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ASSOCIATION BETWEEN PROINFLAMMATORY INTERLEUKIN-1 ß (IL1B) POLYMORPHISM AND HYPERTHYROIDISM IN IRAQI PATIENTS

Talal Murshed Odah al Jaraah[1], Gholamreza Dehghan[2*], Leila Sadeghi[3]

[1,2*,3]Department of Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.

***Corresponding author**:Gholamreza Dehghan
*Department of Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran
Email:-gdehghan@tabrizu.ac.ir

Abstract:
This research examines the relationship between the proinflammatory interleukin-1β (IL1B) single nucleotide gene polymorphism and biochemical parameters in Iraqi hyperthyroid patients. A cohort comprising 220 individuals from Al-Hussein Teaching Hospital in Karbala, Iraq, participated in the study. Factors like smoking habits, residential location, hormone levels (TSH, T3, and T4), lipid profile (TG, TC, LDL, HDL, and VLDL) creatine kinase (CK), and glycemic indicators (HbA1c and FBS) were evaluated. IL1B-511 polymorphisms were detected through polymerase chain reaction (PCR) amplification and gel electrophoresis. Results highlighted a higher smoking prevalence among hyperthyroid patients. Significant differences were observed in T3, T4, and TSH levels between the patient and control groups ($p \leq 0.05$), but not in FBS, HbA1c, CK, TC, LDL, HDL, and VLDL levels ($p > 0.05$). TG levels differed significantly, favoring the control group ($p \leq 0.05$). The IL1B-511 CC genotype was notably linked to hyperthyroidism, with an odds ratio of 4.2 (95% CI: 1.19-14.74, $p = 0.025$). However, hormone concentrations (TSH, T3, T4) didn't significantly differ between C and T allele patients ($p > 0.05$). In conclusion, this study establishes a relationship between IL1B-511 polymorphism and hyperthyroidism in Iraqi patients. The results suggest the CC genotype of IL1B-511 might contribute to hyperthyroidism development.

Keywords: Hyperthyroidism; Hormonal dysregulation; Interleukin-1β; Gene polymorphis

Introduction:

Hyperthyroidism is characterized by excessive thyroid hormone production, which leads to a range of physiological and metabolic changes. This condition is detectable through assessments of thyroid hormones or anomalies detected in various tests such as blood parameters and lipid levels [1], [2]. The way hyperthyroidism is presented clinically can be influenced by the patient's sex, age, and the underlying cause of the condition [3]. The prevalence of hyperthyroidism is relatively high, affecting approximately 2% of women and 0.2% of men [4]. Some studies propose that roughly 79% of the risk of developing hyperthyroidism can be attributed to genetic factors [5]–[7]. Beyond genetics, various environmental factors contribute to the risk of hyperthyroidism. These factors include iodine levels, smoking, alcohol consumption, stress, and infections [8].

Cytokines, a diverse group of polypeptides, play a pivotal role in inflammatory and immune responses. They trigger and coordinate these responses while exerting various effects on non-immunological cells [9].

Within the cytokine family, interleukin-1 (IL-1) consists of two principal agonist molecules, IL-1α and IL-1β, known for their proinflammatory effects [10]. IL-1β, produced by different cell types such as monocytes, macrophages, endothelial cells, neuronal and glial cells, and fibroblasts, induces inflammatory and immune responses [11], [12]. The IL-1B gene, encoding the pro-inflammatory cytokine Interleukin-1β, exhibits a high degree of genetic polymorphism [13], [14]. One such variation is the IL1B-511 polymorphism, located within the promoter region of the *IL1B* SNPs—rs16944 (-511C/T variation) gene on chromosome 2q14 [15].

In the context of hyperthyroidism, investigating the association between the IL1B-511 polymorphism and the disorder can provide valuable insights. By examining the relationship between this genetic variation and hyperthyroidism in a case-control study, we aim to determine if the variant IL1B-511 allele is linked to an increased risk of developing hyperthyroidism. Additionally, we will explore the relationship between the IL1B-511 polymorphism and thyroid hormone levels among hyperthyroid patients to better understand its biological function in the context of thyroid hormone dysregulation.

Subjects and methods

Population study

A consecutive series of 212 individuals aged between 20 and 60 years were recruited for this study from August 2022 to May 2023 at Al-Hussein Teaching Hospital in Karbala. Emphasis was placed on selecting a homogeneous sample with uniform ethnicity, educational levels, and other demographic characteristics. Participants belonging to different groups were assessed using a comprehensive questionnaire encompassing various aspects such as demographic traits (age and gender), lifestyle factors like smoking patterns, personal and familial medical histories, as well as regional disparities (urban or rural residence).

Individuals who had smoked at least 100 cigarettes during their lifetime were categorized as smokers. Current smoking status was determined based on an affirmative response to the question, "Are you presently a smoker?" Conversely, those who responded negatively were classified as non-smokers. Additional data, including the number of cigarettes smoked per day and the duration of smoking in years, were also recorded. A control group of 82 participants, without any clinical manifestations of hyperthyroidism or chronic diseases, was included in the study as apparently healthy individuals.

Exclusion criteria encompassed specific conditions such as hypothyroidism, iodine deficiency, or any other factors causing a reduction in thyroid size (e.g., underactive thyroid). Additionally, individuals with severe chronic ailments, excluding thyroid-related cancers, and those receiving levothyroxine treatment were excluded from participation.

Data collection took place prior to the administration of any treatment or interventions. Written informed consent was obtained from all participants in accordance with the guidelines provided by the research committee of the medical ethics unit under the auspices of the Ministry of Health in Iraq.

Blood sampling

Blood samples were collected using aseptic techniques from the peripheral veins of the participants. The samples were drawn into 10 ml "Venoject" tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. To ensure preservation, the tubes were stored at a temperature of 4 °C until further processing. Each sample was appropriately labeled with the corresponding molecular biology laboratory in Kerbala City to ensure the confidentiality of personal and genetic data. This practice adhered to the ethical guidelines approved by the research committee of the medical ethics unit, Ministry of Health, Iraq (Approval No. 3030.5/5 D. I. M).

Blood sample analysis

Thyroid function blood tests were conducted using enzyme-linked immunosorbent assay (ELISA) to assess the serum concentration of participants, including thyroid stimulating hormone (TSH), triiodothyronine hormone (T3), and thyroxine hormone (T4). All samples were analyzed in triplicate to ensure accuracy, and the final value was determined by averaging the two measurements.

Hematological parameters, including creatine kinase (CK), lipid profile parameters (total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL)), and glycemic parameters (glycated hemoglobin (HbA1c) and fasting blood sugar (FBS)) in the participants' blood samples were evaluated using various laboratory methods. CK levels were evaluated using an enzymatic assay, TC, TG, and HDL were assessed through colorimetric/enzymatic assays, and HbA1c was analyzed using a High-Performance Liquid Chromatography (HPLC). FBS levels were measured based on the glucose oxidase/hexokinase method. All the analywere ses assayed at the molecular biology laboratory in Karbala City according to the guidelines.

Genomic DNA extraction

Genomic DNA was extracted from the collected blood samples using the AddPrep genomic DNA Extraction Kit (Addbio/Korea), a widely used and validated method for DNA isolation. The extraction procedure was performed following the manufacturer's instructions to ensure high-quality DNA samples suitable for subsequent genotyping analysis.

Genotyping of IL1B-511 polymorphism

The genotyping of the IL1B-511 polymorphism, a well-known proinflammatory gene variant, was conducted using conventional PCR. The primer sequences that are used in this study are listed in Table 1, provides the primer names, sequences, and their respective resultant PCR product size on the IL1B-511 gene.

Table 1. Primer sequences employed for PCR.

Primer	Polymorphism	Sequence (5'…….3')	PCR product size
rs16944 G/A	Inner forward	TGGAGGCAATTTTGAGGGGCAGGGA A allele	279 bp
	Inner reverse	TAGGACCCTGGAGGCTGAACCCCGTACCG allele	366 bp
	Outer forward	ACCCAAACACAGGCCTCAGGACTCAACA	600 bp
	Outer reverse	AGTTGGGGACACGCAAGCATGAAGGATA	

PCR amplification

For each genotyping reaction, a total volume of 20 µl was prepared. The reaction mixture consisted of 3 µl of genomic DNA template (at a concentration of 40 ng), 1.5 µl of 10 picomoles of each forward and reverse primer, and 10 µl of the 2X Addbio Mastermix PCR kit (Addbio/Korea). Distilled water was added to reach the final reaction volume (20 µl). The amplification protocol was performed in a Biobase Conventional PCR thermal cycler (Biobase/China), which provided precise temperature control for optimal PCR performance. The cycling conditions were as follows: an initial denaturation step at 95 °C for 15 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 65 °C for 1 minute, and extension at 72 °C for 1 minute.

ARMS-PCR analysis

The genotypes of the IL1B-511 polymorphism, including homozygous patterns and heterozygotes, were determined using the Amplification Refractory Mutation System (ARMS)-PCR analysis.

Agarose gel electrophoresis

Following the PCR amplification, the resulting products were subjected to agarose gel electrophoresis to visualize and analyze the amplified fragments. A 1.5% agarose gel was prepared, and ethidium bromide (0.5 µg/mL) was added to the gel for staining. The PCR products were loaded into the gel

wells, alongside appropriate DNA size markers. Electrophoresis was performed at 70 V for 30 min, allowing the amplified fragments to migrate through the gel matrix based on their molecular weight. Following electrophoresis, the gel was visualized and documented using Gel Doc image analyzer.

Statistical analysis

Statistical analysis involves several tests and measures. Hardy-Weinberg equilibrium (HWE) was assessed in the control group using Fisher's exact test. Proportional differences were determined using the χ^2 test. The distribution of polymorphisms between the control and hyperthyroidism groups was compared, and the odds ratio (OR) with 95% confidence intervals (CI) was obtained through logistic regression. A significance level of $p \leq 0.05$ was considered statistically significant for analyses involving two categories. The statistical software SPSS (IBM SPSS Statistics, SPSS Inc., Illinois, Chicago, USA) version 25.0 was utilized for the analyses.

Results and discussion

Anthropometric analysis

A total of 212 patients, consisting of 105 males and 107 females, were enrolled in the research conducted at Al-Hussein Teaching Hospital in Karbala Governorate from August 2022 to May 2023. The demographic characteristics of both the control and patient groups are summarized in Fig. 1. The participants' ages ranged from 20 to 60 years. Out of the individuals involved in the study, 80 hyperthyroid female patients and 50 hyperthyroid male patients were identified. The age distribution of the specimens is illustrated in Figs. 1a and b. The results reveal an elevated inclination among both female and male participants in the 41-50 age group. Thyroid abnormalities are known to be common in the general population, and some studies have consistently reported a higher prevalence among females [16-18]. In a study conducted in 1977 known as the UK Whickham study, researchers reported that the annual incidence of hyperthyroidism was estimated to be between 100 and 200 cases per 100,000 individuals. They observed a higher prevalence of hyperthyroidism in women, with a rate of 2.7%, compared to a rate of 0.23% in men [19].

We categorized individuals who had smoked at least 100 cigarettes during their lifetime as smokers. Current smoking status was determined by affirmatively responding to the question, "Are you presently a smoker?" Conversely, individuals who responded negatively were classified as Non-smokers. 84 patients were identified as smokers. The distribution of smokers and non-smokers is illustrated in Fig. 1c. The results of our study demonstrate a significant increase in the prevalence of smoking among patients with hyperthyroidism ($p \leq 0.05$). The findings of our study are consistent with the results reported by L. Bartalena, K. Mann, and Hegedüs L [20-22]. Smoking affects thyroid hormone levels through various mechanisms. Tobacco smoke contains toxins like thiocyanate and 2,3-hydroxypyridine. Thiocyanate, a potential goitrogen, inhibits iodide transport and organification while increasing iodide release. 2,3-hydroxypyridine limits the deiodination of thyroxine, temporarily raising serum thyroxine levels before decreasing them by altering deiodinase activity [23]. Also, smoking is associated with a nearly twofold increase in the likelihood of developing Graves hyperthyroidism and an approximately eightfold higher risk of developing Graves ophthalmopathy. Additionally, smokers exhibit a slower response to antithyroid drug treatment [19, 22].

We also assessed the urban and rural populations within our samples. Surprisingly, out of the patients, 86 were from rural areas, which showed a significant variation when compared to the urban population ($p \leq 0.05$). The details are presented in Fig. 1d.

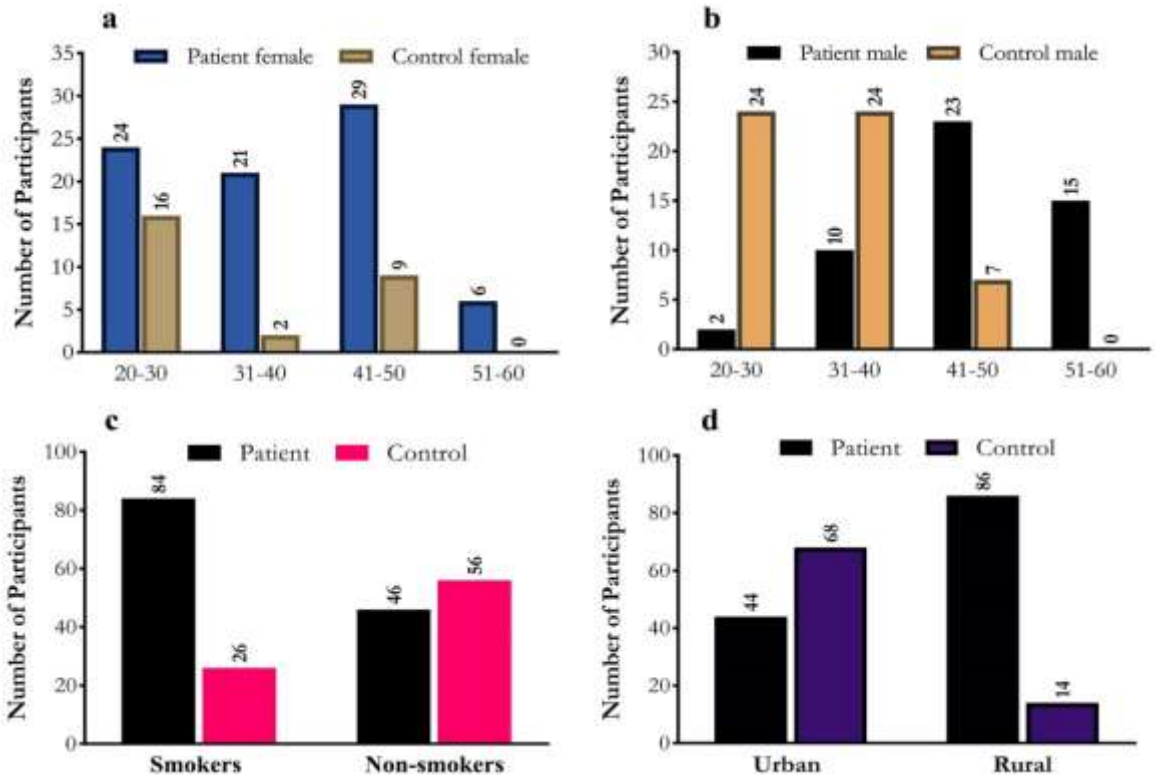

Figure 1. The demographic characteristics of patients and sample groups.

Biochemical parameters analysis

Irregularities in thyroid function including hyperthyroidism cover a wide range of clinical situations. This is a common medical concern, with many patients displaying subtle disruptions in thyroid function that are identified through evaluations of thyroid hormones or abnormal findings in different tests such as blood parameters or lipid levels [24]. A total of 212 samples were analyzed in order to *asses*s the relationship of thyroid hormone levels and hematological parameters between hyperthyroid and healthy groups. The results are shown in Table 2. Upon investigating the thyroid hormone levels, a notable distinction in the values of T3, T4, and TSH was observed between the patient group and the control group, reaching a level of statistical significance at $p \leq 0.05$. Notably, Taskin Senturk also identified a significant variation in thyroid hormone levels between hyperthyroid patients and the control group comprising euthyroid patients [25]. In another study involving patients with thyroid disorders (hyperthyroidism and hypothyroidism) within a sample of Iraqi individuals, a considerable elevation in TSH and T4 levels was documented for both hypothyroidism and hyperthyroidism patients in comparison to the healthy control group. Furthermore, no noteworthy disparity in T3 levels was detected between the groups of patients and their healthy counterparts. [26].

No significant differences were observed between the control and the patient group for CK, TC, HDL, and LDL levels ($p>0.05$). Certain studies indicate reduced levels of LDL cholesterol in individuals with hyperthyroidism [27-30], whereas limited data provide evidence for no significant alteration [31]. Significant differences were noted in TG levels, with the control group reporting value of 161.12 ± 4.93 compared to the patient group ($p \leq 0.05$). Thyroid hormones play a role in all aspects of lipid metabolism, which includes creating, releasing, and breaking down lipids. In cases of hyperthyroidism, the activity of lipoprotein lipase tends to stay the same [32].

During our experiment, we examined the glycemic parameters, including HbA1c and FBS. Interestingly, we did not observe any significant differences between the patient and control groups. Sawer Sabri Ahmeda et al reported a notable correlation between hemoglobin level (Hb) and hyperthyroidism [1].

Table 1. Comparison of thyroid function parameters and hematological properties between the patient and control groups.

Variable	Patient Mean ±SD N=130	Control Mean ±SD N=82	P-Value
Thyroid hormone levels			
TSH	1.96±0.59	0.86±0.82	0.0001
T3	4.55±0.43	2.70±1.52	0.0001
T4	243.65±6.45	213.14±7.67	0.003
Hematological parameters			
CK	40.25±3.13	43.35±2.35	0.84
TC	202.54± 7.98	181.31±6.71	0.13
TG	161.12±4.93	109.28±5.40	0.001
HDL	51.11±5.89	48.75±13.82	0.73
LDL	120.72±7.78	110.23±3.13	0.18
VLDL	25.21±2.77	19.1±1.26	0.074
Hba1c	6.48±0.24	5.33±0.33	9.29
FBS	114.17±18.67	94.21±12.96	4.2

Amplification of IL1B-511 rs16944 gene

The amplification of the IL1B-511 rs16944 gene using conventional PCR resulted in specific product sizes for different alleles. The A allele showed an amplification product size of 279 bp, while the G allele yielded a product of 366 bp. Fig. 2 The PCR products that were subjected to electrophoresis on a 2% agarose gel, along with a 1500 bp DNA ladder, were run at a voltage of 70V/cm for a duration of 30 minutes.

Figure 2. Electrophoresis pattern of PCR product for IL1B-511gene.

Frequency of genotypes

The frequency of IL1B-511 genotypes was assessed for Hardy-Weinberg equilibrium in both the patient and control groups ($p > 0.05$), except for the mutant homozygous genotype (CC). The observed imbalance in this particular genotype deviates from the expected allele frequencies under

Hardy-Weinberg equilibrium, potentially influencing the comparison between cases and controls for this polymorphism.

Table 3 presents the distribution of IL1B-511 genotypes and allele frequencies in both the control and patient groups. The CC genotype showed a significant association with hyperthyroidism in patients, with an odds ratio (OR) of 4.2 (95% confidence intervals (CI): 1.19-14.74, p = 0.025). This finding suggests that individuals carrying the CC genotype may have an increased susceptibility to hyperthyroidism. However, the CT genotype, while more frequent in the patient group compared to the control group, did not reach statistical significance (OR = 2.47, 95% CI: 0.93-6.56, p = 0.067). The allele frequencies of the IL1B-511 polymorphism (C and T alleles) did not differ significantly between the two groups ($p > 0.05$).

These results indicate that the IL1B-511 CC genotype may be a potential genetic risk factor for hyperthyroidism in Iraqi patients. The significant association observed suggests that variations in the IL1B gene, particularly the CC genotype, may influence the development of hyperthyroidism. Numerous genetic investigations have explored the correlation between variations in the IL1B-511 gene and the susceptibility to hyperthyroidism. Different research studies on the IL1B-511 gene variant have been conducted among Tunisian [33], Polish [34], and Iranian [35] populations. Graves' disease (GD), an autoimmune thyroid ailment marked by hyperthyroidism, was the subject of a comprehensive analysis in 2010. This meta-analysis examined a total of 11 case-control studies, revealing that the IL1B-511 gene variant was associated with the risk of GD in diverse populations, including Caucasians and Asians, specifically under the homozygote genetic model (TT vs. CC, OR= 0.86, 95% CI: 0.76–0.97, P-Value = 0.015). However, no significant connections were observed under the dominant and recessive genetic models (TT+TC vs. CC: OR = 0.95, 95% CI: 0.81–1.12, P-Value = 0.553; TT vs. TC+CC: odds ratio = 0.82, 95% CI: 0.60–1.12, P-Value = 0.205, respectively) [36].

Table 2. Genetic distribution of IL1B-511gene polymorphism in the patient and sample groups

IL1B-511 Genotypes/Alleles	Patient (%)	Control (%)	Odd ratio 95%CI	P-value
CC	37(28.02%)	12(16.4%)	4.2 1.19-14.74	0.025
CT	70(53.2%)	38(46.3%)	2.47 0.93-6.56	0.067
TT	23(18.9%)	32 (39.1%)	References	0.095
Allele				
C	70(54.55%)	31(37.8%)	0.48 0.21-1.13	0.097
T	51(62.2%)	60(45.45%)		

Table 4 presents an analysis of thyroid function test concentrations, including TSH, T3, and T4, in patients diagnosed with hyperthyroidism based on their IL1B-511 genotype. The findings revealed that there were no statistically significant differences in hormone concentrations between individuals carrying the C allele and those with the T allele. This comparison was further detailed across patient and control groups. In summary, the analysis indicated that the IL1B-511 genotype was not significantly associated with variations in thyroid function test concentrations in patients with hyperthyroidism. The comparable hormone levels across different genotypes suggest that this particular genetic variation may not strongly influence thyroid hormone levels in the context of hyperthyroidism.

Table 3. Comparison of thyroid function test concentrations in hyperthyroidism patients with T and C alleles

Hormone	Patient		P-Value	Control		P-Value
	T N=60	C N=70		T N=51	CN=31	
TSH	2.2±0.84	1.62±0.35	0.07	2.09± 0.65	1.92± 0.38	0.12
T3	4.61±1.6	4.41±1.34	0.79	4.11± 0.36	4.12± 0.65	0.83
T4	241.35±50.0	239.02±58.3	0.90	224± 22.33	209± 34.65	0.66

Conclusion

In conclusion, our study investigated the association between the proinflammatory IL1B-511 gene polymorphism and hyperthyroidism in Iraqi patients. Our findings revealed significant associations between smoking and hyperthyroidism, as well as significant differences in thyroid hormone levels, indicating hormonal dysregulation in hyperthyroidism. Additionally, hematological parameters, specifically TG levels, were significantly decreased in patient samples. Our results recommended the IL1B-511 CC genotype is linked to increased hyperthyroidism risk in Iraqi patients (OR = 4.2, p = 0.025), while the CT genotype's significance was not reached (OR = 2.47, p = 0.067). Allele frequencies and thyroid hormone concentrations showed no significant differences.

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