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Assessment of Thyroid Hormone Levels, Antioxidant Capacity and Deiodinase Type 1 Gene Polymorphism in Hypothyroid Patients

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ABSTRACT
The thyroid gland is the largest and one of the most important endocrine glands in the body. Hypothyroidism a prevalent endocrine disorder leads to a generalized metabolic slowdown due to insufficient thyroid hormone production. In this study, 60 sample women were collected from patients with hypothyroidism with 30 healthy women as control group, their age ranged between 20-45 years, conducted in Rizgary teaching hospital and Galiawa center in Erbil province from 22nd September 2024 to 25th February 2025. The statistical analysis showed that serum level of TSH, anti-TPO and anti-Tg increased significantly, while T4, T3, FT4 and FT3 decreased significantly in hypothyroid female patients. Serum level of oxidative stress such as MDA increased significantly, while antioxidant parameters such as klotho, selenium and catalase decreased significantly, but GPx and Vit.C increased significantly in hypothyroid group compared to control group. Deiodinase Type 1 gene polymorphism was detected in genotype frequencies in Erbil hypothyroid female patients. The wild type of SNP rs11206244 (C785T) was the most frequent genotype. Gene polymorphisms of DIO1 (C785T) rs11206244 was considered a risk factor for hypothyroidism in Kurdish women population. The CC genotype increase risk of hypothyroidism.
Keywords: *Hypothyroidism, Klotho, Selenium, Deiodinase type 1 gene polymorphism.*

INTRODUCTION

Thyroid gland becomes one of the largest and most sensitive endocrine glands in the body (Salman et al., 2024). Thyroid gland produces three types of hormones: tri-iodothyronine (T3), tetra-iodothyronine (T4), and calcitonin (Assi, 2023). Thyroxine (T4) is a prohormone with low intrinsic activity, secreted by the thyroid gland and subsequently converted by deiodinases in target tissues into triiodothyronine (T3), the biologically active thyroid hormone (Traini et al., 2025). However, a growing body of data suggests that a better indicator of thyroid hormonal balance than TSH concentration is the assessment of free thyroid hormones: free triiodothyronine (FT3) and free thyroxine (FT4) (Łukawska-Tatarczuk and Franek, 2024). Antibodies against thyrocytes transmembrane protein involved in the production of thyroid hormone, known as anti-thyroid peroxidase. The precursor to thyroid hormone thyroglobulin is attacked by anti-thyroglobulin antibodies (Kawther et al., 2022). The regulation of thyroid hormones is associated with a variety of substances such as sodium/iodide symporter (NIS), thyroglobulin, thyroid peroxidase, monoiodotyrosine (MIT), diiodotyrosine (DIT) (Zhang et al., 2024).
Dysfunction in thyroid gland affects approximately 300 million people worldwide, with rates ranging from 4–10%, across different geographic locations. In Iraq, approximately 14.5% of women suffer from hypothyroidism, both iodine deficiency and excessive iodine intake can have adverse health consequences (Berta et al., 2024, Hamim and Almankhee, 2024). Dysfunction is more prevalent in women than in men. Women are 3–5 times more likely to be treated for thyroid disorders than men (Asmelash et al., 2019, Brown et al., 2023).
Functional thyroid disease is mainly divided into hyperthyroidism, marked by excess production or hypothyroidism, characterized by insufficient hormone production (Teja et al., 2024). Hypothyroidism is thyroid inability of synthesis and secrete sufficient amount of thyroid hormones, which stimulates the pituitary to produce thyroid stimulating hormone (Chiovato et al., 2019). Analyses of the impact of overt and subclinical hypothyroidism on producing adverse changes in the lipid profile are also complicated by other factors such as body weight or body mass index and sex (Jonklaas, 2024). Klotho proteins have been shown to exert anti-oxidative, anti-inflammatory, and anti-apoptosis effects and regulate calcium and phosphorus metabolism, T3 significantly increased the expression levels of membrane form of the klotho gene (Zhang et al., 2024).
Human deiodinase type 1 is found in many organs, but it is most abundant in the thyroid, liver, and kidneys. Deiodinase type 1 is thought to play a role in the intrathyroidal production of T3 in the thyroid gland (Sabatino et al., 2021). Deiodinase type 1 is an enzyme that is found in the plasma membrane and is thought

to be the main source of the plasma T3 in humans. The amount of T3 released by this pathway is thought to equilibrate with the plasma pool of T3 (Paragliola et al., 2020). Single nucleotide polymorphisms (SNPs) in the deiodinase genes may influence thyroid hormone levels by interfering with the phenotypic expression of these enzymes (Mohmmed et al., 2023). The aim of this study to evaluation of thyroid function test, determination of antioxidant and oxidative stress and detection of Single Nucleotide Gene Polymorphism of Deiodinase type 1 gene rs11206244 (C785T) Using Polymerase Chain Reaction Protocol.

MATERIALS AND METHODS

Research design and study groups

This research based on a case-control study. The participants enrolled in this study were from Rizgary teaching hospital (oncology department) and Galiawa center in Erbil province from 22nd September 2024 to 25th February 2025. The interview and the structured questionnaire were designed in the data collection process. Patient groups included 60 adult's women with hypothyroidism, their age ranged between 20-45 Years and categorized as 30 patients selected as treated patients with hypothyroid medication and 30 patients selected as patients with hypothyroidism untreated (new case), control group included 30 healthy women without any history, sign and symptoms of hypothyroidism their aged were matched with patient groups.

Collection of blood samples

Seven milliliters of venous blood were drawn from overnight – fasting from each the patients and control group by medical syringes and distributed into three parts. The first part (2ml) of blood was collected in to Ethylene diamine tetra acetic acid (EDTA) tube processed and used in the assessments of hematological parameters. The second part (3 ml) of blood was put into two gel tubes and left at room temperature for nearly twenty minutes for clotting, then centrifuged at 3000 rpm for 15 minutes to separate serum. The third part (2 ml) of blood was collected in to EDTA tube and stored by freezing at -20 C until used for DNA extraction and then performing molecular analyses for single nucleotide gene polymorphism of iodothyronine deiodinase type 1 D1-C785T gene by PCR and related techniques.

Determination of thyroid function test

The level of each of thyroid stimulating hormone (TSH), Triiodothyronine(T3) and Thyroxine (T4), Free Thyroxine (FT4) and Free Triiodothyronine (FT3), Anti-thyroid Peroxidase (Anti-TPO) and Anti-Thyroglobulin antibodies (Anti-TG) in serum are measured by using Cobas e411 analyzer-(Roch-Diagnostic-Germany).

Determination of oxidative stress and antioxidant

Determination of Malondialdehyde

ELISA kit was used for determination of malondialdehyde

Table 3.5 Represent the standard dilution preparation for MDA.

;0 ng/ml	andard No.1	)0µl Original Standard + 150µl Standard Diluents
ł0 ng/ml	andard No.2	)0µl Standard No.1 + 150µl Standard Diluents
!0 ng/ml	andard No.3	;0µl Standard No.2+ 150µl Standard Diluent
) ng/ml	andard No.4	;0µl Standard No.3+ 150µl Standard Diluent
) ng/ml	andard No.5	;0µl Standard No.4+ 150µl Standard Diluent

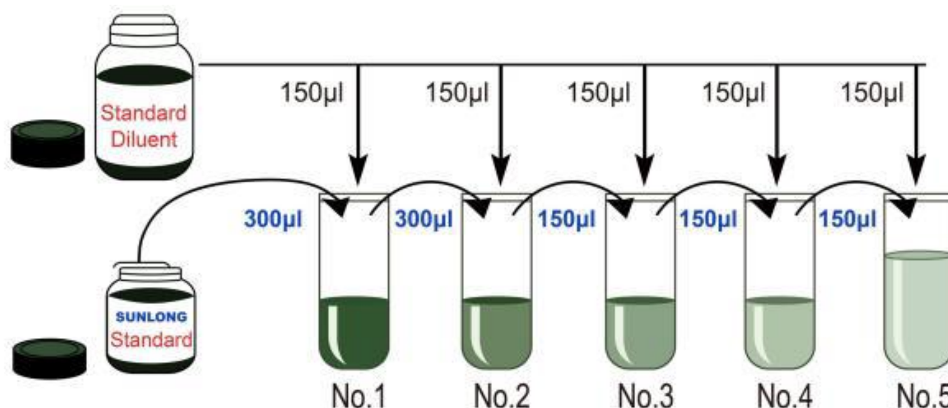

Figure 3.1 A detailed standard solution preparation for MDA.

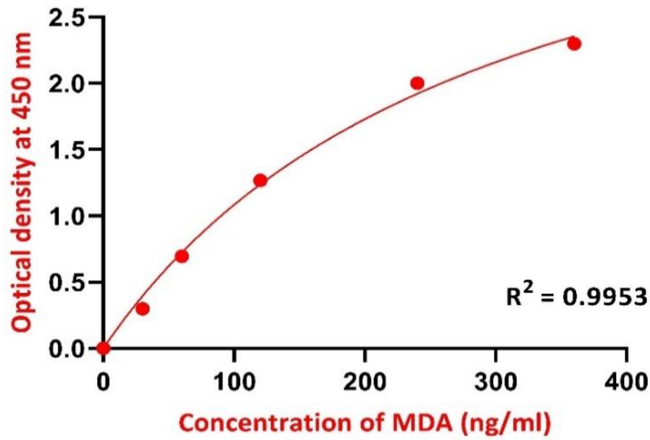

Figure 3.2 Standard curve of MDA.

Determination of Klotho

This kit principle of working is the same as the MDA principle of working as mentioned earlier.

Table 3.6 Represent the standard dilution preparation for Klotho.

andard Concentration	andard No.1	andard No.2	andard o.3	andard o.4	andard o.5
otho (2700 ng/ml)	300 ng/ml	200 ng/ml	00 ng/ml	00 ng/ml	50 ng/ml

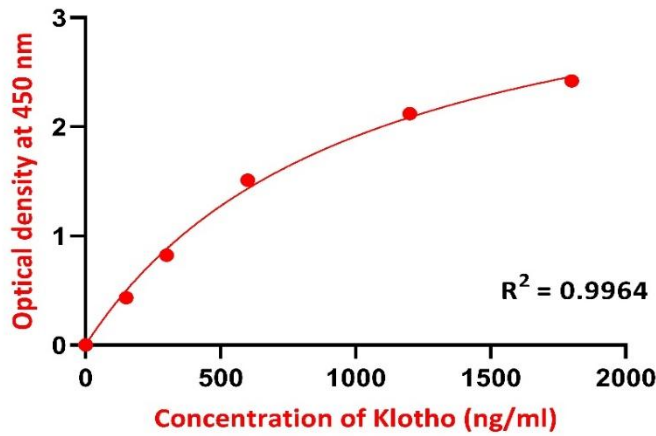

Figure 3.3 Standard curve of Klotho.

Determination of Selenium Binding Protein

This kit principle of working is the same as the MDA principle of working as mentioned earlier.

Table 3.7 Represent the standard dilution preparation for Selenium.

andard Concentration	andard No.1	andard o.2	andard o.3	andard o.4	andard o.5
lenium (1800 ng/ml)	200 ng/ml	00 ng/ml	00 ng/ml	00 ng/ml	00 ng/ml

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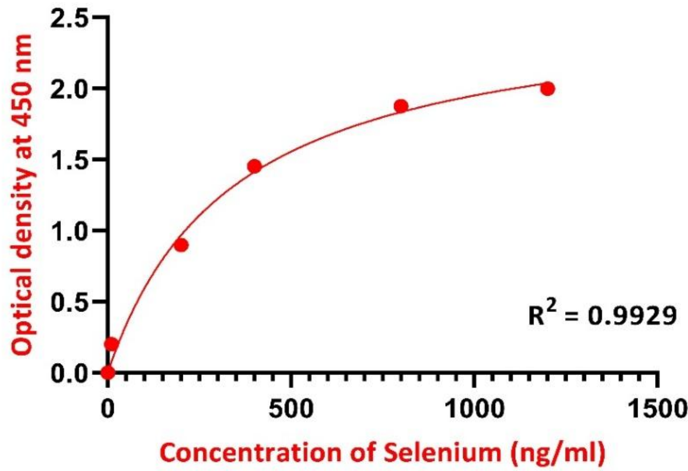


Figure 3.4 Standard curve of Selenium.

Determination of Glutathione Peroxidase

This kit principle of working is the same as the MDA principle of working as mentioned earlier.

Table 3.8 Represent the standard dilution preparation for GPx.

andard Concentration	andard o.1	andard o.2	andard o.3	andard o.4	andard o.5
Px (27 ng/ml)	3 ng/ml	2 ng/ml	ng/ml	ng/ml	5 ng/ml

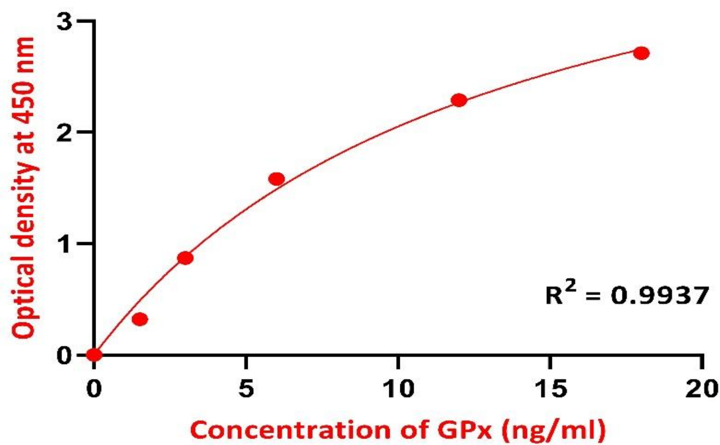


Figure 3.5 Standard curve of GPx.

Determination of Vitamin C

This kit principle of working is the same as the MDA principle of working as mentioned earlier.

Table 3.9 Represent the standard dilution preparation for Vitamin C.

andard Concentration	andard o.1	andard o.2	andard o.3	andard o.4	andard o.5
it.C (45 ng/ml)	) ng/ml	) ng/ml	) ng/ml	ng/ml	5 ng/ml

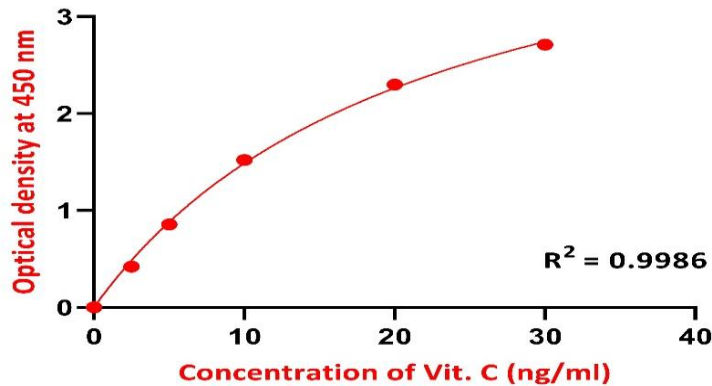


Figure 3.6 Standard curve of Vitamin C.

Determination of Catalase
This kit principle of working is the same as the MDA principle of working as mentioned earlier.

Table 3.10 Represent the standard dilution preparation for catalase.

andard Concentration	andard o.1	andard o.2	andard o.3	andard o.4	andard o.5
atalase (5.4 ng/ml)	6 ng/ml	4 ng/ml	2 ng/ml	6 ng/ml	3 ng/ml

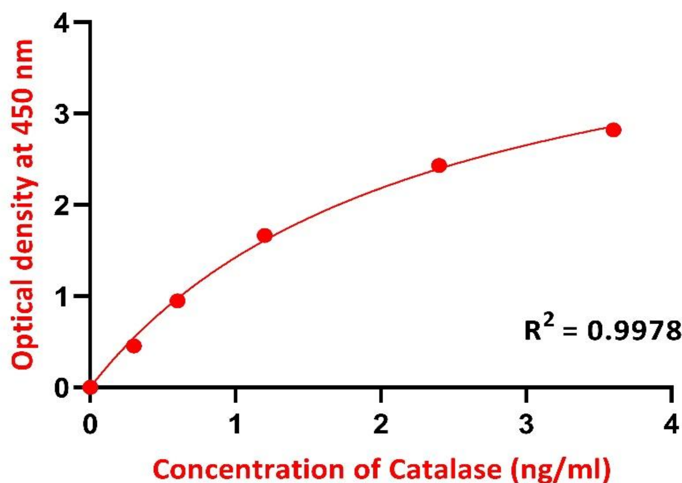


Figure 3.7 Standard curve of Catalase.

Primer design
Primers used in this study were as follow: Forward inner primer (FI): 5´-TCTGGACAGATACCTCAATTCTAGGTTAC-3´ (29mer), Reverse inner primer (RI): 5´-TTGAGAAGCCCTCCCGTGGA-3´ (20mer), Forward outer primer (FO): 5´-TGATTCGTTTCTCTTGCAGGGTAA-3´ (24mer), and Reverse outer primer (RO): 5´-ACAATTTGTCTTGATTGGGTGCTG-3´ (24mer). The product sizes were 191 bp for C allele, 136 bp for the T allele, and 278 bp for the non- allele specific bands.

Molecular assay
Genomic DNA extraction
The total genomic DNA was extracted from a peripheral blood sample. For this purpose, the Genomic DNA Extraction kit (Addbio Prep, South Korea) was used. This kit was designed for the rapid preparation of genomic DNA from up to 200 µL of blood sample like a whole blood.
Assessment of DNA quality (Purification of DNA)

A spectrophotometer (Nanodrop ND1000, thermo scientific, USA) was employed to assess the DNA concentration and purity from contamination. Concentration of DNA was given as ng/μL. Two microliters of each DNA sample was applied in the Nanodrop system and the optical density (O.D.) was measured at 260 and 280nm. Generally, a ratio of A260/A280 of approximately 1.7 – 1.9 indicates pure DNA.

PCR amplification

The amplification refractory mutation system, also termed allele specific PCR, is a simple method for detecting any known mutations involving single base changes which is based on the use of sequence specific PCR primers that allow amplification of the DNA only when the target allele is contained within the sample. Amplification Refractory Mutation System ARMS technique demands only PCR amplification and gel electrophoresis of the amplicons. The targeted regions of Deiodinase Type1 were amplified. A total of 20 μl volume of reaction mixture was prepared to contain 3 μl DNA template, 10 μl of Add Start Taq Master 1μl of each primer and 4 μl double deionized water (ddH2O) in the thermocycler, Applied Biosystem (AB). For Deiodinase Type1 the cycling profile consisted of an initial denaturation step of 95 °C for 5 minutes, then performed 40 cycles of 30 sec. at 95 °C, 35 sec. at 60 °C, 55 sec. at 72 °C, and final extension 72 °C for 5 minutes.

Gel electrophoresis

Agarose gel (2.5%) was used to check the DNA after extraction, gel and DNA preparation and casting were carried as follows. To prepare 1X TBE (Tris-Borate EDTA) buffer, 10 mL of 10 X TBE was pipetted and added to a flask containing 90 mL of distil water. an amount of 2.5 g of agarose powder was weighed and added to the 100 mL of 1X TBE buffer. The solution was dissolved using a microwave and allowed to heating and boiling for 2 minutes until the agarose was completely homogeneous. Then, 10 μL of Red safe stain was added, and mixed gently. Then, it was allowed to cool down to 30 °C. Then, the comb was fixed into the gel tray and the solution was poured into the tray slowly to prevent air bubble formation, and ensuring the tray bottom is fully sealed. Some air bubbles were removed using a pipette and then the gel tray was left to solidify under room temperature (30 minutes). After removing the gel tray and the comb, the solidified gel was first positioned in the tank of electrophoresis and then it was covered with 1X TBE- buffer (approximately 3-5 mm over gel-surface). 10 μl of the PCR product (DND) was loaded into each well. Electrophoresis was carried out at 75 Volts/cm2 for 45 minutes, then the gel was visualized under UV-transilluminator.

Statistical analysis

Graph Pad Prism version 9 MedCalc version 18 computer program were used for the data analysis. Chi square statistics were performed to analyze the demographic characteristics. Ordinary One Way ANOVA was applied to calculated Mean±SE, p value≤0.05 was considered as significant, also the predictive significance of the study was determined severity via Receiver Operator Characteristic ROC Curve analysis and the results were expressed as Area Under Curve AUC values. Also, the WINPEPI (version 11.65) was used for genotype data analysis. Genotypes of Deiodinase Type 1 (C785T) was displayed as percentage frequencies, also, the relative risk (RR), etiological fraction were determined to illustrate how a genotype is associated to a disease. The Hardy Weinberg equilibrium (HWE) was estimated using the H-W calculator for two alleles.

Ethical approval

The participants were fully informed regarding the objectives of the study and ensured them was used only for particular academic purposes, and their participation in the research in voluntary, also we ensured ethics remained a top priority throughout the study (SU2025HREC/12).

RESULTS

Thyroid function test and Hypothyroidism

The mean serum levels of Thyroid-Stimulating Hormone (TSH), Total Thyroxine (T4), Total Triiodothyronine (T3), Free Thyroxine (FT4), Free Triiodothyronine (FT3), Anti-Thyroid Peroxidase Antibodies (Anti-TPO), and Anti-Thyroglobulin Antibodies (Anti-Tg) differed significantly among the three groups ($p < 0.0001$). Serum TSH level was 2.45±0.15 in control group, 6.18±0.50 in treated group and 9.02±0.68 in untreated group. Statistically, control group had lower serum level than those with hypothyroid groups with significant difference, and the treated group showed a significant increase compared to control group. Conversely, serum T4 level was significantly lower in the untreated group 82.41±2.10 compared to treated 93.61±2.24 and control group 103.70±3.07. Similarly, serum T3 level was significantly lower in the untreated group 1.55±0.06 compared to treated 1.846±0.08 and control group 2.14±0.07.

Serum FT4 level decreased significantly in the untreated group 11.67±0.20 compared to both the treated 13.18±0.19 and control group 14.42±0.35. Also, serum FT3 level was significantly lower in the untreated

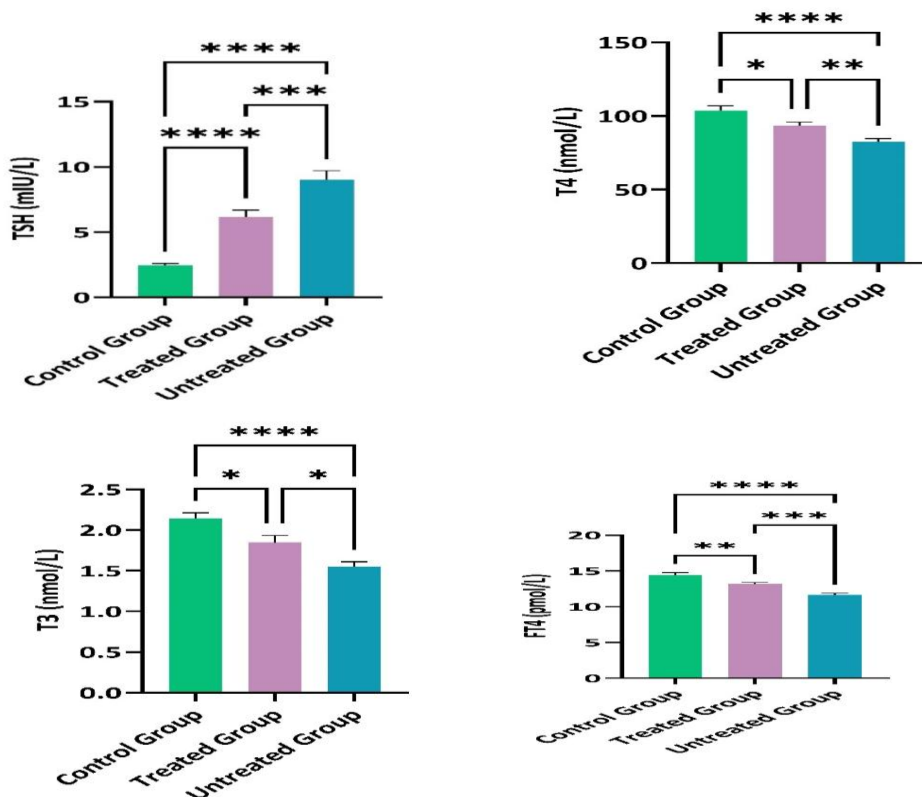
group 3.92±0.07 compared to treated 4.343±0.05 and control group 4.71±0.14. Furthermore, Serum anti-TPO level was 14.32±0.32 in control group, 17.10±0.35 in treated group and 19.05±0.27 in untreated group. Statistical analysis indicated that significant increase level of anti-TPO in the untreated group compared to both treated and control groups, also in the treated group relative to control group.

Serum anti-Tg level was 21.31±0.65 in control group, 25.18±0.92 in treated group and 29.52±0.97 in untreated group. Statistically, serum anti-Tg level elevated significantly in the untreated group compared to the control group. Additionally, there was a slight but statistically significant increase in the untreated group compared to the treated group, as well as a slightly significant increase in anti-Tg levels in the treated group compared to the control group. Table 4.2 Figure 4.3

The results recorded in figure 4.4 (A, B, C, D, E, F and G) emphasize the importance of those parameters: TSH and anti-TPO which showed AUC values of 0.958, p= 0.001 and 0.914, p= 0.001 these values indicate their potential as valuable indicators in the assessment of hypothyroid progression. AUC value of T4, T3, FT4, FT3 and anti-Tg also showed a good biomarker for diagnosing of hypothyroidism which were 0.762, 0.735, 0.831, 0.729, 0.822 respectively and the p-value of these parameters were p= 0.001. Sensitivity were 90.00, 75.00, 41.67, 55.00, 95.00, 78.33, 80.00 and specificity were 100.00, 66.67, 96.67, 100.00, 53.33, 90.00, 70.00 respectively. Figure 4.4

Table 4.2 Thyroid hormones, Anti-TPO and Anti-Tg in studied group.

ıyroid Hormones	ɔntrol Group	ɾeated Group	ntreated Group	value
SH (mIU/L)	45±0.15	18±0.50	02±0.68	0001
4 (nmol/L)	03.70±3.07	.61±2.24	.41±2.10	0001
3 (nmol/L)	14±0.07	846±0.08	55±0.06	0001
T4 (pmol/L)	.42±0.35	.18±0.19	.67±0.20	0001
T3 (pmol/L)	71±0.14	343±0.05	92±0.07	0001
nti-TPO (IU/mL)	.32±0.32	.10±0.35	.05±0.27	0001
nti-Tg (IU/mL)	.31±0.65	.18±0.92	.52±0.97	0001



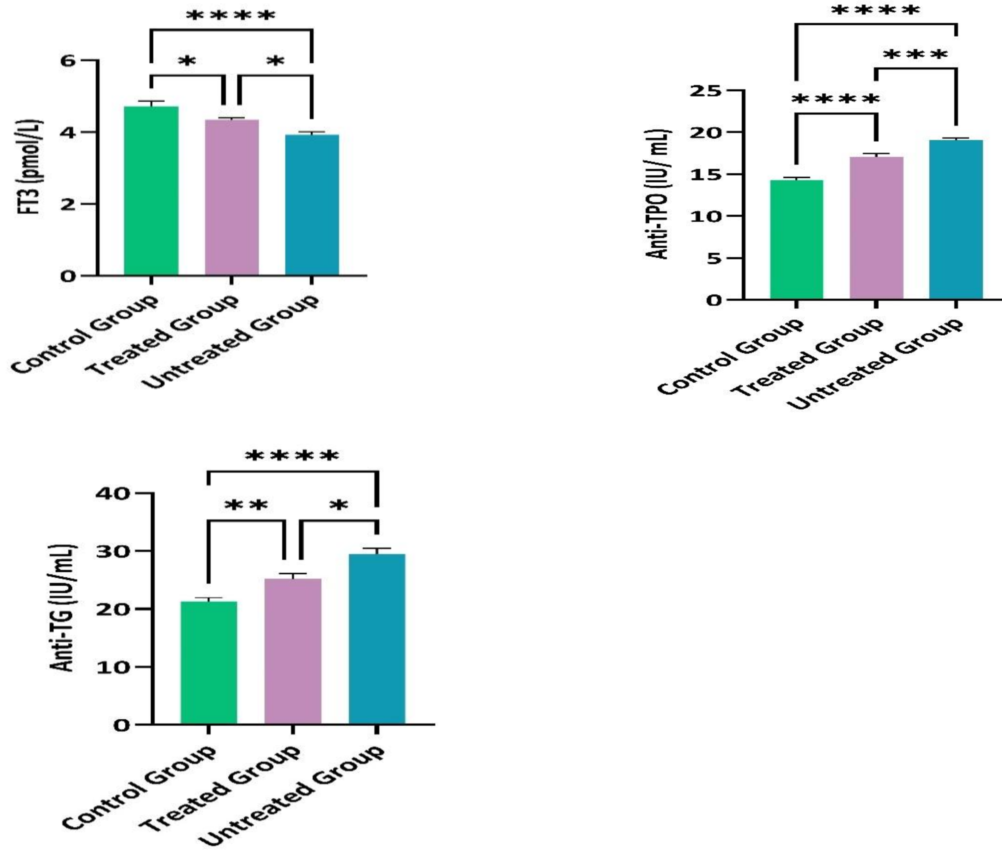
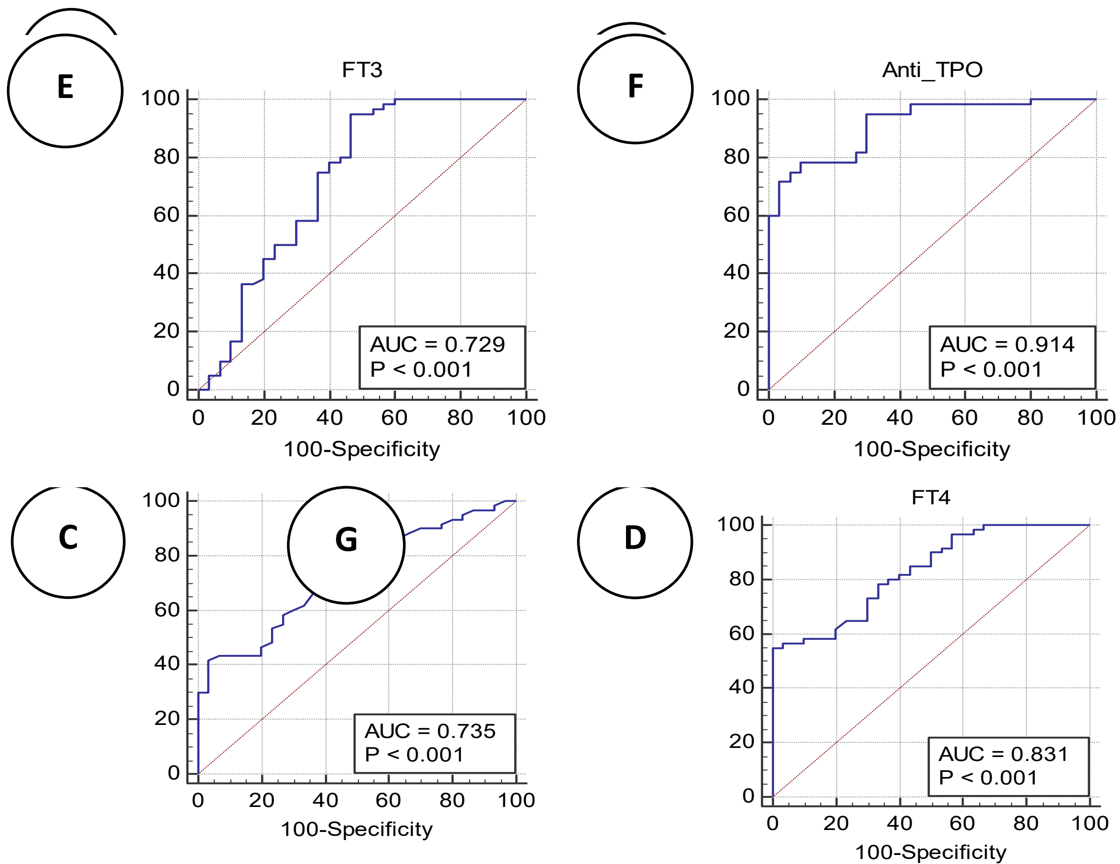


Figure 4.3 Thyroid hormones, Anti-TPO and Anti-Tg in studied group.



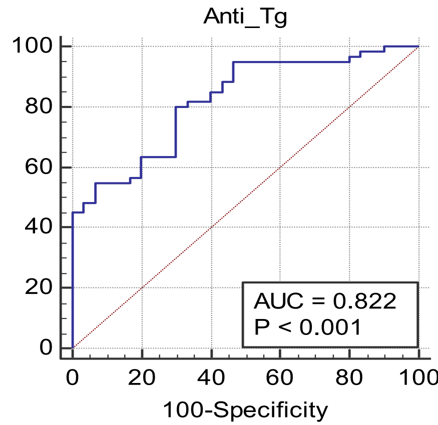


Figure 4.4 The AUC values of Thyroid hormones, Anti-TPO and Anti-Tg in studied group.

Oxidative stress and antioxidant parameters related with Hypothyroidism

According to the analysis of oxidative stress and antioxidant parameters, serum malondialdehyde level was significantly elevated in treated 143.60±4.70 and untreated group 161.70±6.38 compared to control group 121.70±2.90, and in the untreated group exhibited a significant increase compared to the treated group. Serum klotho level lowered significantly in treated 731.50±19.21 and untreated group 686.10±22.05 compared to control group 812.20±18.55, while the decrease in the untreated group compared to treated group was non-significant. Serum level of selenium was 292.20±9.99 in control group, 247.10±10.51 in treated group and 212.30±7.97 in untreated group, which significantly reduced in treated and untreated group compared to control group. In addition, selenium level was decreased significantly in the untreated group compared to the treated group.

Serum Glutathione Peroxidase (GPx) level markedly elevated in treated 6.938±0.33 and untreated group 8.43±0.33 compared to control group 5.53±0.23, with significant increase between two hypothyroid group. Serum level of vitamin C level was 13.69±0.46 in control group, 15.61±1.04 in treated group and 18.38±1.27 in untreated group. Statistically, a significant increase in untreated group compared to control group, with increase level in the untreated group compared to treated group, also increase in the treated group compared to control group was non-significant. Serum catalase level was lowered significantly in untreated group compared to control group. Decrease in the treated group compared to control group also untreated group to treated group was non-significant. Table 4.4 Figure 4.7

According to AUC values, MDA was considered potential biomarker for diagnostic disease. Klotho, selenium, and GPx were identified as good biomarkers, while vitamin C and catalase were classified as satisfactory markers for hypothyroidism. The AUC of MDA level was 0.804, klotho was 0.756, selenium was 0.796, GPX was 0.790, Vit.C was 0.657, catalase was 0.602, and p-value= 0.0001, 0.0001, 0.0001, 0.0001, 0.0093, 0.1225 and sensitivity were 61.67, 75.00, 76.67, 96.67, 85.00, 90.00, and specificity were 90.00, 70.00, 73.33, 56.67, 46.67, 33.33 respectively. Figure 4.8 (A, B, C, D, E and F).

Table 4.4 Oxidative stress and Antioxidant parameters in studied group.

xidative stress and ntioxidant parameters	ontrol Group	eated Group	ntreated Group	value
DA (ng/ml)	1.70±2.90	3.60±4.70	1.70±6.38	0001
otho (ng/ml)	2.20±18.55	1.50±19.21	36.10±22.05	0001
lenium (ng/ml)	2.20±9.99	7.10±10.51	2.30±7.97	0001
Px (ng/ml)	53±0.23	938±0.33	43±0.33	0001
it.C (ng/ml)	.69±0.46	.61±1.04	.38±1.27	0046
atalase (ng/ml)	57±0.07	46±0.03	35±0.03	0128

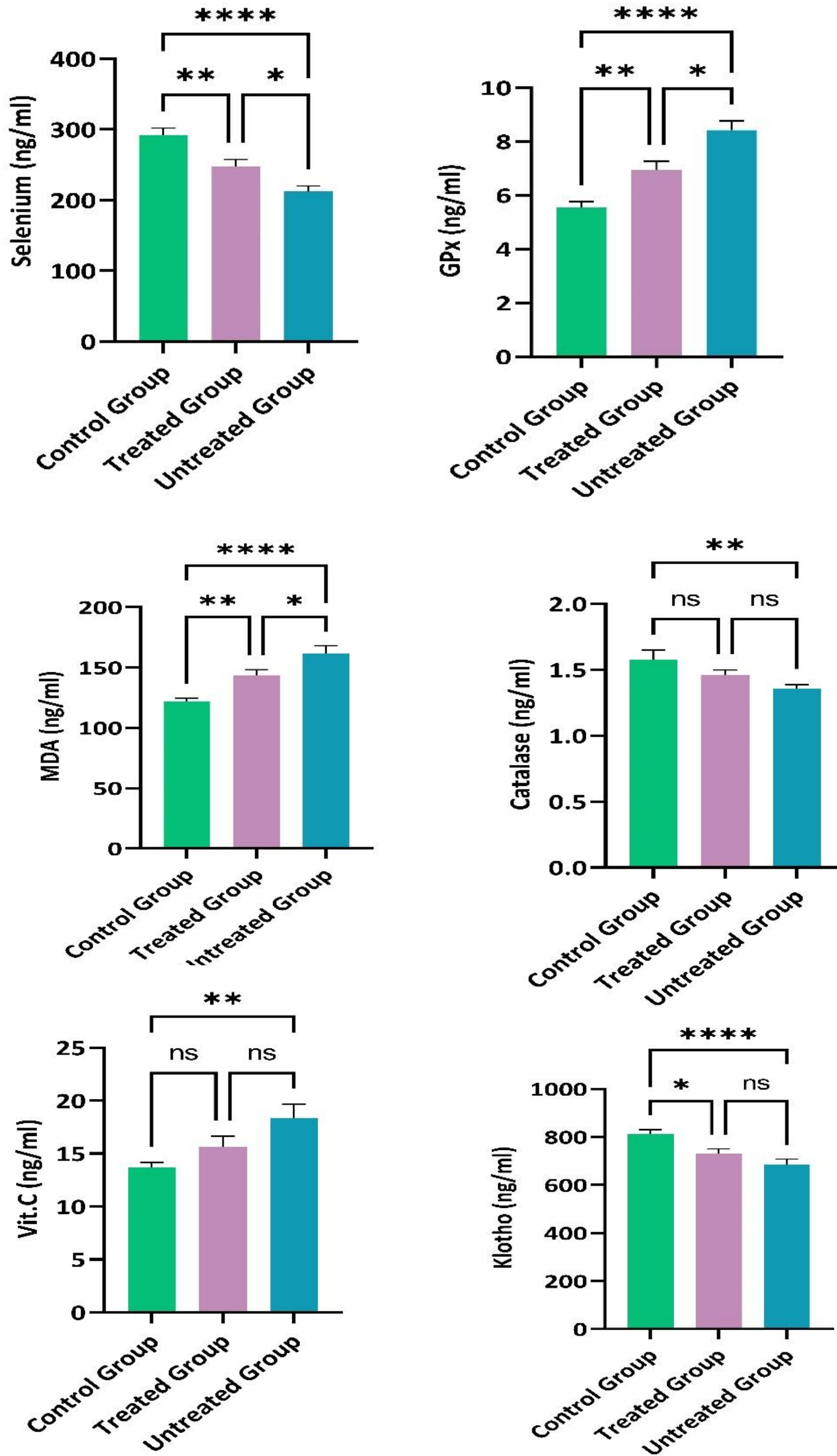


Figure 4.7 Oxidative stress and Antioxidant parameters in studied group.

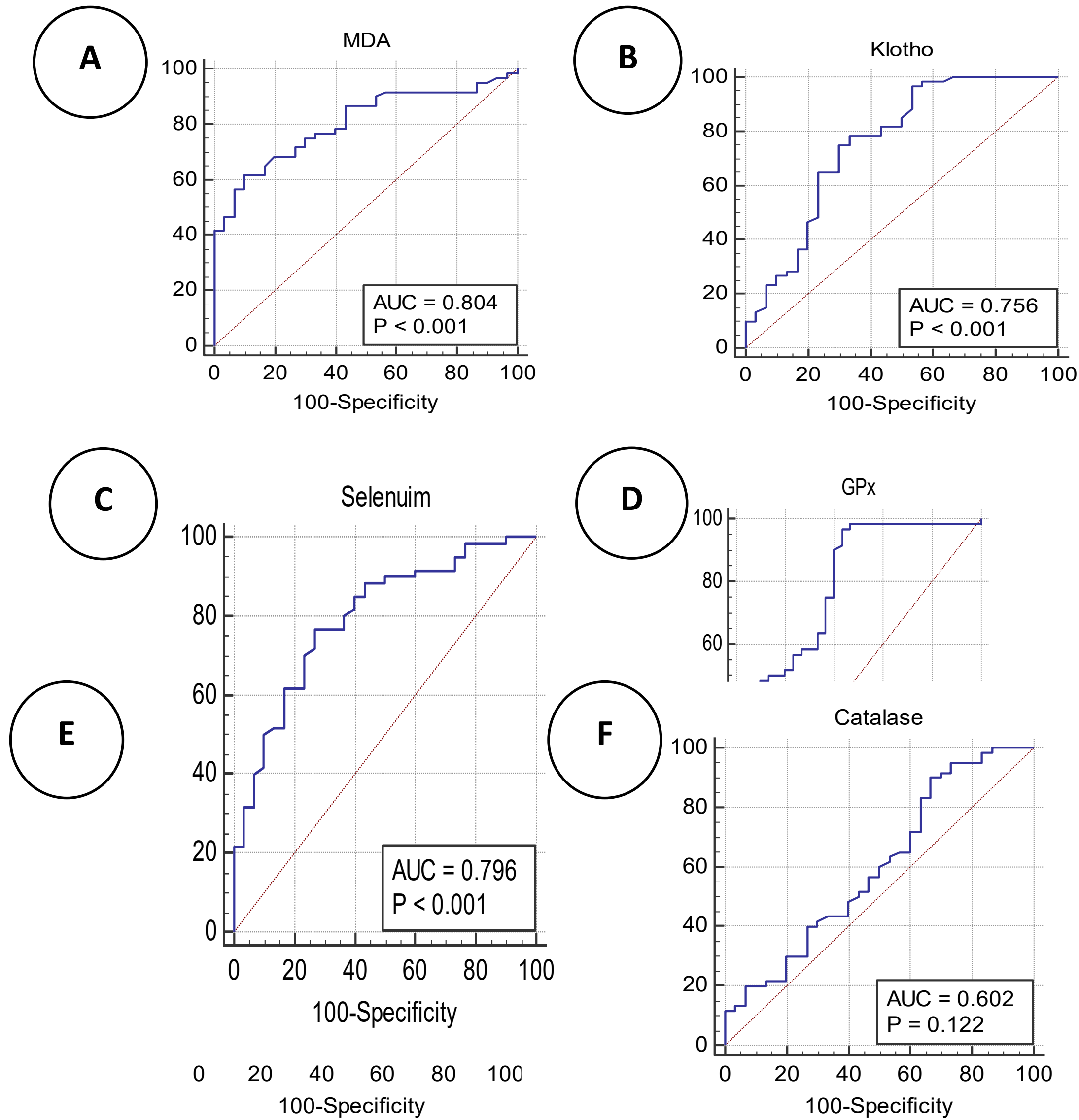


Figure 4.8 The AUC values of oxidative stress and antioxidant parameters in studied group.

Deiodinase Type 1 (C785T) genotype

The amplification of the Deiodinase Type 1 (C785T) gene was carried out, and the amplicons were gel electrophoresed. The CC (278/191 bp) genotype implies normal homozygous, the CT (278/191/136 bp) genotype indicates heterozygous, while TT (278/136 bp) genotype implies mutant homozygous. Figure 4.19

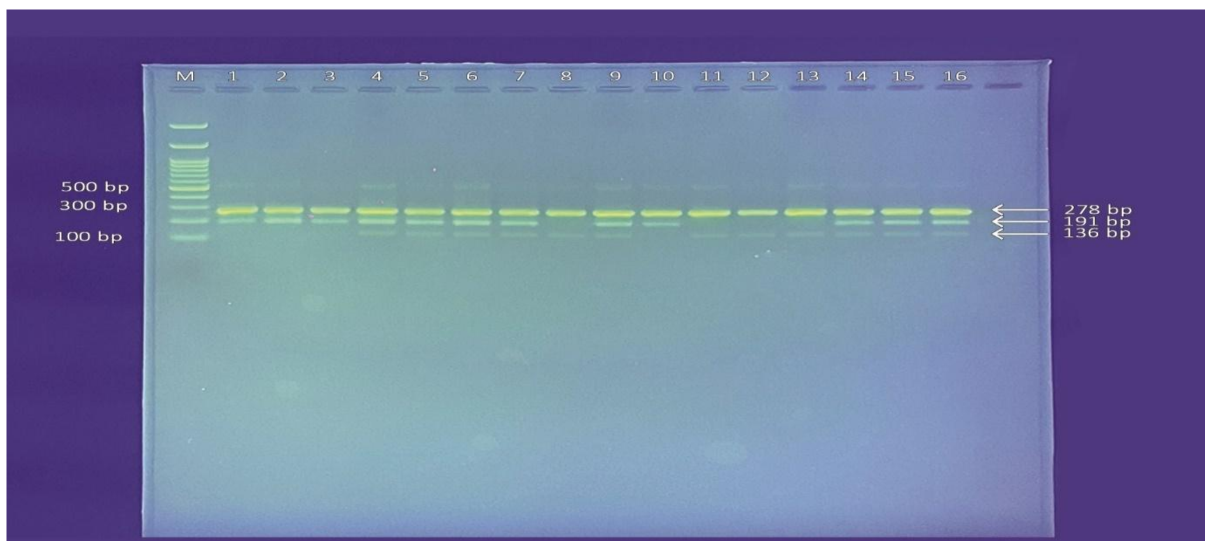


Fig 4.19 Agarose gel electrophoresis illustrating PCR products for the Deiodinase type 1 rs11206244 (C785T). Ethidium bromide used to make the DNA visible under UV light with PCR products for (C785T) SNP. M stands for DNA Marker (Ladder) 100 bps, CC: Wild type homozygous, CT: Mutant heterozygous, TT: Mutant homozygous.

Association of Deiodinase Type 1 (C785T) genotype distributions and allele frequencies in hypothyroidism

Table 4.11 summarize the distribution of Deiodinase Type 1 (C785T) rs11206244 variants in hypothyroidism and control group. Among hypothyroid group the frequencies of individuals of CC, CT and TT genotypes were, 47 (78.33%), 11 (18.33%) and 2 (3.33%) in hypothyroid group and 22 (73.33%), 5 (16.66%) and 3 (10%) in control group, respectively. The analysis of deiodinase type 1 genotype CC and CT showed a 1.31 and 1.12 times risk for hypothyroidism with p= 0.606 and 1.000 respectively while the genotype frequency of TT showed protective factor for hypothyroid patients with non-significant.

The frequencies of major allele C compared to the minor allele T in hypothyroidism and control group were 105 (87.5%) and 15 (12.5%) in the case hypothyroid group and 49 (81.67%) and 11 (18.33%) in the control group, respectively. The rs11206244 variant was also associated with a higher risk of hypothyroidism in the recessive model (CC+CT) vs TT 58 (81.70%) in patient group and 27 (77.15%) in control group. (RR= 3.22, p= 0.328)

Table 4.11 The genotypes and allele frequencies of Deiodinase Type 1 C/T in hypothyroid patients and control group.

eiodinase Type 1 :/T)	ıtient %	ɔntrol %	elative isk R)	iology or reventive action	kact Fishers robability)	% ɔnfidence ıtervals I)
enotype						
C	(78.33%)	(73.33%)	31	18	606	48 – 3.58
T	(18.33%)	(16.66%)	12	02	000	36 – 3.53
T	(3.33%)	(10%)	31	06	328	05 – 1.92
C+CT) vs TT	(81.70%)	(77.15%)	22	66	328	52 – 19.98
T+CT) vs CC	(18.30%)	(22.85%)	76	06	606	28 – 2.07
llele						
allele	05 (87.5%)	(81.67%)	57	31	368	68 – 3.65
allele	(12.5%)	(18.33%)	64	06	368	27 – 1.48

The genotype frequencies of deiodinase type 1 among the categories of hypothyroidism was assessed by the HWE calculation. The differences in frequency of CC, CT and TT genotype, wild type (CC) between observed and expected in hypothyroid group were 47 (78.33%), 45.94 (76.56%); 11 (18.33%), 13.13 (21.88%) and 2 (3.33%), 0.94 (1.56%). In control group 22 (73.33%), 20.01 (66.69%); 5 (16.66%), 8.98 (29.94%) and 3 (10%), 1.01 (3.36%) respectively. The variance between the observed and expected values of patient genotype frequencies was statistically non-significant, while the variance between the observed and expected values of control genotype frequencies was statistically significant. Table 4.12

Table 4.12 Hardy-Weinberg equilibrium (HWE) test for the genotypes and allele distributions of Deiodinase Type 1 C/T in hypothyroid patients and control group.

ıse Categories		eiodinase Type 1 gene at position C785T (rs11206244)					
		enotypes			WE value	lleles	
		C	T	T			
ypo yroid Patients	bserved	(78.33%)	(18.33%)	(3.33%)	209	05 (87.5%)	(12.5%)
	xpected	.94 6.56%)	.13 1.88%)	94 .56%)		A	
ɔntrol	bserved	(73.33%)	(16.66%)	0%)	015	(81.67%)	(18.33%)

	xpected	).01 6.69%)	98 9.94%)	01 .36%)		A
A: Not applicable						

DISCUSSION

Thyroid hormones are essential for the proper regulation and performance of diverse bodily functions. Consequently, understanding the factors that can influence the levels of TSH and thyroid hormones is of paramount importance. While genetic determinants account for approximately 65% of the variability in TSH and thyroid hormone concentrations among individuals, a multitude of environmental factors are also known to impact thyroid function. These include lifestyle choices such as smoking, alcohol intake, dietary habits, and physical activity, as well as exposure to environmental pollutants like chemicals and heavy metals. An excessive intake of iodine, a crucial dietary micronutrient, has been observed to increase TSH level while reducing thyroid hormone levels. Among the various pollutants studied, exposure to perchlorate has been commonly associated with a reduction in thyroid hormone levels (Babić Leko et al., 2021).

The cornerstone of thyroid function testing today is the serum thyroid stimulating hormone (TSH) concentration, which can be measured with an adequate sensitivity assay. For untreated groups of people that are at risk for primary thyroid dysfunction, a normal TSH concentration almost certainly rules out an anomaly. Nevertheless, serum TSH can provide a false signal of thyroid state in a number of crucial circumstances, most notably pituitary disorders and the early therapy of thyroid dysfunction (Fröhlich and Wahl, 2017).

Geographically, thyroid disorders are more pronounced in iodine-deficient areas. Inadequate consumption of iodine is linked to reduced production of thyroid hormones and an increased prevalence of hypothyroidism (Voulgari et al., 2022).

Scientists recognized the crucial role of TSH in FT3 conversion, and more studies have shown that temporarily stopping oral levothyroxine increases plasma TSH levels and alters FT3/FT4 ratios significantly within three days. This suggests a direct correlation between TSH levels and the conversion of FT3 and FT4. The lower TSH levels observed in hypothyroidism patients undergoing therapy across treatment duration may be attributed to the TSH-lowering effect of the treatment (Duntas and Jonklaas, 2019). Johnson (2019) investigated the duration of L-thyroxine therapy and its impact on thyroid function status and metabolic pathways aiming to optimize levothyroxine therapy.

A significant increase in the plasma patients TSH comparing to the healthy control people, indicating potential thyroid dysfunction. Additionally, elevated levels of hypothyroidism patient's plasma FT3 level indicated hyperactivity of thyroid biological activity. Unlikely, FT4 was dramatically lower in the patients, signaling potential disruptions in free thyroxine regulation (Miran et al., 2024). Somayaji et al. (2024) and Khambalkar and Jadhav (2025) recorded that the TSH level elevated significantly and FT3 with FT4 decreased in hypothyroid patients compared to control group, these results are in line with the present study. Some studies reported that increase in T3 and FT3 plasma levels in hypothyroidism patients appear to be a strong positive relationship between serum TG and serum TSH/FT3-FT4, while FT4 showed a negative association (Saldarriaga et al., 2020, Huang et al., 2022). According to the American Thyroid Association, patients who are hypothyroid will have an elevated T3 level. In some individuals with a low TSH, only the T3 is elevated and the FT4 or FT3 is normal (Paczkowska et al., 2020).

Al-Fatlawi (2022) showed significant decrease of T4 in cases compared to control group, and this decrease most often manifests by decrease physical and mental activity due to reduction in metabolic function. While Miran et al. (2024) suggested problems with the regulation of free thyroxine, along with increased TSH, FT3 and TT3 levels, which indicated thyroid dysfunction and hyperactivity. Despite the fact that only 1% of people have Autoimmune Thyroid Disease (AITDs), 15% of people with normal thyroid function may have subclinical and localized thyroiditis and circulating antithyroid antibodies (Cárdenas Roldán et al., 2012). According to a study done by Siriwardhane et al. (2019) showed anti-TPO antibodies were more prevalent than anti-Tg antibodies in all outcomes.

The role of anti-TPO as predictive markers for thyroid autoimmunity (Rudijanto and Wijaya, 2024). According to one theory, T cells develop self-tolerance when blood triglyceride levels are within the normal range, whereas B cells do not. B lymphocytes that identify TG limit their movement in the T cell zone of peripheral lymphoid organs, even when they do not engage with CD4 helper populations. A lack of contact induces death in the B cells and inhibits their migration from the T cell zones to the follicles. Due to the actions of B cells, individuals in good health typically have minimal quantities of anti-TG Abs, which are

below the threshold for detection (Hattori et al., 2017). Amin et al. (2018) no significant differences were shown in T3 levels while contrary highly significant increase was shown in TSH, anti-Tg and anti-TPO levels between healthy subjects and unhealthy patient groups.
Oxidative stress is an impairment of the equilibrium between reactive oxygen species (ROS) and antioxidants on behalf of reactive oxidants. ROS act by causing pathological changes in the cellular membrane, cellular organelles, and DNAs through the oxidation of proteins, lipids, and carbohydrates. As a result of this, functional impairment or cell death may develop, and tumors may develop by gaining mutant features (Zhu et al., 2020).
Excessive oxidative stress controls the immune system and contributes to the pathogenesis of autoimmune disorders such as increased inflammation, proapoptotic effect and breaking down the immunological tolerance (Somayaji et al., 2024). The mitochondrial antioxidant defense system is considerably influenced by the thyroid status of the body. Thyroid hormones might be able to regulate the activities of antioxidant enzyme systems in the various organs (Joshi et al., 2018). Hypothyroidism is associated with increased oxidative stress response. Treatment with L-thyroxine is effective in bringing reduction in the level of stress markers (Chakrabarti et al., 2016), and this result was in line with present study.
Malondialdehyde estimates the damage caused by reactive oxygen species. Peroxidation of polyunsaturated fatty acids in biological membranes produces MDA, a highly reactive aldehyde. The MDA level has increased due to increasing oxidative stress (Somayaji et al., 2024). Previous study has found MDA to be useful in studying oxidative stress and it does not vary across the age and gender of the study population. MDA level was found to be higher in the patients as compared to controls suggesting increased oxidative stress in hypothyroid subjects (Jhansi Lakshmi et al., 2013).
Klotho, an anti-aging, anti-inflammatory and anti-oxidative stress effects. In humans, the serum levels of klotho proteins decrease with age from the age of 40 years. Klotho proteins are highly expressed in the kidneys, cerebral choroid plexus, parathyroid glands, pituitary gland, thyroid gland, aorta, ovaries, and testes (Yang et al., 2020). The soluble form acts as a circulating hormone exhibiting diverse activities, such as anti-inflammatory, anti-oxidative stress, tumour-suppressive, and proteolytic cleavage activity (Hajare et al., 2025). The protein αKlotho (hereafter referred as Klotho) has been found to be related to the appearance of oxidative stress. Thus, Klotho deficiency has been shown to increase endogenous ROS generation and accentuate oxidative stress (Izbeki et al., 2010); conversely, Klotho administration effectively reduces oxidative stress and preserves mitochondrial function (Miao et al., 2021).
Moreover, many studies on Klotho in elderly-related diseases indicate that a decrease in serum Klotho levels may contribute to the prevalence of certain chronic conditions in the elderly population (An et al., 2023). Therefore, it is speculated that a decrease in Klotho level is unfavorable for inhibiting aging, and serum Klotho level is negatively correlated with the prevalence of hypothyroidism in elderly individuals (Zhang et al., 2023). Zhang et al. (2024) disagree with present study, that showed serum klotho level increased significantly in hypothyroid patients compared to control group. Selenium is a microelement with its highest concentration in thyroid gland (Błażewicz et al., 2021). The population's dietary characteristics and their geographical location (mainly soil composition) affect selenium levels (Ventura et al., 2017).
Selenium acts as an essential cofactor for most GPx isoforms; therefore, selenium deficiency can alter GPx activity, increasing the risk of diseases related to oxidative stress (Nirgude and Choudhary, 2021). In fact, it was found that selenium deficiency decreases the synthesis of thyroid hormones, as it decreases the function of selenoproteins, in particular iodothyronine deiodinases (DIOs), which are responsible for the conversion of T4 to T3. This decreased production of thyroid hormones leads to the stimulation of the hypothalamic-pituitary axis due to the lack of negative feedback control, increasing TSH production. TSH stimulates the DIOs to convert T4 to T3, with consequent production of hydrogen peroxide, which is not adequately removed by less active glutathione peroxidases (GPx) and accumulates itself in the thyroid tissue causing thyrocyte damage with subsequent fibrosis (Ventura et al., 2017).
In another study, higher selenium levels (both in the soil and in the serum) were correlated with lower prevalence of subclinical and overt hypothyroidism, autoimmune thyroiditis, enlarged thyroids and a higher prevalence of subclinical hyperthyroidism (Wu et al., 2015). Higher serum Se level were associated with consumption of meat, eggs, and green tea. Those results were hindered by gender imbalance, indirect measurement of Se levels by questionnaires, lack of consideration for other factors, such as genetic predispositions, diet, and environment (Chaberska et al., 2024). Milanović et al. (2024) showed that selenium level decreased significantly in hypothyroid patients compared to control group, this result was in agreement with present study.

Glutathione peroxidase (GPx) is a crucial enzyme that protects cells from oxidative damage by catalyzing the reduction of hydrogen peroxide and organic hydroperoxides by using reduced glutathione as a substrate. There are multiple isoforms of GPx, each exhibiting distinct substrate specificities and tissue distributions (Nirgude and Choudhary, 2021). (Omon and Ajay, 2023, HAMIDY et al., 2024, Festus et al., 2025) these studies disagree with present study, showed that GPX level decreased significantly in hypothyroid patients compared to control group. Vitamin C (ascorbic acid) is a water-soluble vitamin, that plays a key role in the prevention and treatment of scurvy. As vitamin C is an antioxidant and thyroid function may be affected and may affect vitamin C levels (Behnoush et al., 2023).

Study by (Jubiz and Ramirez, 2014) which assessed the effect of vitamin C on the concentration of TSH, T3 and T4 in hypothyroid cases with gastrointestinal abnormalities and elevated TSH levels, concluded that vitamin C can increase T3, T4 and subsequently reduce TSH. While there is no clear explanation of vitamin C's effect on levothyroxine malabsorption, there has been a suggestion that high gastric pH may interfere with levothyroxine absorption, and therefore, decreasing pH via vitamin C can enhance the drug's absorption. This idea could be supported by a recent systematic review which demonstrated an increase in TSH levels in concomitant use of levothyroxine and proton pump inhibitors (PPIs) (Guzman-Prado et al., 2021). However, this might cause problems in hypothyroid patients in need of PPI use, and increased gastric pH while consuming oral ascorbic acid with levothyroxine may have potential benefits in increasing the drug's absorption.

Erdamar et al. (2008) showed no statistical difference in vitamin C levels between untreated hypothyroid patients and healthy control at baseline; however, treatment with levothyroxine in hypothyroid cases had a significant association with an increase in vitamin C levels. As vitamin C is among non-enzymatic antioxidants, it was concluded that reactive oxygen species were increased in hypothyroidism, suggesting the oxidative stress caused by this condition. The study done by (Antúnez and Licht, 2011) investigated the effect of vitamin C administration in hypothyroid patients with normal TSH levels. The TSH level was measured before and after treatment with 1 g/day of vitamin C administered with levothyroxine, while the levothyroxine dose was constant, and it was found that the patients had statistically lower TSH levels after the intervention.

Catalase is one of the most important antioxidant enzymes, it is present in almost all aerobic organisms (Nandi et al., 2019). Catalases, heme enzymes which catalyze decomposition of hydrogen peroxide to water and molecular oxygen, are important members of the antioxidant defense system of cells of almost all aerobic organisms (Gebicka and Krych-Madej, 2019). Omon and Ajay (2023) agree with present study who showed that catalase level decreased significantly in hypothyroid patients compared to control group. Festus et al. (2025) disagree with present study, catalase level increased non-significantly in hypothyroid patients compared to control group.

In the present study, the results demonstrated that deiodinase type 1 (DIO1) C785T rs11206244 polymorphism was not significantly associated with the increased risk of hypothyroidism. Philibert et al. (2011) revealed that the DIO1 rs11206244 genotype was associated with lifetime major depression in female subjects.

DIO1 mutations seem to be rare, DIO1 SNPs have been identified and also found to be associated with alterations in the free T3 to free T4 ratio, the C-allele in this SNP correlated with increased D1 function, resulting in elevated free T3/T4 ratio and free T3 levels, as well as decreased free T4 and rT3 levels. Genetic modifications in DIO1 may also explain anecdotal reports that the T3/rT3 ratio could be used to assess the effectiveness of therapy with LT4, given that rT3 is cleared from the circulation via D1, any impairment in D1 activity can result in an accumulation of circulating rT3, decreasing further the T3/rT3 ratio (Penna et al., 2024).

Gałecka et al. (2016) demonstrated that the distribution of demographic trials for the various genotypes of rs11206244 (C785T) SNP did not show any detectable variations. Arici et al. (2018) found that the fT3 and fT4 level in CC, CT, and TT groups are not significantly varied, however tT4 was higher among the wild type group, but to a non-significant level, also observed that the values of TSH, fT3 and fT4 were in normal range in patients with variant and wild alleles of DIO1 rs11206244. Van Der Deure et al. (2009) demonstrated that the free T4 (FT4) and reverse T3 (rT3) levels were higher in the T allele than in the C allele of this polymorphism, also showed that no considerable variation in fT3 and fT4 levels were found among the wild and the mutant types groups during his study on the Dutch population.

Peeters et al. (2003) revealed that reverse T3 is produced by DIO3-catalyzed IRD of T4 and removed by DIO1-catalyzed outer ring deiodination (ORD). The favored substrate for the DIO1 is rT3. Peeters et al. (2005) reported that the DIO1 efficiency in the ORD of rT3 was 100 times greater than that of the ORD of

T4. This indicates the significance role of DIO1 in the breakdown of thyroid hormone. As a result, polymorphisms in DIO1 could be expected to be associated with rT3 levels. This could explain why the rs11206244 (C785T) SNP variation had no significant association with plasma T3 and T4 but did have a high association with rT3 levels in the study sample. Cooper-Kazaz et al. (2009) showed that among SNPs in DIO1, only the DIO1-785T genotype was associated with the efficacy of LT3 supplementation (DIO1-785T has reduced activity).

As far as we know, this is the first study in the Kurdistan region that investigate the effect of the Deiodinase-1 (DIO1) gene polymorphism rs11206244 (C785T) Single Nucleotide Polymorphism on the clinical management of levothyroxine-treated and untreated hypothyroid Kurdish female patients. Deiodinase Type 1 enzyme expression in the kidney, liver, pituitary and thyroid, but not in vascular smooth muscle (Paragliola et al., 2020). DIO1 catalyzes the monodeiodination of T4 to T3 (Wang et al., 2023). The human carriers exhibit impaired metabolism of iodothyronines with elevated rT3 and higher rT3/T3 ratios presumably due to reduced clearance of rT3 by DIO1 (Hernandez et al., 2021).

Genes involved in thyroid hormone metabolism, such as the DIOs enzyme gene, may have genetic variations that alter the protein levels, expression, or activity, as well as the outcomes of various pathways (Gałecka et al., 2016). The rs11206244 (C785T) is one of the polymorphisms that has been recognized as a potential candidate for physiological and pathological states in humans. The rs11206244 polymorphism is located in the mRNA's 3`-untranslated region of DIO1 gene (Verloop et al., 2014). The DIO genetic polymorphisms have received the most attention in patients with thyroid disorders, such as hypothyroidism and thyroid cancer (Bunevicius et al., 2019).

It is expected that the modifications in the DIO1 genes will result in abnormalities in the hormone metabolism since DIO1 functions as a scavenger by eliminating iodo group from both the inner and outer rings (AlRasheed et al., 2019). Deiodinase type1 (DIO1) gene encodes for the DIO1 enzyme that regulates the metabolism of thyroid hormones and LT4 as well. Polymorphisms in this genes could be responsible for altered DIO1 enzymatic activity and accordingly altered response to LT4 (Jawad et al., 2022). Clinical evidence in the community indicates that patients with hypothyroidism who were administrating thyroid hormone replacement therapy continue to experience disease-related symptoms. Not all patients receiving treatment have the same standards for quality of life (Hegedüs et al., 2022).

Single nucleotide polymorphisms (SNPs) in the DIO1 gene may change the amount of thyroid hormone in the body by changing how the enzymes act (Dora et al., 2010). Complex feedback loops are the base of thyroid hormone homeostasis. Thyroid hormone feedback, for instance, inhibits the release of TSH, and evidence suggests that, in addition to controlling thyrotropin-releasing hormone (paracrine action), TSH also controls its secretion autocrine action (Gottwald-Hostalek and Kahaly, 2021). The thyroid hormone levels in serum are regulated by thyroid function, the capacity of thyroid hormone binding and deiodinase activity (Peeters et al., 2005). The ratio of iodothyronines in serum describes the various deiodinases activity most accurately due to the confusing influence of different quantities of thyroid hormone-binding proteins. Because the ratios of the various iodothyronines are more indicative of peripheral thyroid hormone metabolism rather than variations in serum thyroid hormones, the differences in serum iodothyronines ratios could be more indicative of the impact of polymorphisms in DIO1 (Peeters et al., 2005, Peeters et al., 2006).

Van Der Deure et al. (2009) agree with present study, revealed that wild type CC was more common, where the distribution of CC genotype was more than CT and TT. (Panicker et al., 2008, Gałecka et al., 2016, Arici et al., 2018) These studies were performed on hypothyroid patients where the wild type CC is predominant followed by the heterozygous mutant type CT and homozygous mutant type TT. On the other hand, De Jong et al. (2007) found that the distribution of CT was more than other genotypes CC and TT. Also, revealed that the T-allele carrier patients had higher plasma rT3 levels. Which reported a significant correlation between T allele and a decreased in T3/rT3 ratio.

The C-allele in this SNP correlated with increased D1 function, resulting in elevated free T3/T4 ratio and free T3 levels, as well as decreased free T4 and rT3 levels. The minor genotype of a few DIO1 SNP (rs11206244) were associated with reduced psychological wellbeing, but not those for DIO2 or DIO3 (Young Cho et al., 2017). The finding of Nuñez et al. (2021) study suggest that T3 supplementation, in association with genetic variation in DIO1-C785T which functionally reduces peripheral conversion of T4 to T3, may attenuate a relative functional deficit in carriers of the minor allele (CT + TT). Oxidative stress, which is also characteristically observed in depression is an important factor that may affect and reduce DIO1 levels (Czarny et al., 2015).

(Chen et al., 2016) suggest that oxidative stress may reduce expression of DIO1 on the mRNA level and downregulates the conversion of T4 to T3 through the function of DIO1. The most important human plasma

T3 source has been the subject of debate for a very long time. Even though it was first believed that DIO1 generated the major amount of plasma T3 in humans, but recent researches indicate that DIO2 had a significant contribution. Accordingly, PTU therapy (which blocks DIO1 activity) only results in a 30% drop in T3 in individuals receiving constant exogenous T4 doses for primary hypothyroidism, indicating a potential significant role for DIO2 in the synthesis of plasma T3. This finding could be a possible explanation for why that the rs11206244 (C785T) SNP does not significantly affect the T3 level (Paragliola et al., 2020).

CONCLUSION

In this research study, on comparison of hypothyroid patients with healthy women we found statistically significant increase in TSH, anti-TPO and anti-Tg in hypothyroid female patients, with significant decrease in T4, T3, FT4 and FT3 in hypothyroid patients compared to control group. However, there are significant increase in oxidative stress with significant decrease in antioxidant parameters such as klotho, selenium and catalase but serum level of GPx and Vit.C significantly increased in hypothyroid patients compared to control groups. This research study also detected Deiodinase Type 1 (DIO1) gene polymorphism in genotype frequencies in Erbil hypothyroid female patients. The wild type of SNP rs11206244 (C785T) was the most frequent genotype. Gene polymorphisms of Deiodinase Type 1 (C785T) rs11206244 was considered a risk factor for hypothyroidism in Kurdish women population. The CC genotype increase risk of hypothyroidism.

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