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# THE EFFECT OF THYROID PEROXIDASE (TPO) GENE POLYMORPHISM SNP RS732609 ON THE THYROID-STIMULATING HORMONE AND ANTI-TPO ANTIBODY IN A SAMPLE OF IRAQI PATIENTS WITH HYPOTHYROIDISMS DISORDER.

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## Abstract

**Background & Objective :** The purpose of this study was to look at the relationship between the level of severity of the medical condition and SNPs in the TPO gene (rs732609), anti-TPO stages, and TSH levels of Iraqi patients suffering from auto immune hypothyroidism.

**Method:** Blood samples were taken from 50 a hypothyroid patient as well as fifty people in good health with the same condition in order to look for DNA. The SNP rs732609 was subsequently identified using the RT-PCR-HRM (High Resolution Melting) technique with the latest version of Eva green the pigment . Also, Chemical analyses were carried out on the Cobus E411 device, which uses electro chemiluminescence (ECL) technology to transform electrical energy into light (radiative energy), for determining the amounts of Thyroid stimulating hormone and anti-TPO antibodies.

**Result**: Regarding the TPO rs732609 A/C polymorphism, patients with hypothyroidism had significantly higher CC genotypes of rs732609 A/C and the C allele; the p-value for the (AA+AC) CC genotype is 0.0001, the odd ratio (95% CI) is 16.9, and the level of TSH was significantly higher than expected at p-value (0.05), and the level of anti-TPO ab in serum was also significantly higher at p-value (0.04).

**Conclusion**: Our findings show a link among anti-TPO antibodies levels along with a variety of genotypes in hypothyroid people, as well as a link between the rs732609A/C variations and auto immune hypothyroidism. We also found a link among the severeness of the condition and the rs732609A/C polymorphisms.

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

## Introduction

The most common clinical condition is thyroid hormone deficiency, which, if untreated, can have serious health consequences for several organ systems, the cardiovascular system being the most studied. (1) Thyroid-stimulating hormonee (TSH) and thyroxinee (T4) levels that are higher than the reference range are considered signs of apparent primary hypothyroidism. Subclinical hypothyroidism, defined by elevated TSH levels but within normal T4 ranges, is sometimes regarded as an early indicator of thyroid insufficiency. (2).

Hypothyroidism is classified as primary, central, or peripheral based on whether it affects the pituitary, hypothalamus, thyroid, or peripheral organs. (3). The most common type of hypothyroidism is acquired primary hypothyroidism, which is caused by a severe iodine deficiency. However, prolonged autoimmune thyroiditis is more common in areas where iodine levels are high (4) (5). Thyroglobulin tyrosine residues are iodinated and then coupled by thyroid peroxidase (TPO), a glycoprotein with enzymatic activity in thyroid metabolism. T4 and T3 thyroid hormones are produced as a result of this process. (6) which are crucial for regulating metabolic differentiation, controlling growth, and nearly all physiological processes in human tissues. which are crucial for regulating metabolic differentiation, controlling growth, and nearly all physiological processes in human tissues. (7).

The human TPO gene, which has 17 exons and a length of about 150 Kb, is found on chromosome 2p25. TPO is a 102 kDa glycoprotein that is membrane-bound and exists in dimers. Usually, TPO mutations are inherited as autosomal recessive characteristics.(8, 9).(10). The majority of hypothyroidism patients have high levels of TPOAb and TSH in their sera, which are helpful indicators of autoimmune thyroiditis (11) .It is with great caution that we report that TPOAb levels in hypothyroidism patients are highly and perhaps related to thyroid destruction (12).To elucidate the relationship between TPO gene polymorphisms and the onset and prognosis of hypothyroidism, we genotyped one single nucleotide polymorphism (SNP) in this study.

## Materials and Methods

### The subjects

A total of fifty female patients were enrolled in this study from three Baghdadi hospitals. As controls, fifty healthy females were also added. We assessed each participant's height, weight, and body mass index (BMI) in addition to performing thyroid function tests such as measuring thyroid-stimulating hormone (TSH) and measuring immunological parameters such as anti-TPO antibody for two groups: one for hypothyroidism patients and the other for apparently healthy controls. Two tubes were used to hold the samples. Fifty female patients in all were gathered for this study from three Baghdadi hospitals. As controls, fifty healthy females were also added. The participants' height, weight, and body mass index (BMI) were recorded, along with thyroid function tests such as TSH and immunological parameters like anti-TPO antibody. **First tube**: EDTA tube (2 ml): For the molecular genetic investigation, two millilitres of blood were stored at -20 C. **Second tube**: The remaining 3 millilitres of venous blood were transferred to gel serum separation tubes (5 millilitres) and a clot activator. Serum is produced by putting blood samples in gel tubes and allowing them to stand at room temperature (20 to 25 °C). Centrifugation was then used to separate the serum for 15 minutes at 3000 rpm. Using the internationally patented ECL technology, sera were collected to perform the following biochemical tests: Thyroid-stimulating hormone (TSH) and Anti-Thyroid Peroxidase Antibody (Anti-TPO Ab) at the Cobas E411/Roche system with high accuracy, speed, and efficiency.

### DNA extraction and genotyping of TPO gene

The extraction of genomic DNA was done using the Easy Pure® Genomic DNA Kit. The extracted DNA's concentration and purity were evaluated using a Quantus Fluorometer. EVA

Green, I type, master mix, and HRM genotyping primers were utilised. Genetic variants for the SNP rs732609 A/C were examined using amplification and high-resolution melting curve (HRM) analysis. Table 1 summarises the forward and reverse primer sequences, PCR-HRM conditions, and table 2 displays the heat profile of HRM genotyping. By using an electrophoretic UV transillumination method, DNA fragments were visible. after ethidium bromide staining electrophoresis gels. The device took pictures and saved them to the computer using special software.

**Table (1): The study's designed primers.**

| **SNP** | **Primer** | **Sequence From 5-3** | **Product size bp.** | **Company origin** |
|---|---|---|---|---|
| **TPO gene/Exon Rs732609 A> C** | Forward | **AAATTCCCCGAAGACTTTGA** | **76** | Alpha DNA-Canda<br><br>Primers designed using Primer3Plus And Primer Explorer V4 |
| | Revers | **GAGGAAAGGTTTCCCTCCAG** | | |

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

| **Step** | **Temperature (ºC)** | **Time (sec.)** | **Cycles** |
|---|---|---|---|
| **Enzyme activation** | **94** | **30** | **1** |
| **Denatturation** | **94** | **5** | **40** |
| ***Annealing RS 732609** | **(54)** | **15** | |
| **Extension** | **72** | **20** | |
| **HRM** | **55-95** | **0.2sec for 1 degree** | |

**Statistical analysis**

The statistical software SPSS 25 (SPSS Inc., Chicago, IL, USA) was used for all analyses. The mean ± standard deviation is used to express all normally distributed qualitative data, and the

t-test was used to compare two groups. ratios of odds (OR). P-values were deemed significant if they were less than 0.05. The mean ± standard deviation is used to express qualitative data, and the t test was used to compare two groups. The odds ratios, or OR, were computed. P-values that were less than 0.05 were regarded as significant.

**Results**

Table 3 displays the participants' demographic information. Between the control and patient groups, there was no discernible difference in age or BMI (P=0.4 for age and P=0.5 for BMI). Each of the aforementioned auto-antibody anti-TPO ab was positively correlated with hypothyroidism in a statistically significant way. Both the p value of TSH and the p value of having high anti-TPOAb were 0.0001. The SNP (rs732609) genetic analysis results showed that 32% (n=16) of the hypothyroid patients had the heterozygous (AC) genotype, 16% (n=8) had the wild (AA) genotype, and 52% (n=26) of the hypothyroid patients were homozygous (CC). Comparably, 66% (n=36) of the control group had wild-type AA genotypes, 22% (n=11) heterozygous AC genotypes, and 6% (n=3) homozygous CC genotypes. The genotype CC The homo-mutant genotype rs732609 CC was found to be at a higher risk of hypothyroidism than the wild type rs732609 AA, and it was also demonstrated to be a risk factor for hypothyroidism (Odds ratio = 39) when compared with those carrying the wild-type AA. The frequency was significantly higher in hypothyroid patients than in apparently healthy control (p=0.0001). The risk was 6.5 times higher in those with the heterozygous mutation (rs732609 AC genotype) than in the wild-type AA (p=0.0007). According to this study, hypothyroid patients had a higher frequency of TPO SNP (rs732609) C carriers (AC + CC genotypes) than the control group, with an odds ratio of 13.5 (odds ratio= 13.5) for carrying any mutant allele. (Table 4).

The relationship between the various rs732609 genotypes (AA, AC, and CC) and serum laboratory values measurements for TSH and Anti-TPO ab was examined in this study. Three genotypes, AA, AC, and CC, and anti-TPO levels were found to differ significantly (p=0.05). According to Fig. 2(a), the genotypes CC, AC, and AA had respective means (±SDs) of 150.3027 ± 210.477, 159.9782 ± 325.356, and 159.037 ± 120.1697). Furthermore, a noteworthy distinction was observed between the three genotypes and TSH levels. In a similar vein, TSH and the three genotypes showed a significant correlation (P=0.04). According to Fig. 2(b), the genotypes CC, AC, and AA had mean (±SD) values of 34.4462 ± 27.4709, 32.8906 ± 23.1327), and 24.6750 ± 19.22905, respectively.

**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 | |
| | patients | 1.666 ±1.2909 | 0.4 |

| | | | |
|---|---|---|---|
| Age | control | 17.333 ±4.1633 | |
| TSH | patients | 32.3850 ± 24.77310 | 0.0001** |
| | control | .6412 ±. 32453 | |
| Anti -TPO | patiants | 239.008 ± 183.9025 | 0.0001** |
| | control | 15.382 ± 34.7040 | |

Means having with the different letters in same column different significantly. **(p<0.01)

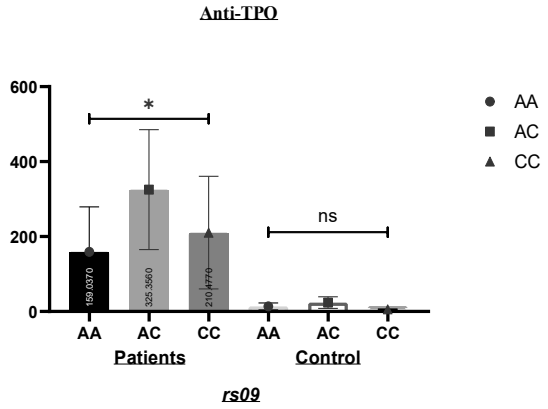
**The genotypes and allele frequency distributions for TPO gene SNP rs732609 are shown in Table (4).**

| ***SNP2*** ***Rs09*** | **Frequencies (%)** | | **P value** | **Odd ratio (95% CI)** |
|---|---|---|---|---|
| | **Patients (n= 50)** | **Control (n= 50)** | | |
| **Co-dominant** | | | | |
| **AA** | **8 (16%)** | **36 (66%)** | **---** | **1.00 (Reference)** |
| **AC** | **16 (32%)** | **11 (22%)** | **0.0007**** | **6.5 (2.2127 to 19.362)** |
| **CC** | **26 (52%)** | **3 (6%)** | **0.0001**** | **39 (9.4308 to 161.279)** |
| **Dominant** | | | | |
| **AA** | **8 (16%)** | **36 (66%)** | **---** | **1.00 (Reference)** |
| **AC+ CC** | **42 (84%)** | **14 (28%)** | **0.0001**** | **13.5 (5.0865 to 35.830)** |
| **Recessive** | | | | |
| **AA +AC** | **24 (48%)** | **47 (94%)** | **---** | **1.00 (Reference)** |
| **CC** | **26 (52%)** | **3 (6%)** | **0.0001**** | **16.9 (4.6611 to 61.799)** |
| **Allele** | | | | |
| **A** | **0.32 (32)** | **0.83 (83)** | **---** | **1.00 (Reference)** |
| **C** | **0.68 (68)** | **0.17 (17)** | **0.0001**** | **10.3 (5.3094 to 20.273)** |

*=Significant (p<0.05).

**=Highly significant (p<0.01).

Ns= Not significant.



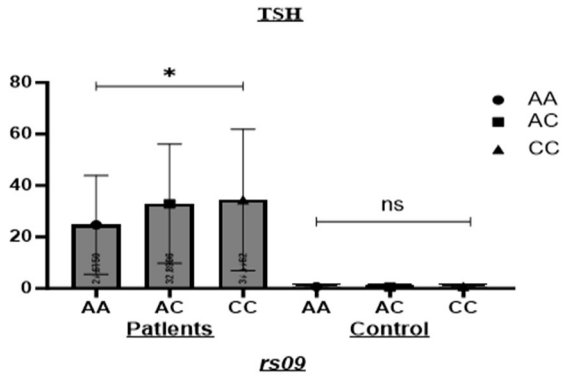




Fig 2.a

Fig 2.b

*=significant ($p<0.05$)

Fig. 2. (a) The relationship between serum anti-TPO antibody levels and the AA, AC, and CC genotypes of the rs732609 polymorphism (b) The relationship between serum TSH levels and the AA, AC, and CC genotypes of the rs732609 polymorphism.

**Discussion:**

The current study assesses the possible correlation between the TPO gene and hypothyroidism in Iraqi patients. The main finding of this study was that TPO gene polymorphisms might be a significant contributor to Iraqi hypothyroidism. In the state of Iraq, thyroid conditions are very common. Since TPO is an essential enzyme for the synthesis of thyroid hormones, a TPO gene mutation may cause serious abnormalities in the production of thyroid hormones. The findings showed that the C/C genotype is a risk genotype linked to hypothyroidism, as is the C allele of Thr725Pro (rs732609). The secondary structure of the TPO protein may be disrupted by the substitution of proline (Pro) for threonine (Thr) in Thr725Pro. When proline is positioned between regular secondary structure elements like beta sheets and alpha helices, it disrupts the structure. The protein's phosphorylation site, threonine, is present simultaneously and is crucial for the protein's activation (9).Changes to this amino acid could therefore affect the TPO enzyme's activity, which could ultimately lower the enzyme's functional efficacy. This SNP has not yet undergone functional analysis, which ought to be looked into in our upcoming research. These findings are consistent with an Egyptian study (13) , which discovered that the frequency of hypothyroidism in patients with C carriers (AC + CC genotypes) was statistically significantly higher than that of control subjects (p-value= 0.001). The A (wild-type) allele frequencies were 93.5% in hypothyroidism patients compared to 76.5% in controls who appeared to be in good health. The C allele (variant) frequencies in hypothyroidism patients were 60.5%, which was significantly higher (p=0.001) than in the apparently healthy control group, which had 23.5% of the allele. However, our findings conflicted with those of a study by Tomari (14). which discovered that in patients with hypothyroidism and the control group, the C allele and the CC genotype of rs732609 A/C were nonsignificant frequent . Thus, the findings of this study are consistent with those of previous research from Egypt and India(15), which demonstrated that allele A of TPO

(rs732609 A>C) protects against the hypothyroidism disease, while allele C is a risk factor for the condition. Moreover, Thr725Pro polymorphism has been linked to an increased risk of hypothyroidism (13). A significant correlation was found between hypothyroidism and TSH, higher titers of Anti-TPO ab, and the studied parameters across all participants when the relationship between hypothyroidism and these parameters was tested. Table (6) shows that there was a significant positive correlation between TSH and anti-TPO antibodies in both the patient and the healthy control group. This positive outcome is consistent with other earlier research that revealed similar conclusions and that serum anti-TPO levels(16) shown that the prevalence of antiTPOAb in the Iranian population as a whole was 14.9% ,which is comparable to the findings of the current study (13.23%). whose thyroid dysfunction is most likely not brought on by an autoimmune condition; for these individuals, the diagnosis of hypothyroidism occurred early (before the age of 15)(17). The most common causes of primary hypothyroidism at birth are disorders of thyroid hormone biosynthesis (dyshormonogenesis) or problems with thyroid gland development (dysgenesis)(18). Thyroid dysgenesis and dyshormonogenesis account for about 85% and 15% of permanent cases of congenital primary hypothyroidism, respectively, when hypothyroidism is present from birth (congenital hypothyroidism) (19).

**Table 5: TSH and ANTI TPO Ab serum levels correlated with rs732609 polymorphism**

**Correlations**

| | | TSH | Anti-TPO | rs09 |
|---|---|---|---|---|
| **TSH** | **Pearson Correlation** | 1 | 0.377** | 0.467** |
| | **Sig. (2-tailed)** | | .000 | .000 |
| **Anti-TPO** | **Pearson Correlation** | 0.377** | 1 | 0.389** |
| | **Sig. (2-tailed)** | .000 | | .000 |
| **rs09** | **Pearson Correlation** | 0.467** | 0.389** | 1 |
| | **Sig. (2-tailed)** | .000 | .000 | |

**. Correlation is significant at the 0.01 level (2-tailed).

**Conclusion**

In summary, our research discovered a correlation between anti-TPO antibody levels and various genotypes in hypothyroid patients, as well as a relationship between rs732609A/C polymorphisms and auto immune hypothyroidism. Additionally, we discovered a correlation between the severity of the disease and the rs732609A/C polymorphisms. and a connection was discovered between the various genotypes of anti-TPO antibodies in the Iraqi population and the levels of these antibodies in hypothyroid patients. Our study was limited by the small sample size; therefore, a larger population should be investigated to determine the frequency of TPO genetic polymorphisms. To validate the current findings, more research on different nationalities and ethnicities is required.

**Ethical statement**

On November 28, 2022, the College of Institute of Genetic Engineering and Biotechnology for Postgraduate Studies/University of Baghdad's ethical committee approved this study (No. 2922). Prior to inclusion, all participants gave written consent. The research followed the guidelines set forth in the 2013 Declaration of Helsinki.

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