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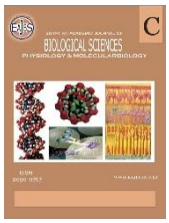

The Relationship Between the E12 Polymorphism of The Thyroglobulin Gene and Hyperthyroidism Diseases in Affected People in Samarra City

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ABSTRACT

The study included (90) samples, including (60) samples infected with hyperthyroidism and (30) samples representing the healthy control group. The samples were collected during the period from November 10, 2021, until February 10, 2022, within the laboratories of the city of Samarra. The study included observing the phenotypic symptoms of hyperthyroidism.

The results showed that 85% of all patients with hyperthyroidism suffered from bulging eyes, 92% of all patients suffered from increased appetite and weight loss, and 95% of the sample of patients suffered from high temperature in the extremities and high sensitivity to temperature.

The study also included identifying the E12 polymorphism of the TG gene, which is responsible for encoding the production of thyroid hormones. Genetic profiling of the E12 polymorphism was conducted. It was observed that there was a difference in the allelic frequency between the affected group and the control group, in addition to a higher incidence of phenotypic symptoms in people carrying the homozygous mutant genotype. GG and the impact of some of their biochemical characteristics, as the results of the study showed the significant impact of hyperthyroidism on the level of blood lipids (CHO, triglycerides (TRI), high-density lipoproteins (HDL-C), low-density lipoproteins (LDL-C), and very low-density lipoproteins (VLDL)) It was observed that there was a decrease in all lipid variables in people with hyperthyroidism, as the arithmetic mean for them was (142.98-107.42-23.28-98.21-21.48), respectively. As for the control group, the arithmetic mean was (190.07-177.3-46.6-108.31-35.46) respectively.

INTRODUCTION

The thyroid gland is one of the most important endocrine glands in the organism and has an important role in regulating metabolism within the body, and any disturbance that occurs in this function leads to imbalances and disorders in all parts of the body and vital processes (Keith *et al*.,2021), as Disorders that occur in the thyroid gland are one of the diseases that are widespread among members of society. They affect about one person out of every twenty members of society and are more widespread among females than males(Al-Tekreeti *et al.*,2017).

These disorders are represented by a decrease in the secretions or production of this gland, or an increase and Its excessive production, which may also be called thyrotoxicosis (Abdelaleem *et al.*, 2018).

Hyperthyroidism is one of the diseases that still to increased over the recent period, This disease results in an increase in the concentration of thyroid hormones (T3) and (T4) in the bloodstream beyond normal limits, causing disturbances in the vital processes within the body and the occurrence of malfunctions in the internal organs. And perform its functions (Al-Samarrai.,2012). This disease results in some signs or symptoms through which the disease can be inferred, such as weight loss despite increased appetite, feeling and feeling very hot, heart palpitations, trembling limbs, (Abdulbaqi *et al.*,2018)

bulging eyes, anxiety, and lack of sleep. And excess energy, These symptoms appear as a result of abnormal functioning of the immune system of the affected person, and the TSH hormone cannot control the process of excess hormone secretion from thyroid cells, as happens in people who do not suffer from hyperthyroidism (Ibrahim.,2008).

There are many types of hyperthyroidism, such as Graves' disease (GD), thyroid cancer, and nodular hyperplasia of thyroid cells. Graves' disease is the most common type and represents about three-quarters of hyperthyroidism cases that result from infection. this disease can affect all ages and is widespread among women, with a rate of every three infections in females compared to one infection in males (Al-Qaisi.,2011).

Hyperthyroidism is considered one of the diseases that can appear due to some environmental conditions that contribute to the infection, as well as the occurrence of genetic changes or immune disorders that affect the body and that are transmitted from one generation to another genetically through the occurrence of some genetic mutations that occur in the Thyroglobulin gene (TG) which is responsible for the functioning of the thyroid gland and the production of its hormones (Abdullah *et al*.,2019)
, which is located on chromosome 24q8, as it encodes for the production of the hormone thyroglobin (TG), which is a glycoprotein with a weight of 660 kilodaltons and represents 75-80% of the total thyroid protein (Chu and Yeh.,2020).

Wang and others have indicated that functional disorders and autoimmune diseases that affect the thyroid gland are linked to the polymorphism of the gene (TG), as they mentioned that the association with Hashimoto's thyroiditis (HT) and Graves' disease (GD), which are It is one of the most common thyroid diseases due to polymorphisms of the TG gene (Wang *et al*.,2020).

MATERIALS AND METHODS

The Study Groups:

The first group consists of (60) samples, which is a group of people with hyperthyroidism of both sexes. The number of females was (40) and males were (20) diagnosed by the specialist doctor their ages were between (20-85) years.

The second group consists of (30) samples representing the control group without hyperthyroidism(M.T. *et al.*,2019)
. The total number of females in this group was (18) samples, while the number of samples for males within this group was (12).

Second: Collecting Samples:

Samples were collected by drawing blood from a vein using a disposable syringe. The amount of blood drawn was approximately (10 ml) for each sample. The blood was divided based on the type of examination into two parts for each sample (Hussain *et al.*,2018)
, the first part for measuring thyroid hormones as well as measuring Serum lipids using a gelatin tube After placing the blood in it and centrifuging it for 5 minutes at 8000 rpm, As for the second part of the blood, it was used for molecular tests(Mahmood Z. F. *et al.*,2023)

Third: Hormonal Assay:

Estimating the concentration of the level of thyroid hormones, triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) in the blood serum of study samples by using the ready-made kit prepared by the German company "Human"(Hussein *et al*.,2019)

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2-Estimation of Cholesterol Concentration: The concentration of lipid profile was estimated by using the enzymatic colorimetric method

Molecular Analysis: Molecular studies were conducted in the laboratories of the Center for Scientific Progress in Baghdad, which included extracting DNA and measuring its concentration and degree of purity, based on the Relia Prep™ Blood gDNA Miniprep System method, which is equipped by the American company Promega(Nouri *et al.*,2015).The TG gene responsible for the production of the thyroglobin hormone was genotyped in relation to polymorphism Formal (E12). The reaction was performed using a test kit PCR-Master mix prepared by the American company Promega, with a final volume of 25µL, as well as the use of specialized primers, according to what was mentioned before (Mohammadi *et al*.,2011).

Forward primer: 5'- CAG AGC CCA CAC AGA GCA GG -3'

Reverse primer: 5'- AAA AAG GGG TGT CAC TTG GC -3'

RESULTS AND DISCUSSION

The results of the current study showed the effect of hyperthyroidism on hormonal variables, as Table (1) indicates the values of the arithmetic mean and standard deviations for the group of patients and their comparison with the control group (Nemat J. A. *et al.*,2015), which shows that there are significant differences between the two groups at the probability level ($P \leq 0.05$), and the first of these variables is Studied thyroid-stimulating hormone (TSH).

The value of the arithmetic mean and standard deviation for the patient group was equal to (0.22±0.09), while the values for the control group were (2.66±0.38), as it was noted that there was a significant decrease in the level of thyroid-stimulating hormone concentration in people with hyperthyroidism above the normal limit when compared with the control group.

The results in Table (1) also indicate an increase in the arithmetic averages of the two types of thyroid hormones (T3 and T4) for the infected group when compared with the control group, where the values of the hormone (T3) for the infected group were equal to (0.35 ± 4.21), while the results were The control group was (2.48 ± 0.38), while the T4 hormone results for the infected group were (159.03 ± 9.02), compared to the control group, which was (93.72 ± 2.12).

There are many reasons that lead to a decrease in TSH. The defect may be due to inflammation or hypertrophy in the cells of the pituitary gland that produce this hormone, or due to disorders affecting the thyroid gland (Cheng *et al*.,2010), as autoimmune diseases lead to Such as Graves' disease, due to a decrease in the level of the hormone (TSH) as a result of the presence of antibodies in the blood circulation that inhibit the function of the pituitary gland, reduce production, and stimulate the thyroid gland to increase production.

Also, the enlargement of the thyroid gland due to the decrease in the amount of absorption of iodine present in the blood circulation may lead to the occurrence of these disorders as a result of the amount of accumulated thyroids (Gule *et al*.,2011).

In autoimmune diseases, such as Graves' disease, these antibodies play the role of stimulating cells and thus contribute to increasing production, as their effect is longer lasting than that of thyroid-stimulating hormone (TSH) (Song *et al*.,2019). This increase in the proportion of hormones is also attributed to Thyroid disease and it can lead to diseases that affect the gland, such as nodular hyperplasia, which results from a decrease in the rate of inorganic iodine absorbed from the foodstuffs that the individual eats Likewise, thyrotoxicosis causes a dysplasia of the gland that results in an increase in the number of hormone-producing cells, and the enlargement resulting from this dysplasia can be observed in the upper neck area (Negro and Stagnaro.,2014).

Table 1: Shows the results of hormone titration tests for the two study groups.

The variables		N0	Mean±SD	SIG.
TSH	control	30	0.38 ± 2.66	0.000
	patients	60	0.09 ± 0.22	
T3	control	30	0.30 ± 2.48	0.000
	patients	60	0.35 ± 4.21	
T4	control	30	2.12 ± 93.72	0.000
	patients	60	9.02 ± 159.03	

The results of the fat test shown in Table (2) also indicate that there is a significant difference at the probability level ($P \leq 0.05$), as the test results obtained for all fat standards for the infected group had a smaller arithmetic mean compared to the control group.

Thyroid hormones play a major role in the fat metabolism process, as they affect the process of building and catabolizing all types of fats, thus affecting their percentage in blood circulation in terms of increase and decrease.The phenomenon of low blood fat levels and the effect of thyroid hormones on them can be explained by the regulatory effect caused by the T3 and T4 hormones on the special key enzymes responsible for the fat metabolism process, which contribute in imbalance in vital functions.

Thyroid hormones stimulate the action of the enzyme lipoprotein lipase (LPL), which plays a major role in the process of decomposing HDL-2 and converting it to HDL-3, thus contributing significantly to the oxidation of LDL, leading to a decrease in its percentage in the blood circulation (Sharma *et al*.,2017).

The increase in thyroid hormones also causes the bile to secrete cholesterol, as well as an increase in gene expression for LDL receptors, which accelerates the process of decomposition and catabolism of LDL particles through these receptors(Seddiq *et al.*,2022).

The increase in thyroid hormones also causes an increase in the percentage of (cholesterylester transfer protein CETP), which causes an increase in the process of transferring cholesterol esters from HDL to VLDL and an increase in the process of catabolism of HDL, and thus its percentage in the blood circulation decreases. The decomposition of fats in various tissues also increases when thyrotoxicosis occurs, and free fatty acids, glycerol, and glucose rise in the blood, and this increase is linked to the mechanism of beta catecholamine receptors (El Kawkgi *et al*.,2021), and although the percentage of cholesterol, LDL, and TG decreases to the lowest level in the blood in people with hyperthyroidism, which does not cause atherosclerosis, it can cause serious diseases of the vascular system. As in myocardial infarction and coronary artery disease, recurrent pulmonary asphyxia also occurs (Ibrahim., 2008).

Table 2 : Lipid tests for the two study groups.

The variables		N0	Mean±SD	SIG.
VLDL	Control	30	1.01 ± 35.46	0.000
	Patients	60	3.92 ± 21.48	
LDL	Control	30	8.13 ± 108.31	0.000
	Patients	60	5.31 ± 98.21	
HDL	Control	30	3.65 ± 46.6	0.000
	Patients	60	2.44 ± 23.28	
TRI	Control	30	5.05 ± 177.3	0.000
	Patients	60	19.58 ± 107.42	
CHO	Control	30	9.59 ± 190.07	0.000
	Patients	60	3.76 ± 142.98	

The results of the multiplex reaction (PCR) of the DNA sequence showed using the specialized primer EXON 12 within the (TG) gene that regulates the action and secretion of thyroid hormones. This primer was used with the PCR test reaction mixture for a group of samples of people with hyperthyroidism and adult diseases. Their number is (60) samples, as with the control group, which is (30) samples. This is based on the program that was described by (15) and, after transferring the results of the PCR test for the genomic DNA samples onto a 2% agarose gel and photographing the products using a UV Transilluminator device, all bands of genomic DNA resulting from replication appeared for all samples, as shown in Figure (1).

Many scientific studies indicate that the bands resulting from the duplication of this specialized primer without the addition of cutting enzymes are of a molecular size equal to (241 bp) (Barberio *et al*.,2017, Chu and Yeh., 2020).

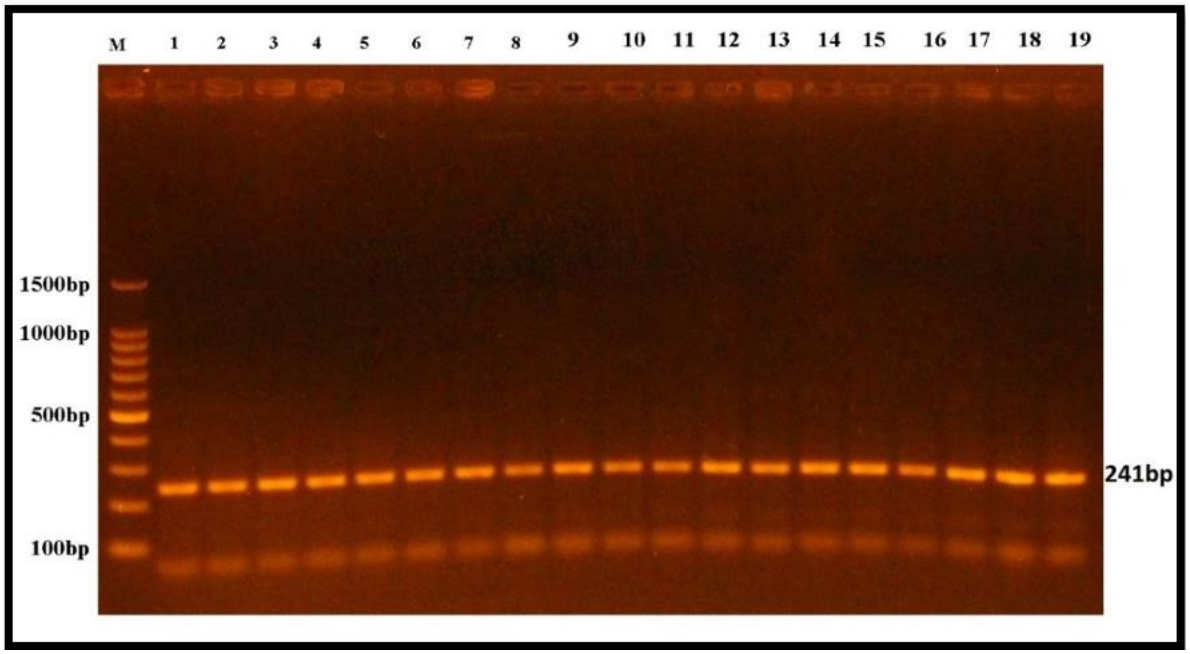

Fig. 1: electrophoresis result of replication of the exon 12-specific primer without the cutting enzymes for the study samples on a 2% agarose gel.

After obtaining these results, specialized cutting enzymes were added, represented by the enzyme BsaAI, which works on the E12 bases. The result was the emergence of three different genotypes, where the first type was the Normal Homozygous (AA) genotype, which is represented by the band with a molecular size of (241 bp). As for the second heterozygous genotype (AG), represented by the main band with a molecular size of (241bp) and the second and third bands with a molecular size of (136 bp) and (105 bp), respectively, The last resulting genotype is the Mutant Homozygous (GG) type, which is represented by only two mutant bands, the first (136 bp) and the second (105 bp), as in Figure (2).

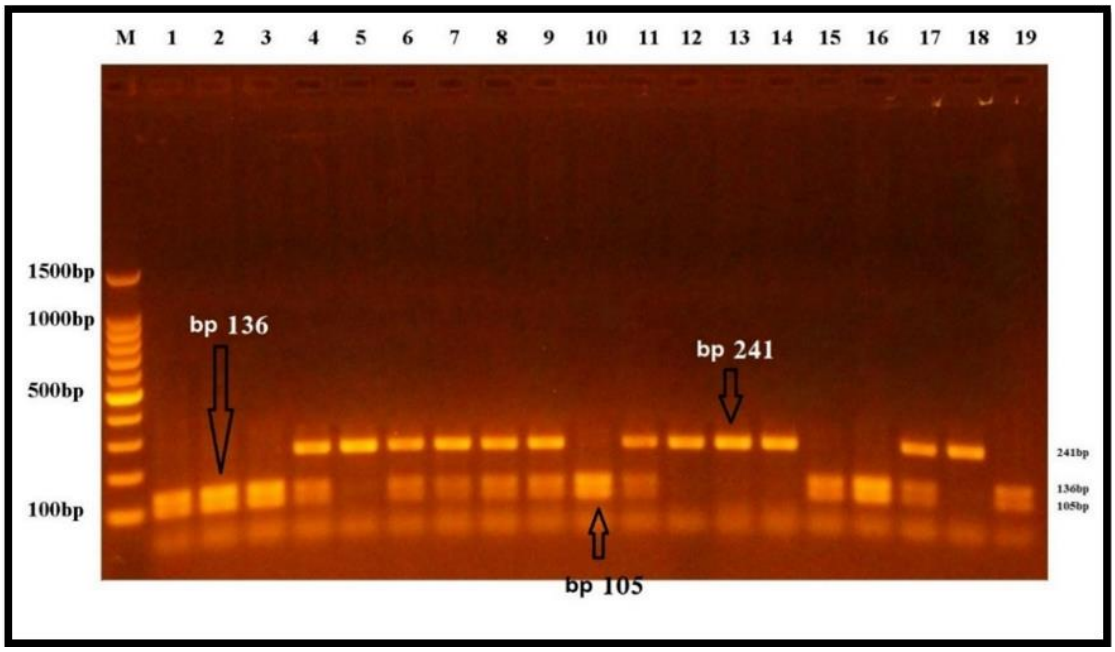

Fig. 2: Electrophoresis results of the specialized primer exon 12 after adding the cutting enzyme BsaAI to samples of people with hyperthyroidism on a 2% agarose gel.
Samples: (18,14,13,12,5) represent normal homozygous (AA) genotypes.
Samples: (17,11,9,8,7,6,4) represent heterozygous genotypes (AG).
Samples: (19,16,15,10,3,2,1) represent homozygous mutant genotypes (GG).

The mutation of the substitution of the nitrogenous base adenine "A" for the nitrogenous base quinine G at nucleotide No. 3082 in exon No. E12 of the TG gene led to a substitution in the genetic code for amino acid No. 1028 within the polypeptide chain of the hormone thyroglobin, which led to the production of the amino acid valine. The code (GUG) was replaced by the amino acid methionine code (AUG). This change led to the appearance of restriction sites for the BsaAI cutting enzyme.

Therefore, when the PCR products are treated with this enzyme, it will cut the main band resulting from the reaction with a molecular size of 241bp into only two mutant bands, the first (136 bp) and the second (105 bp). The appearance of a band with a main molecular size of 241bp indicates normal genotypes (AA) that are not affected by this enzyme.

As for the asymmetric genotype (AG), it contains three bands, the first being the main one, in addition to two mutant bands with a molecular size of (136,105 bp), which were produced by the restriction enzyme BsaAI, which cut the main band with a molecular size of (241 bp). If the person is healthy and does not carry the mutation in both alleles, his genotype will be normal and homozygous (AA), and the weight of the resulting packet when relaying the PCR-RFLP results will be 241bp.

If the person has the heterozygous (AG) genotype, which means that one of the two alleles is healthy and has a molecular size of 241bp, and the other allele is mutant and has a molecular size of 136bp and 105bp the enzyme was able to cut, but if the person is a carrier of the mutant homozygous genotype (GG) This means that both alleles are mutant, and the restriction enzyme BsaAI was able to cut them, resulting in only the two mutant bands, the first (136 bp) and the second (105 bp), respectively.

We assume that p represents the frequency of the non-mutant allele A while q represents the mutant allele G. Therefore, in a population in Hardy-Weinberg equilibrium, the sum of the allelic frequencies is $p + q = 1$, while the frequency of the genotypes (genetic frequency) is (p2) AA)+2pq+(AG)+ q2 (GG)=1). Therefore, the results of profiling for the polymorphism of E12 of the TG gene

for the study sample of people with hyperthyroidism showed that the observed number of infected people carrying the healthy, homozygous normal genotype AA (AA) is (11) and with a frequency of (0.183), While the number of observations for the heterozygous genotype AG was (30) with a frequency of (0.5), while the number of observations for the homozygous mutant genotype GG was (19) with a frequency of (0.316), it is clear from these results that the frequency of the A allele is (0.43) while the frequency of the A allele is (0.43), G equals (0.57), Tables (3 and 4).

Table 3: Observed and expected number and frequency of genotypes and alleles of the E12 polymorphism of the TG gene for a sample of people with hyperthyroidism**.**

Genotype expected (frequency)	Viewed number (Frequency)	The expected number (frequency)
AA (p2)	11 (0.183)	11 (0.183)
AG (2qp)	30 (0.5)	29 (0.483)
GG (q^2)	19 (0.316)	20 (0.333)
Total	60	60
2□ □Calculated = 0.084 < □2 Tabulation 5.99 At a probability level 0.05 ، d.f=2		

Table 4: Allelic frequency of the E12 polymorphism in the TG gene for a sample of people with hyperthyroidism.

Allele	Replication
A (p)	**0.43**
G (q)	**0.57**
Total (p+q)	**1.000**

The results showed when the chi-square test was applied to the group of people with hyperthyroidism that the calculated chi value reached (0.084), which is less than the tabular chi value (5.99) at 2 degrees of freedom and a significance level of 0.05. This indicates that the group of people with hyperthyroidism was balanced with regard to the E12 polymorphism of the TG gene, so the Hardy-Weinberg law of balance applied to it.

The results of our study agreed with Jeng *et al.* (2007) when they studied the association between polymorphisms in the TG gene with the risk of developing autoimmune diseases that affect the thyroid gland in Taiwan, where the study sample taken from affected people, amounting to 215 affected samples, appeared to be balanced when studying the mutation occurring in exon 12. Our results also agreed with Belguith *et al* (2008) when they studied the association of polymorphisms of the TG gene and the pathogenesis of autoimmune thyroid diseases in the capital, Tunis.

While there was disagreement with the results of Basman *et al*. (2021) when she studied genetic polymorphisms of the TG gene in patients with autoimmune thyroiditis among residents of the Babil and Karbala governorates, as she indicated a lack of balance in the sample of those infected.

The results of profiling for the E12 polymorphism of the TG gene for the sample of the control group showed that the observed number of people carrying the normal homozygous genotype AA was (24) samples with a frequency of (0.8), while the number of observations of the heterozygous genotype AG was (6) samples and with a frequency of (0.2). The number of observations for the homozygous mutant type TT was (0) and with a frequency of (0.0), as is clear from these results that the frequency of the A allele is (0.9) while the frequency of the G allele is (0.1), Tables (5 and 6).

Table 5: Observed and expected number and frequency of genotypes and alleles of the E12 polymorphism of the TG gene for the control group sample.

Genotype expected (frequency)	Viewed number (Frequency)	The expected number (frequency)
AA (p^2)	24 (0.8)	24.3 (0.81)
AG (2qp)	6 (0.2)	5.4 (0.18)
GG (q^2)	0 (0.0)	0.3 (0.01)
Total	30	30
2□□Calculated = 0.369 < □2 Tabulation 5.99 At a probability level 0.05 ، d.f=2		

Table 6: Allelic frequency of the E12 polymorphism in the TG gene for the control group sample**.**

Allele	Replication
A (p)	**0.9**
G (q)	**0.1**
Total (p+q)	**1.000**

The results showed when applying the chi-square test □2 to the control group that the calculated chi value is (0.369) less than the tabular chi value (5.99) at a degree of freedom of 2 and a significance level of 0.05. This indicates that the control group was balanced in terms of polymorphism. E12 is the TG gene, so the Hardy-Weinberg Law of equilibrium applies to it.The results shown in Table (6) indicate an increase in the frequency of the mutant genotype GG in the group of people with hyperthyroidism compared to the control. The results of Table (7) show an increase in the frequency of the mutant allele G compared to the healthy allele A. This indicates the role that the mutation plays in A to G in exon 12 at nucleotide position 3082 activators. the BsaAI binding sites for this site in the TG gene, which causes a functional defect in the work of the gene through an increase in its concentration.

The process of changing the nitrogenous base adenine to quanine leads to the formation of the amino acid valine, which is a stimulant and catalyst for metabolic processes within the body, which leads to an increase in the concentration and effectiveness of thyroid hormones and thus causes thyroid diseases (Khalilzadeh *et al*.,2010).

The results shown in Table (7) showed the arithmetic averages of the biochemical trait values and their relationship to the E12 polymorphism of the TG gene. Patients carrying the healthy normal genotype AA had the highest values of (VLDL, HDL, TRI, CHO) in the subjects, as these values reached (0.32). (21.93, 24.27, 109.64, and 144.09) respectively, and as shown in Table (7).

The results of our study agree with (Ulloa *et al*.,2014) when they studied the relationship between some biochemical variables and polymorphisms in exon 8 and exon 12 in the TG gene. People carrying the homozygous mutant genotype GG had the highest values (T3, TSH, T4) when compared to the genotype AG and AA. The substitution process occurring in exon 12 plays an important and sensitive role in the process of building and forming thyroid hormones and their effectiveness, as it causes an increase in the concentration of thyroglobin as well as affecting thyroid receptors and stimulators (Gallo *et al*.,2022).

Indicated that the substitution process in exon 12 leads to a change in the receptors found on the follicular cells of the thyroid gland, which are responsible for

producing the hormone, causing increased production and hypertrophy of the gland's tissue, which leads to hyperthyroidism. It also leads to This leads to an increase in the production and synthesis of autoantibodies against TSH receptors, which causes an interaction between the TSHR and the specific antibody, causing an immune response that results in the enlargement of the gland and an increase in the production and synthesis of the hormone (Ulloa *et al.*,2014, Gallo *et al*.,2022).

Table 7: Arithmetic averages of biochemical trait values for genotypes of the E12 polymorphism of the TG gene for hyperthyroidism patients.

The variables	Genotype E12			The mean
	AA N= 11	AG N= 30	GG N= 19	
TSH	4.13	4.10	4.43	
T3	153.35	156.17	166.84	4.22
T4	0.12	0.25	0.32	158.79
VLDL	21.93	21.73	20.83	0.23
LDL	97.89	98.09	98.59	21.50
HDL	24.27	23.50	22.37	98.19
TRI	109.64	108.67	104.16	23.38
CHO	144.09	AG N= 30	GG N= 19	107.49

REFERENCES

Abdelaleem, M. M., El-Tahawy, N. F. G., Abozaid, S. M. M., & Abdel-Hakim, S. A. B. (2018). Possible protective effect of curcumin on the thyroid gland changes induced by sodium fluoride in albino rats: light and electron microscopic study.*Journal of Endocrine regulations,* 52(2), 59-68.

Al-Qaisi, Amal Mahrouz Abdullah (2011) Study of a number of physiological and biochemical parameters for patients with thyroid disorders, Master's degree / College of Science / Tikrit University.

Al-Samarrai, Sahar Abdel-Wahab (2012), Study of some physiological and biochemical variables in people with thyroid disorders in the city of Samarra and its environs, Higher Diploma / College of Science / Tikrit University.

Abdulbaqi, N. J. and Dheeb, B. I. and Irshad, R. (2018).Expression of Biotransformation and Antioxidant Genes in the Liver of Albino Mice after Exposure to Aflatoxin B1 and an Antioxidant Sourced from Turmeric (Curcuma longa). Jordan Journal of Biological Sciences. 11(2) 89 – 93.

Abdullah, H.I., Hammadi, S.Y., Hussein, A.S., Dheeb, B.I. (2019) . Investigation of genetic diversity and relationships among the clinical candida species using random amplified polymorphic DNA (RAPD) analysis. Research Journal of Biotechnology. 14(Special Issue 1), pp. 6–13.

Al-Tekreeti, A. R., Al-Halbosiy, M. M. F., Dheeb, B. I., Hashim, A. J. and Al-Zuhairi, A. F. H. (2017). Molecular identification of clinical Candida isolates by simple and randomly amplified polymorphic DNA-PCR. Arab Journal of Scicne and Engring. DOI 10.1007/s13369-017-2762-1.

Barberio AM, Hosein FS, Quinonez C, McLaren L,(2017), Fluoride exposure and indicators of thyroid functioning in the Canadian population: implications for

community water fluoridation. *Journal Epidemiol Community Health*, 71(10):1019–1025.

Basman A. Nadhim, Abbas G. Hammed, Hayder O. Hashim, Nazar J. Metib (2021) Molecular Study Of The TG Gene Polymorphisms ?In Patients With Hashimoto's Thyroiditis Among Iraqis Population. Thesis,college of education. Baghdad Unversity.

Belguith-Maalej, S., Kacem, H. H., Rebai, A., Mnif, M., Abid, M., & Ayadi, H. (2008). Thyroglobulin polymorphisms in Tunisian patients with autoimmune thyroid diseases (AITD). *Immunobiology*, *213*(7), 577-583.

Cheng, S.Y., Leonard, J.L. and Davis, P.J. (2010) Molecular Aspects of Thyroid Hormone Actions. *Endocrine Reviews*, 31, 139-170.

Chu, Y.D.; Yeh, C.T. (2020), The Molecular Function and Clinical Role of Thyroid Stimulating Hormone Receptor in Cancer Cells. *Cells*, 9, 1730.

Dahham, M.T., Omar, A.F., Dheeb B.I. (2019). Synergistic effect of tea tree oil on fungi causing vaginal thrush in pregnant women. Journal of Biotechnology Research Center 13 (2)35-44.

El Kawkgi, O. M., Ross, D. S., & Stan, M. N. (2021). Comparison of long-term antithyroid drugs versus radioactive iodine or surgery for Graves' disease: a review of the literature. *Clinical Endocrinology*, *95*(1), 3-12.

Gallo, D., Mortara, L., Veronesi, G., Cattaneo, S. A., Genoni, A., Gallazzi, M., Piantanida, E. (2022). Add-on effect of selenium and vitamin D combined supplementation in early control of Graves' disease hyperthyroidism during methimazole treatment. *Frontiers in endocrinology*, 13, 886451.

Gule, M. K., Chen, Y., Sano, D., Frederick, M. J., Zhou, G., Zhao, M., ... & Lai, S. Y. (2011). Targeted therapy of VEGFR2 and EGFR significantly inhibits growth of anaplastic thyroid cancer in an orthotopic murine model. *Clinical Cancer Research*, *17*(8), 2281-2291.

Hussein, H.S., Dheeb B.I., Hamada,T.A.. Studying the candida resistance and sensitivity for some antifungals (2019). Journal of Biotechnology Research Center 13 (2)25-34.

Hussain, A.F., Sulaiman, G.M., Dheeb, B.I. , Hashim, A.J .(2018). Histopathological changes and expression of transforming growth factor beta (TGF-β3) in mice exposed to gliotoxin .Journal of King Saud University – Science. 27, 193–197.12.

Hashim S. S, Mahmood Z. F, Abdulateef S. F, Dheeb B. I. (2023).Evaluation Cytotoxicity Effects of Centaurea Cineraria Extracts Against some of Cancer Cell Lines. Biomedical and Pharmacology Journal ;16(1).223-225

Ibrahim, Shaima Razzaq (2008). The relationship between patients with hypothyroidism and insulin resistance in terms of some inflammatory indicators. Master's thesis/College of Science/University of Baghdad.

Keith C. Bible, Electron Kebebew, James Brierley, Juan P. Brito, Maria E. Cabanillas, Thomas J. Clark Jr., Antonio Di Cristofano, Robert Foote, Thomas Giordano, Jan Kasperbauer, Kate Newbold, Yuri E. Nikiforov, Gregory Randolph, M. Sara Rosenthal, Anna M. Sawka, Manisha Shah, Ashok Shaha, Robert Smallridge, and Carol K. Wong-Clark. (**2021)**. American Thyroid Association Guidelines for Management of Patients with Anaplastic Thyroid Cancer. *Thyroid journal* . (31) 3. 15-18.

Khalilzadeh, O., Anvari, M., Esteghamati, A., Momen-Heravi, F., Rashidi, A., Amiri, H. M., ... & Amirzargar, A. (2010). Genetic susceptibility to Graves' ophthalmopathy: The role of polymorphisms in anti-inflammatory cytokine genes. *Ophthalmic genetics*, journal 31(4), 215-220.

Mohammadi-asl, J., Larijani, B., Khorgami, Z., Tavangar, S. M., Haghpanah, V., Kheirollahi, M., & Mehdipour, P. (2011). Qualitative and quantitative promoter hypermethylation patterns of the P16, TSHR, RASSF1A and RARβ2 genes in papillary thyroid carcinoma. *Journal of Medical oncology*, 28, 1123-1128.

Negro, R., & Stagnaro-Green, A. (2014). Clinical aspects of hyperthyroidism, hypothyroidism, and thyroid screening in pregnancy. *Endocrine practice*, *20*(6), 597-607.

Nouri, M. A., Al-Halbosiy, M. M. F., Dheeb, B. I. and Hashim, A. J. (2015). Cytotoxicity and genotoxicity of gliotoxin on human lymphocytes in vitro. Journal of King Saud University – Science. 27, 193–197.

Rassin, N. K., Nemat J. A, Dheeb, B. I. (2015). Molecular Identification of Aspergillus fumigatus Using ISSR and RAPD Markers. Iraqi Journal of Science. 56 (4A), 2788-2797.

Salih, I.I., Seddiq, S.H., Hashim, S.S., Dheeb, B.I. (2022). Application of Omics and Proteomics in Fungi. AIP Conference Proceedings. 6(3).23-25.

Sharma, A., Kaur, J., Kaur, A., Uppal, N., & Kaur, M. (2017). Evaluation of serum lipid profile in patients with hyperthyroidism. *International Journal of Clinical Biochemistry and Research*, 4(2), 126-128.

Song, Y. S., Kim, M. J., Sun, H. J., Kim, H. H., Shin, H. S., Kim, Y. A., ... & Park, Y. J. (2019). Aberrant thyroid-stimulating hormone receptor signaling increases VEGF-A and CXCL8 secretion of thyroid cancer cells, contributing to angiogenesis and tumor growth. *Clinical Cancer Research*, *25*(1), 414-425.

Tomari, S., Watanabe, M., Inoue, N., Mizuma, T., Yamanaka, C., Hidaka, Y., & Iwatani, Y. (2017). The polymorphisms in the thyroid peroxidase gene were associated with the development of autoimmune thyroid disease and the serum levels of anti-thyroid peroxidase antibody. *Endocrine journal*, *64*(10), 1025-1032.

Ulloa-Aguirre, A., Reiter, E., Bousfield, G., Dias, J. A., & Huhtaniemi, I. (2014). Constitutive activity in gonadotropin receptors. *Advances in Pharmacology*, 70, 37-80.

Wang, M., Liu, L., Li, H., Li, Y., Liu, H., Hou, C., ... & Wang, A. (2020). Thyroid function, intelligence, and low-moderate fluoride exposure among Chinese school-age children. *Journal of Environment international*, 134, 105229.