İfadesi Üzerine Etkisi

Hatice Nur ŞEFLEK[1] Elif GÜLBAHÇE MUTLU[2] Gökhan CÜCE[3] Sabiha Serpil KALKAN[3]

[1] *Department of Histology and Embryology, Faculty of Medicine, KTO Karatay University, KONYA, TÜRKİYE*
[2] *Department of Medical Biology, Faculty of Medicine, KTO Karatay University, KONYA, TÜRKİYE*
[3] *Department of Histology and Embryology, Faculty of Medicine, Necmettin Erbakan University, KONYA, TÜRKİYE*

ABSTRACT

Objective: Hypothyroidism is a problem of deficient production of thyroid hormones. These hormones regulate metabolism. Therefore, the current health status of the person is adversely affected due to thyroid hormone deficiency. Coenzyme Q10 (CoQ10) is a vitamin-like substance with strong antioxidant properties. The aim of this scientific study is to investigate the effects of CoQ10 on hypothyroidism at sera level with important biomarkers glial cell line-derived neurotrophic factor (GDNF), promyelocytic leukaemia zinc finger protein (PLZF), high mobility group box transcription factor 3 (SOX3) and thymocyte differentiation antigen 1 (THY1).

Material and Methods: Four experimental animal groups were formed: Control group (n:7); hypothyroidism group (n:7); CoQ10 group (n:7); hypothyroidism + CoQ10 group (n:7). On the thirty-first day, sera of the animals were collected and *Gdnf, Plzf, Sox3, Thy1* expression levels were analyzed in the blood.

Results: Significant results occurred in all four biomarkers. As a final result, both hypothyroid pathology was associated with all biomarkers, and CoQ10 positively affected hypothyroidism.

Conclusion: The effect of coenzyme Q10 on gene expression levels of *Plzf, Gdnf, Thy1, Sox3* at sera level in rats with experimental hypothyroidism was shown by molecular analyses. Coenzyme Q10 regulates sera gene expression levels during treatment.

Keywords: *Gene expression, hypothyroidism, coenzymes, metabolism.*

ÖZ

Amaç: Hipotiroidizm, tiroid hormonlarının eksik üretilmesi sorunudur. Bu hormonlar metabolizmayı düzenler. Dolayısıyla tiroid hormon eksikliğine bağlı olarak kişinin mevcut sağlık durumu olumsuz etkilenir. Koenzim Q10 (CoQ10) güçlü antioksidan özelliklere sahip vitamin benzeri bir maddedir. Planlamış olduğumuz bu bilimsel çalışma, CoQ10'un hipotiroidizm üzerine etkilerini, önemli biyobelirteçler olan glial hücre kaynaklı nörotrofik faktör (GDNF), promyelositik lösemi çinko parmağı (PLZF), high mobility group box transcription factor 3 (SOX3) ve timosit farklılaşma antijeni 1 (THY1) ile serum düzeyinde araştırmaktır.

Gereç ve Yöntemler: Dört deney hayvanı grubu oluşturuldu: Kontrol grubu (n:7); hipotiroidi grubu (n:7); CoQ10 grubu (n:7); hipotiroidi + CoQ10 grubu (n:7). Otuz birinci günde hayvanların serumları alındı ve kanda *Gdnf, Plzf, Sox3, Thy1* ekspresyon düzeyleri analiz edildi.

Bulgular: Dört biyobelirteçte de anlamlı sonuçlar elde edildi. Nihai sonuç olarak, hem hipotiroid patolojisi tüm bu biyobelirteçlerle ilişkilendirildi hem de CoQ10'un hipotiroidizmi olumlu yönde etkilemiştir.

Sonuç: Deneysel hipotiroidizmli sıçanlarda koenzim Q10'un serum düzeyinde *Plzf, Gdnf, Thy1, Sox3*'ün gen ifade düzeyinde etkisi moleküler analizlerle gösterilmiştir. Koenzim Q10, tedavi sırasında serum gen ifade seviyelerini düzenlemektedir.

Anahtar Kelimeler: *Gen ekspresyonu, hipotiroidizm, koenzimler, metabolizma*

Correspondence / Yazışma Adresi: **Dr. Hatice Nur ŞEFLEK**
Department of Histology and Embryology, Faculty of Medicine, KTO Karatay University, KONYA, TÜRKİYE
Phone / Tel: +905072249885 **E-mail** / E-posta: haticenur.seflek@karatay.edu.tr
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INTRODUCTION

Hypothyroidism is an endocrine disorder defined as an inadequate thyroid gland function or an inability of thyroid hormones to act on target tissues (1). The clinical presentation of patients can vary from asymptomatic disease to myxedema coma (2). Hypothyroidism is characterized by low thyroid hormones (triiodothyronine and tetraiodothyronine) and elevated thyroid-stimulating hormone (TSH) (3). Thyroid hormones are important in the activity of metabolism (4). These hormones are active in the functioning of many organs related to the heart, brain, intestines, and reproductive system (5). Thyroid hormones exert a major influence from the fetal period to adulthood by controlling gene and protein expression in almost every tissue (6).

Coenzyme Q10 (CoQ10, ubiquinone) is a substance in the cell membrane with antioxidant properties. It is essential for cellular energy production (7). It interacts with free radicals and acts as an antioxidant, preventing lipid peroxidation and biomolecular damage. Other functions of CoQ10 include membrane stability, cell signaling, gene expression, cell growth, and apoptosis control (8). There are numerous medical studies on the potential therapeutic benefits of CoQ10 supplementation with its antioxidant properties in treating various diseases, including endocrine diseases: immunological, neurological, diabetes, cardiovascular, muscular, male infertility and dental (9).

Looking at levels in human plasma, it has been shown that the concentration of CoQ10 is different in hypothyroid subjects compared to healthy subjects and that there is a significant inverse correlation between CoQ10 levels and thyroid hormone levels (10). Sera CoQ10 levels in hyperthyroid patients are considerably lower than in hypothyroid individuals (11).

Recent studies have provided a lot of information about biomarkers. Many studies have focused on "biomarkers", which are measurable indicators of a biological state or condition (12). Glial cell line derived neurotrophic factor (gdnf), promyelocytic leukaemia zinc finger protein (PLZF), high mobility group box transcription factor 3 (SOX3) and thymocyte differentiation antigen 1 (THY1) are only four of these biomarkers.

The aim of this study was to investigate the changes in the gene expression profile of some transcription factors and regulators *(Thy1, Plzf, Gdnf, Sox3)* in sera levels in rats with experimentally induced hypothyroidism and to determine the effect of CoQ10 supplementation on sera *Plzf, Thy1, Gdnf, Sox3* gene expression levels in comparison with other study groups. As a result of this study, it is aimed to elucidate the etiology of hypothyroidism at gene expression levels and to examine the relationship between hypothyroidism and CoQ10 at gene expression levels.

MATERIALS AND METHODS

The study experiment protocol was discussed by the Necmettin Erbakan University KONÜDAM Experimental Medicine Application and Research Centre Directorate Animal Experiments Local Ethics Committee at its meeting dated 15.09.2023 and numbered 2023-046 and decided to be ethically appropriate. All experimental procedures were performed according to the protocol guide. The experimental parts of the research including molecular and biochemical analyses were performed at KTO-(Konya Chamber of Commerce) Karatay University, Faculty of Medicine, Konya, Türkiye.

Experimental Groups

Twenty-eight adult male Wistar albino rats weighing between 255 and 304 g were used in the study. Rats were kept in stainless steel cages. Throughout the study, the rats were kept in the department laboratory at room temperature (22±2°C), 40-50% humidity and 12 h light/12 h dark cycle. Rats had free access to food and water. The weights of the animals were measured at the beginning and end of the experiment. In this study, rats were housed in 4 groups of 7 male rats each and randomly divided into groups and subjected to the relevant treatments as follows.

Control group (G1): 7 adult male *Wistar albino* rats in this group received no treatment for 30 days.

Hypothyroidism group (G2): In this group, 7 adult male *Wistar albino* rats were given 0.05% w/v 6-n-propyl-2-thiouracil (PTU) (Sigma-Aldrich, USA) in their drinking water for 30 days to induce hypothyroidism (13).

CoQ10 group (G3): In this group, 7 male adult *Wistar albino* rats were administered intraperitoneally (*i.p.*) with 10 mg/kg CoQ10 (Coq brand, China)' dissolved in 10% dimethyl sulfoxide for 30 days. 15

Hypothyroidism + CoQ10 group (G4): In this group, 7 adult male *Wistar albino* rats were given 0.05% w/v PTU in their drinking water for 30 days to induce hypothyroidism. Each rat was administered 10 mg/kg CoQ10 dissolved in 10% dimethyl sulfoxide i.p. for 30 days (14).

On day 31, after ketamine-xylazine (50 mg/kg, Sigma-Aldrich) anaesthesia, 4-6 ml of blood was collected from the heart by syringe into heparinized tubes. Blood samples collected in the tubes were centrifuged at 3000 rpm for 10 minutes and sera were collected. Sera samples were stored at -80ºC until molecular and biochemical analyses. TSH, fT3 and fT4 were measured for thyroid function tests. Gene expression levels for *GDNF, PLZF, THY-1, SOX3* were determined by

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quantitative real-time polymerase chain reaction (RT-PCR).

Biochemical Analysis

TSH (Elabscience, E-EL-R0976), fT3 (Elabscience, E-EL-0079), fT4 (Elabscience, E-EL-0122) levels were determined in sera collected at the end of the experiment using commercially available ELISA kits. ELISA measurements were performed on an Allsheng AMR-100 ELISA reader. Serum TSH levels were calculated as ng/mL, fT3 and fT4 levels as pg/mL.

RNA isolation, cDNA synthesis and RT-PCR analysis

Total RNA was isolated from sera using a commercially available RNA isolation kit (Roche, Germany; Cat. No: 11828665001). The purity and amount of isolated RNA was determined using a spectrophotometer (Thermo Scientific Multiskan Sky; USA). The mRNAs were then analyzed using the OneScript Plus cDNA synthesis kit (ABM, Canada) to obtain cDNAs.

Primers for expression analysis of target genes at the mRNA level were obtained from Oligomer (Türkiye). *Gapdh* was used as a reference gene (Table 1).

Table 1. Primers used for RT-PCR

Gene	Primer Sequence (5'→3')	TM (°C)
Gdnf Forward	CCTTCTGAATGGAAGGTGAAGA	58
Gdnf Reverse	TGGAGACAGTGCTAGGAGTTA	58
Plzf Forward	TTGATTGGACTTGATGCCCC	57
Plzf Reverse	ACCATGATGACCACATCGCA	57
Sox3 Forward	CTCGAGAGAACGCATCAGGT	59
Sox3 Reverse	TCGGAGTTGTGCATCTTGGG	59
Thy1 Forward	TGTATGGGGACACGGGTACT	59
Thy1 Reverse	AGAACCAAAGGCTGTCCTCG	59

Gdnf: Glial cell line-derived neurotrophic factor, *Plzf*: Promyelocytic leukaemia zinc finger, *Sox3*: High mobility group box transcription factor 3, *Thy1*: Thymocyte antigen-1, TM: Melting temperature.

The primers used in the study were analyzed at approximately 50 ng in each reaction. A commercial kit (BlasTaq 2X qPCR Master Mix, ABM, Canada) was used for the PCR reactions. Reactions were performed in a total volume of 20 µl. After primer optimization, PCR conditions were as follows: enzyme activation step at 95°C for 3 minutes, denaturation at 95°C for 10 seconds, binding and polymerization at 60°C for 1 minute, and the number of cycles was 40. All reactions were performed on a LightCycler*96 (Roche, Germany). The results of the qPCR analysis were evaluated using the 2-ΔΔCT method developed by Livak and Schmittgen (15).

SPSS 26.0 was used for statistical analysis. Arithmetic means, and standard deviations were calculated for all parameters. "The Shapiro-Wilk test was performed to determine the homogeneity of the data and normal distributions were found. "One-way analysis of variance (ANOVA) was used to determine differences between groups, and the Tukey test was used to determine which group was responsible for the differences. Differences at the $p<0.05$ levels were considered significant.

RESULTS

Weight Results

The weights of the animals in all four groups were compared at the beginning and end of the experiment and the results of the weight control are shown in Table 2. The difference between the weights at the start and end of the experiment in group 2 ($p<0.004$) and group 4 ($p=0.002$) was considered statistically significant. Group 1 ($p=0.000$) and group 3 ($p=0.058$) were not considered statistically significant.

Biochemical Results

The results of thyroid function control tests (TSH, fT3, fT4) are shown in Table 2. TSH levels, fT3 results and fT4 levels of rats in group 1 were within normal reference values. An increase in TSH was observed in group 2. Due to PTU-induced hypothyroidism, fT3 and fT4 levels were low. In group 3, low TSH levels and increased fT4 and fT3 levels were observed in group 3. In group 4, a significant decrease in TSH and a significant increase in fT4 and fT3 were observed. There were differences between the groups.

Table 2. Morphological and biochemical results of experimental animals

Groups	Start of exp.	End of exp.	TSH±SD	fT3±SD	fT4±SD
	AW ±SD	AW ± SD			
Group 1	288.42^{a}±17.84	310.57^{a}±21.4	2.03±1.06	10.05^{a}±3.57	5.74^{a}±1.33
Group 2	255.14^{b}±14.79	297.85±26.11	7.46^{a}±1.94	6.85^{a}±2.21	1.42^{b}±0.4
Group 3	304^{a}±14.71	322.42±20.15	1.32±1.06	18.32^{b}±6.66	8.15^{c}±1.63
Group 4	266.8^{b}±17.18	300.14±15.38	3.53±3.1	8.44^{a}±2.91	2.19^{b}±0.71

AW: Animal weight, TSH: Thyroid-stimulating hormone, fT3: Freetriiodothyronine, fT4: Freethyroxine, SD: Standard deviation.

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Molecular Results

We used PCR to investigate the expression of *Gdnf, Plzf, Sox3*, and *Thy1* genes in rats with hypothyroidism. *Gdnf* gene expression findings revealed that the lowest *Gdnf* expression was observed in rats with hypothyroidism (G2). In contrast, *Gdnf* expression was increased in the remaining three groups, as shown in Figure 1. *Gdnf* gene expression was found to be significant between all groups ($p \leq 0.05$).

The results obtained from the analysis of *Plzf* gene expression showed that the lowest *Plzf* expression was observed in hypothyroid rats (G2). In contrast, *Plzf* expression was upregulated in other groups, as shown in Figure 2. *Plzf* gene expression level was found to be meaningful between all groups ($p \leq 0.05$).

The lowest *Sox3* expression was observed in hypothyroid rats (G2). In contrast, *Sox3* expression increased in the remaining groups, as shown in Figure 3. The level of expression of the *Sox3* gene was found to be significantly different between the groups to a considerable extent ($p \leq 0.05$).

Thy1 expression was lowest in hypothyroid rats (G2). In contrast, as shown in Figure 4, *Thy1* expression was increased in the other groups. *Thy1* gene expression was significant ($p \leq 0.05$).

Sera *Gdnf*, *Plzf*, *Sox3*, and *Thy1* gene expression levels were found to be significant ($p \leq 0.05$) between all groups.

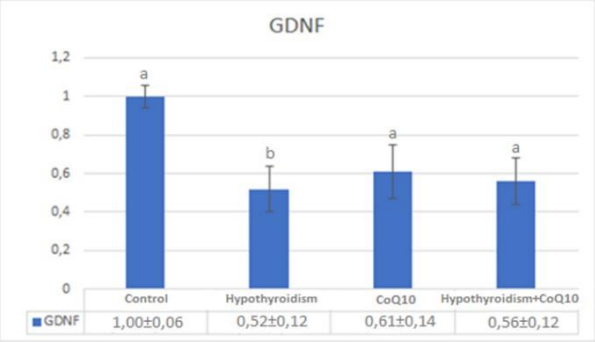

Figure 1. Graph showing *Gdnf* gene expression results in all experimental groups

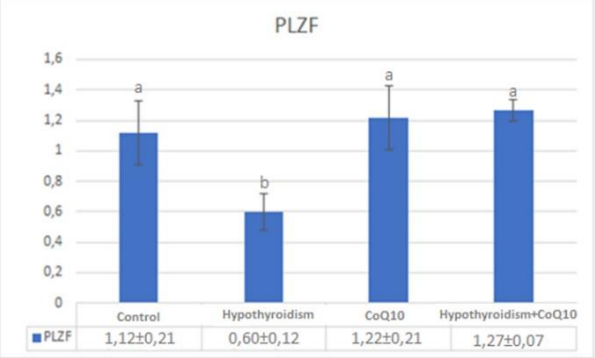

Figure 2. *Plzf* gene expression findings in all experimental groups

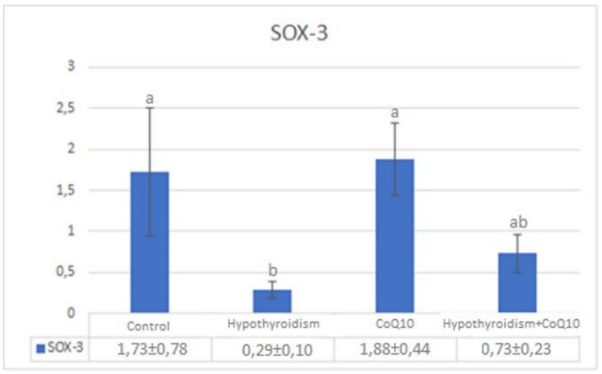

Figure 3. Graph showing *Sox3* gene expression values in all groups

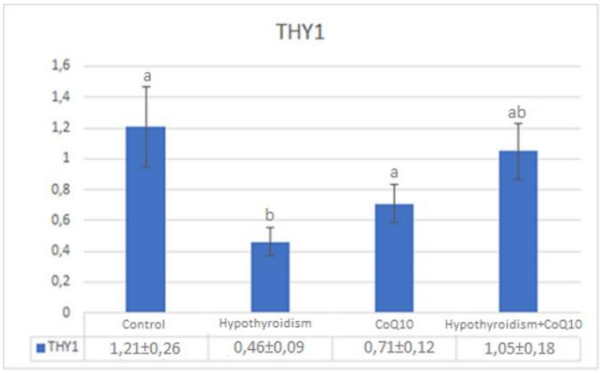

Figure 4. Graph reflecting *Thy1* gene expression values in the experimental groups

DISCUSSION

The expression of *Gdnf* and *Gdnf* receptors (*GFRA1*, *RET*) has been detected in thyroid tissue. RET (rearranged in transfection) receptor is expressed in parafollicular cells, whereas *Gfra1* (GDNF family receptor alpha-1) receptor is more commonly expressed in follicular cells (16). Thyroxine (T4) hormone has been shown to increase *Gdnf* expression by induction of RET tyrosine kinase (17). *Gdnf* gene has been associated with thyroid carcinoma (18). However, in 2023, a group of researchers reported the expression and prognostic value of the *Gdnf* gene in thyroid cancer (19). In the literature review, a limited number of studies have been conducted on other thyroid gland diseases compared to studies on thyroid cancer and *Gdnf* expression. These data are as follows; Bilous et al. reported that GDNF level was significantly lower in patients with thyroiditis (20). In another study, Kamyshna et al. reported that *Gdnf* expression was significantly reduced in patients with primary hypothyroidism and that vitamin D was a regulator of *Gdnf* expression (21). In our study, serum GDNF level was evaluated. It was observed that *Gdnf* was significantly down-regulated in the group of hypothyroid rats and its expression was increased in the other groups. Therefore, it can be predicted that hypothyroidism inhibits *Gdnf* expression. The GDNF receptor RET has been reported to mediate the growth, differentiation and migration of neural crest-derived

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cells and may directly or indirectly regulate transcriptional up-regulation of integrins (22). In light of these data, GDNF levels were found to be decreased in the hypothyroidism group in our study. This decrease suggests that GDNF, which cannot be expressed sufficiently, does not bind to its receptor, thus it may cause disruption in the regulation of integrins and may cause cell loss. Although there are studies on GDNF levels in patients with hypothyroidism in the literature, they are few and our study is the first study to examine its relationship with CoQ10, which is known to be a powerful antioxidant. It has been reported that there is a strong positive correlation between serum GDNF concentrations in healthy individuals (23). In line with these data, serum GDNF level is likely to be an indicator of metabolism. Based on these findings, *Gdnf* expression may be a potential marker of the pathogenesis of hypothyroidism and finally, CoQ10-targeted therapies may have a potential future therapeutic impact on hypothyroidism.

Expression of *Plzf* has been demonstrated in human tissue (24). Matsuzawa et al. confirmed that *Plzf* is expressed in the thyroid gland and reported that *Plzf* expression was higher in patients with papillary thyroid cancer compared with adenomatous lesions and normal thyroid (25). However, in contrast to this study, Chen et al. reported that *Plzf* expression was decreased in sera and thyroid tissue in patients with Graves' disease (26). Compared with thyroid cancer, *Plzf* expression levels exhibit the opposite pattern in Graves' disease. In Graves' disease, TSH receptor (TSHR) expression and *Plzf* expression in human thyroid cells gave an antagonist effect. *Plzf down-regulated Tshr* gene expression. This was associated with the transcriptional repressor property of *Plzf* (27). When the *Plzf* gene expression level was analyzed in our study, low expression was observed in the hypothyroidism group, which may be due to suppression of target gene expression in thyroid hormone deficiency. Taken together, our results may serve as a potential target for thyroid pathology in the future. It provides important information about patients with *Plzf* expression. In conclusion, to the best of our knowledge, this is the first study to characterize *Plzf* expression in hypothyroidism at the serum level, and CoQ10 supplementation increased *Plzf* expression by regulating thyroid hormone regulation.

Sox3 gene has been associated with hypopituitarism and it has been shown that SOX3 is required for the formation of the hypothalamus-pituitary axis in the embryonal period (28). The roles of SOX3 in subsequent development and most organs/tissues are still waiting to be investigated. Our analyses here suggest that *Sox3*, which is involved in the development of the hypothalamic-pituitary axis, may be important in hypothyroidism. The effect of SOX3 proteins in hypothyroidism is unclear. So far, only one study has been associated with our research topic. It has been reported that T3 hormone activates the *Sox3* gene and SOX3 is regulated by T3 (29). Different from the *Sox3* gene, it has been reported that TSH affects *Sox9* gene expression which belongs to the SOX family (30). TSH elevation due to hypothyroidism suppresses the expression of *Sox9* genes (31). No study on the relationship/interaction between hypothyroidism and SOX3 was found in the literature. As far as we know, this study provides the first evidence that *Sox3* is down-regulated in hypothyroidism. When the therapeutic effects of the SOX family in thyroid cancer were investigated, some SOX (SOX4, SOX11, SOX9, SOX17) proteins were reported as potential molecular markers for cancer prognosis and putative potential therapeutic targets, and *Sox* expressions were found to be higher in thyroid tumour tissue compared to normal tissue (30,32-34). In contrast to thyroid cancer cases, our study showed that *Sox3* was poorly expressed in the sera of hypothyroid experimental animals. Furthermore, the data suggest that CoQ10 supplementation promotes up-regulation of *Sox3*. In conclusion, the importance of SOX family members in the pathology of hypothyroidism is still a complex, unexplored area of research and remains largely unknown. Considering that SOX proteins are involved in the regulation of specific biological processes, research in this area is therefore warranted.

Horiguchi et al. revealed the expression of THY1 proteins in the anterior pituitary lobe. THY1 was shown to be present in TSH immunopositive cells (thyrotropes). THY1 appeared to represent a novel marker for thyrotropes. As a result, THY1 is a potent thyrotrope marker (35). Smith et al. reported that thyroid tissue fibroblasts express *Thy1* (36). THY1 has previously been shown to be present in serum in a soluble form (37). In our study, serum *Thy1* level was evaluated. No study on the relationship/interaction between hypothyroidism and THY1 was found in the literature. To our knowledge, this is the first study documenting that *Thy1* is down-regulated in hypothyroidism. The data also suggest that CoQ10 supplementation promotes up-regulation of *Thy1*. Overall, the conclusion of this study is that THY1 can be proposed as a marker for the progression of hypothyroidism. It is also suggested that CoQ10 may be a promising candidate to aid in treatment.

In this study, the effects of CoQ10 supplementation in adult Wistar albino rats with experimental hypothyroidism were investigated using molecular methods. Hypothyroidism may affect the expression of *Gdnf, Plzf, Sox3* and *Thy1* genes in a gene-specific manner, and these changes in gene expression may play

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a role in the development of complications associated with thyroid pathology. Detection of these genes at the blood sera level may be used as an important minimally invasive prognostic marker in thyroid pathology. We believe this study will make an important contribution to the literature in analyzing the effects of CoQ10 in hypothyroidism. Further research is needed in this area. Our study has several limitations that need to beconsidered. The fact that many organs, especially the thyroid gland, were not analyzed in the gene expression study and only serum levels were examined can be considered as a limitation of this study. In addition, the literature search was limited by the fact that there were very few articles similar to the subject of this study. Although there are studies on gene expression levels in hypothyroid patients in the literature, they are few and this study is one of the first studies to examine the relationship with antioxidants. In parallel with the results of this study, we plan to conduct future studies to analyze the role, mechanism and gene regulation of genes studied by genetic studies.

Conflict of Interest: The author have indicated no conflicts of interest regarding the content of this article.
Researchers' Contribution Rate Statement: Concept/Design: HNŞ, EGM, GC, SK; Analysis/Interpretation: HNŞ, EGM; Data Collection: HNŞ, EGM; Writer: HNŞ, EGM; Critical Review: HNŞ, EGM, GC, SK; Approver: HNŞ, EGM, GC, SK.
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