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The Effect of Thyroid Peroxidase (TPO) Gene Polymorphism at rs1126797 SNP on the Thyroid-Stimulating Hormone and Anti-TPO Antibody Levels in a Sample of Iraqi Patients with Hypothyroidisms Disorder

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Abstract: Thyroid peroxidase (TPO) gene mutation leads to a change in enzyme-built structure resulting in the anti-TPO autoantibodies production that may cause thyroid destruction. The aim of study to evaluate the association of single nucleotide polymorphisms (SNP) of the TPO gene (rs1126797) and anti-TPO levels and TSH level in Iraqi patients with autoimmune hypothyroidism and correlate them with the disease severity. **Methods**: DNA has been obtained from the blood specimens of 50 hypothyroid patients and 50 apparently healthy people with the disorder, and the SNP rs123797 was identified employing the RT-PCR-HRM method. Chemical analyses were carried out for the amounts of serum TSH and anti-TPO ab using the Cobase E411. **Result**s: As regards TPO rs112797 C/A polymorphism , the AA genotypee of rs112797 C/A and the A allele were non-significantly increased in patients with hypothyroidisme, the p-value of (CC+CA) AA genotype is 0.4 and the odd ratio (95% CI) is 0.6 **Conclusion**: There is no correlation between TSH and anti-TPO levels with different genotype of rs 112797 C/A polymorphism, and no association with the disease severity.

Keywords: Hypothyroidism, thyroid peroxidase gene, single nucleotide polymorphism, Anti -Tpo.

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Introduction

Thyroid hormone deficiency is the most common clinical condition and, if left untreated, can have serious adverse health effects on multiple organ systems, with the cardiovascular system being the most studied target. Apparent primary hypothyroidism is defined as elevated thyroid - stimulating hormone (TSH) concentrations and thyroxine (T4) concentrations below the reference range(1). Subclinical hypothyroidism is often considered an early sign of thyroid insufficiency , defined as elevated TSH levels but T4 levels within reference ranges(2). Hypothyroidism is classified as primary , central , or peripheral , depending on the pathology of the thyroid, pituitary or hypothalamus , or peripheral tissues. Acquired primary hypothyroidism is the most common form and can be caused by severe iodine deficiency, but chronic autoimmune thyroiditis is more

common in iodine-supersaturated areas (3, 4). Thyroid peroxidase (TPO), a glycoprotein with enzymatic activity in thyroid metabolism , TPO catalyzes the iodination and subsequent coupling of tyrosine residues in thyroglobulin , resulting in the synthesis of the thyroid hormones T4 and T3(5). which play an important role in growth control, regulation of metabolic differentiation and almost all physiological functions of human tissues(6). In humans, the TPO gene is found on the chromosome 2p25 and spans close to 150 kb, via 17 exons. TPO is an enclosed by the membrane protein glycoprotein (102 kDa) that is present as a dimer form. (7). TPO mutations are typically inherited as autosomall recessive traits.(6). Most hypothyroidism patients' serums contain significant quantities of TPOAb and TSH, which are useful markers of autoimmune thyroiditis. (8). We perilously reported that the TPOAb levels in patients with hypothyroidism were highly and may be associated with thyroid destruction (9). In this study, we genotyped one single nucleotide polymorphisms (SNP) in the TPO gene to clarify the association of TPO gene polymorphisms with the development and prognosis of hypothyroidisme.

Materials and methods

Sample collection

In this study, fifty female patients were recruited from three Baghdad hospitals. In addition, fifty healthy females served as controls. We measured participants' height, weight, and body mass index (BMI), as well as thyroid function tests such as thyroid-stimulating hormone (TSH), and immunological parameters such as anti-TPO antibody, for two groups: hypothyroidism patients and apparently healthy controls. The samples were separated into two tubes. **1st tube**: EDTA tube (2 ml); Two ml of blood was placed in an EDTA tube and kept at -20 C^{o} to be used in the molecular genetic study. **2nd tube**: The remaining (3 ml) venous blood was transferred to a clot activator and gel serum separation tubes. Serum is obtained by placing blood samples in a gel tube and allowing them to stand at room temperature (20 to 25°C). The serum was separated by centrifugation at 3000 rpm for 15 minutes. TSH and Anti-TPO Ab tests were performed using the Cobas E411/Roche system, which uses patented ECL technology for high accuracy, speed, and efficiency.

DNA extraction and genotyping of TPO gene

The EasyPure® Genomic DNA Kit was used for genomic DNA extraction. The concentration and purity of the extracted DNA were assessed using a Quantuse Fluorometer . HRM genotyping primers, EVA Green I, and a master mix were used. Amplification followed by high-resolution melting curve (HRM) analysis was used to investigate genetic variants for the SNP rs112797(10). Table 1 summarises the forward and reverse primer sequences, as well as the RT-PCR-HRMe conditions used, while Table 2 shows the thermal profile of HRM genotyping. DNA fragments were visualised by UV transillumination electrophoresis . After staining electrophoretic gels with ethidium bromide , special software was used to save the photos captured by the device to the computer.

Table (1): Primers used in the present study

SNP	Primer	Sequence From 5-3	Product size bp.	Company origin
TPO gene/Exon Rs1126797 C>A	Forward	CCCTGTTTGCCTGTCTCATT	85	Alpha DNA-Canda Primers designed using Primer3Plus and Primer Explorer V4
	Revers	TGAAGGAAGACGCTCTGGAT		

Table (2): The thermal profile of HRM genotyping. *Dye activation stage

Step	Temperature (ºC)	Time (sec.)	Cycles
Enzyme activation	94	30	1
Denaturation	94	5	40
*Annealing RS 112797	65	15	
Extension	72	20	
HRM	55-95	0.2sec for 1 degree	

Statistical analysis

All statistical analyses were carried out using the SPSS 25 software (SPSS Inc., Chicago, IL, USA). Qualitative data are normally distributed and expressed as mean ± SD. The t-test was used to compare two groups. Odds ratio (OR). P-values less than 0.05 were considered significant. Qualitative data is presented as mean ± standard deviation, and two groups were compared using the t test. The odds ratios (ORs) were calculated. P-values < 0.05 were considered significant.

Results and discussion

The Table 3 shows the demographic characteristics of the participants. There was no statistically significant variance in BMI or age between the control and patient groups (P = 0.5 for BMI and P = 0.4 for Age). There was a statistically significant positive correlation between hypothyroidism and the anti-TPO antibody. The p value of having high anti-TPOAb was 0.0001, and the p value of TSH was 0.0001. Results of genetic analysis of SNP (rs112797) revealed that 12% (n=6) of the hypothyroid patients to be homozygous (AA), 50% (n=25) to be heterozygous (CA), and 38% (n=19) to be of wild (CC) genotype. Similarly, in the control group, 50% (n=25) were of wild-type CC, 32% (n=16) heterozygous CA, and 18% (n=9) homozygous CC genotypes (Table 4). The AA genotype frequency was non-significantly higher in hypothyroid patients than in apparently healthy controls (p=0.4), indicating that the homoe-mutant genotype rs112797 AA was not associated with hypothyroidism when compared to the wild-type rs112797 CC and was not a risk factor for hypothyroidism (Odds ratio =0.9) when compared to those carrying the wild-type CC. Our study discovered that the frequency of TPO SNP (rs112797) A carriers (CA + AA genotypes) was normal in hypothyroid patients compared to the control group, with an odds ratio of carrying any mutant allele (both homozygous and heterozygous) = 1.6. In our study there was no association between different genotypes of rs112797 (CC, CA, and AA) polymorphism and serum

laboratory values measurements for Anti -TPO Ab and TSH , no significant differences were found among anti-TPO levels and three genotypes, namely CC, CA, and AA (p=0.1). The mean (±SD) in CC, CA, and AA genotypes was (253.147± 195.1431), (260.180± 181.5286), and (106.017± 70.3096) respectively (Figure 1 (a)). Also, there was no significant difference found between TSH levels and the three genotypes. Similarly, a nonsignificant correlation was observed between TSH and the three genotypes (P=0.9). The mean (±SD) values for CC, CA, and AA genotypes were (32.2789± 17.3067), (32.2260± 14.5044), and (33.3833± 19.1268) respectively (Figure 1 (b)).

Table (3): The demographic characteristics of participants

Parameters	Group	Mean ±Std. Deviation	P-value
BMI	patients	16.666 ±8.6216	0.5
	Control	16.666 ±7.0945	
Age	patients	1.666 ±1.2909	0.4
	Control	17.333 ±4.1633	
TSH	patients	32.3850 ± 24.77310	0.0001**
	Control	.6412 ±. 32453	
Anti -TPO	patients	239.008 ± 183.9025	0.0001**
	Control	15.382 ± 34.7040	

Means having with the different letters in same column differed significantly.** (P≤0.01).

Table (4): The genotypes and allele frequency distributions of SNP rs112797 of the TPO gene.

SNP Rs1126797	Frequencies		P value	Odd ratio (95% CI)
	Patients (n= 50) (No, %)	Control (n= 50) (No, %)		
Co-dominant				
CC	19 (38%)	25 (50%)	---	1.00 (Reference)
CA	25 (50%)	16 (32%)	0.1	2.05 (0.8650 to 4.8868)
AA	6 (12%)	9 (18%)	0.8	0.9 (0.2661 to 2.8917)
Dominant				
CC	19 (38%)	25 (50%)	---	1.00 (Reference)
CA+AA	31 (62%)	25 (50%)	0.2	1.6 (0.7361 to 3.6163)
Recessive				
CC +CA	44 (88%)	41 (82%)	---	1.00 (Reference)
AA	6 (12%)	9 (18%)	0.4	0.6 (0.2033 to 1.8986)
Allele				
C	0.63 (63)	0.66 (66)	---	1.00 (Reference)
A	0.37 (37)	0.34 (34)	0.6	1.1 (0.6385 to 2.0355)

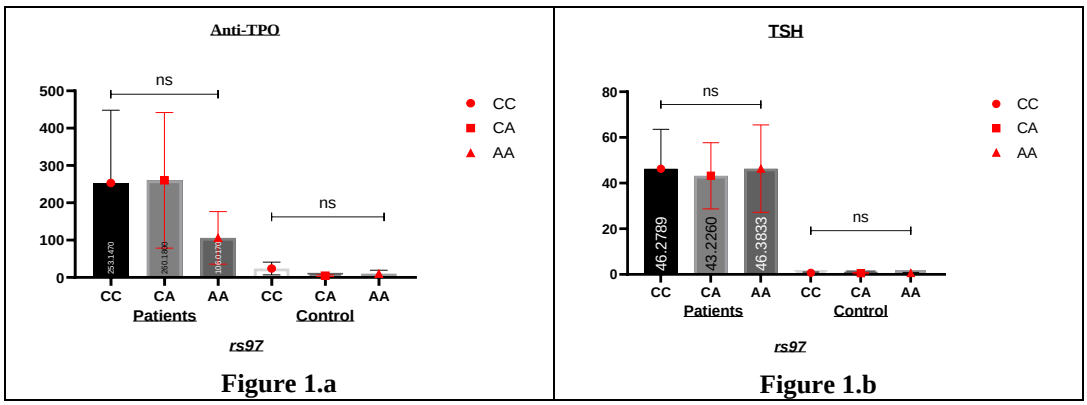

Figure 1.a **Figure 1.b**

Figure (1): (a) The association between the CC, CA and AA genotype of the rs112797 polymorphism and serum anti-TPO antibody levels (b) The association between the CC, CA and AA genotype of the rs112797 polymorphism and serum TSH levels.

A previous study identified over 50 TPO gene mutations caused by DNA nucleotide base deletion, insertion, or sequence changes (11). Mutations that alter the enzyme's normal location or three-dimensional shape can have an effect on its activity. As a result, thyroid hormone production was affected, causing the appearance of thyroid disorder symptoms..(12) The present study evaluates the potential association of TPO gene in patients with hypothyroidisms in Iraq., TPO gene defect can potentially lead to severe defects in thyroid hormone production . The primere was created for rs 1123797 based on the data recorded on the NCBI website. These data showed the variatione that may occur in rs 1123797 on giving three forms of the alleles C>T, C>A, and C>G. The previous studies available regarding this SNP (rs 1123797) were only three citations and they tested the change C>T while in this study the RT-PCR -HRM was detect variation in allele C that change to A allele (C>A) to make rs 1123797 (ASP666GLU) instead (ASP666ASP). The results revealed that the A allele of Asp666Glu (rs112797) (c.1998A allele) may be a protective allele towards the disease with hypothyroidism. It's not significant interaction of hypothyroidism with rs1123797 polymorphisms frequency of A carriers (CA + AA genotypes) patients than in control subjects (p-value= 0.2). The C (wild-type) allele frequencies were 66% in apparently healthy control but in hypothyroidism patients 63%. The A allele (variant) frequencies were 34% in apparently healthy control, and in hypothyroidism patients 37%, was non-significant (p=0.6) in hypothyroidism patients than in apparently healthy control. So, result of this study showing that allele A of TPO (rs112797 C>A) is considered non a risk factor for the hypothyroidism disease. Alteration of Aspartic acid to Glutamic acid occurred by missense variant that make this variant may be not affected to protein structure because both aspartic acid (Asp) and glutamic acid (Glu) are acidic amino acids (13)Therefore, there might not be a drastic change in the charge of the amino acid at that position in the proteine. Moreover, When the correlation between the hypothyroidism and the studied factors was tested in all participants, it revealed a negative correlation between hypothyroidism and TSH titer, Anti-TPO ab (14). Table 5

shows that there was a significant negative correlation between TSH and anti-TPO antibodies in both patients and healthy controls. This negative result is consistent with previous studies, which found that serum anti-TPO levels are negatively correlated with hypothyroidism. These antibodies are thought to be involved in the pathogenesis of autoimmune hypothyroidism.(15, 16) The prevalence of anti-TPO Abe in the whole Iranian the population was found to be 14.9%. (15) Which was close to the results of our current study (13.23%). whose thyroid dysfunction is most likely not caused by an autoimmune disease, and for whom the diagnosis of hypothyroidism occurred early (before the age of 15 years). Primary hypothyroidism at birth is typically caused by a problem with thyroid gland development (dysgenesis) or a disorder of thyroid hormone biosynthesis (dyshormonogenesis). When hypothyroidism is present at birth, thyroid dysgenesise and dyshormonogenesise account for approximately 85% and 15% of permanent cases of congenital primary hypothyroidism , respectively (4).

Table (5): The correlation between serum levels of TSH, ANTI TPO AB and rs1123797 polymorphism

		TSH	Anti-TPO	Rs1123797
TSH	**Pearson Correlation**	1	0.377**	0.033
	Sig. (2-tailed)		.000	0.747
Anti-TPO	**Pearson Correlation**	0.377**	1	-0.081
	Sig. (2-tailed)	0.000		0.422
Rs1123797	**Pearson Correlation**	0.033	-0.081	1
	Sig. (2-tailed)	0.747	0.422	
**. Correlation is significant at the 0.01 level (2-tailed).				

Conclusion

In conclusion, our study discovered no association between rs1126797 C/A polymorphisms and auto immune hypothyroidism, as well as a correlation between anti-TPO antibody levels and genotypes in hypothyroid patients. Furthermore, there was no correlation found between anti-TPO antibody levels in hypothyroid patients and the various genotypes in the Iraqi population. Our study's limitation was the small sample size, and the frequency of TPO genetic polymorphisms should be determined using a larger population. Additional research on other nationalities and ethnicities is required to validate the current findings.

Ethical statement

The ethical committee of the College of Institute of Genetic Engineering and Biotechnology for Postgraduate Studies/University of Baghdad approved this study (No. 2922) on November 28, 2022. Prior to inclusion, all participants provided written consent. The study followed the principles of the Declaration of Helsinki, 2013.

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