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Immunogenetic Association of TNFAIP3 rs10499194 Polymorphism with Susceptibility to Immune Thrombocytopenia in Iraqi Patients

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Abstract

Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by increased platelet destruction and impaired production. This study evaluated the association of the *TNFAIP3* rs10499194 polymorphism and serum TNFAIP3 levels with ITP susceptibility. This case–control study included 120 Iraqi patients with ITP and 60 healthy controls. *TNFAIP3* rs10499194 (C>T) was genotyped using TaqMan real-time PCR, and serum TNFAIP3 levels were measured by ELISA. Genotype and allele distributions and Hardy–Weinberg equilibrium was assessed. The distribution of TNFAIP3 rs10499194 genotypes differed significantly between ITP patients with healthy controls ($p < 0.001$). The CT genotype was more frequently observed among patients, whereas the CC genotype predominated in the control group. Allelic analysis demonstrated a significantly higher frequency of the T allele in patients compared with controls, indicating an association between rs10499194 and susceptibility to ITP. Serum TNFAIP3 concentrations were significantly elevated in ITP patients compared with healthy controls (638.63 ± 62.11 pg/mL vs. 401.94 ± 45.32 pg/mL, $p < 0.001$). Moreover, serum TNFAIP3 levels differed significantly according to rs10499194 genotype, with higher concentrations observed among carriers of the T allele. *TNFAIP3* rs10499194 was significantly associated with ITP susceptibility and elevated serum TNFAIP3 levels, suggesting a potential role in ITP pathogenesis.

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1. Introduction

Immunological thrombocytopenia (ITP) is an acquired autoimmune disorder resulting from immune-mediated platelet destruction in the absence of other identifiable causes [1, 2]. Although current treatments including corticosteroids, rituximab, thrombopoietin receptor agonists (TPO-RAs), and splenectomy can achieve sustained platelet count stabilization in over two-thirds of adults with chronic ITP, a subset of patients remains refractory or relapses following therapy. This highlights the urgent need for more effective and targeted therapeutic strategies [3, 4]. The immunopathogenesis of ITP remains incompletely defined, involving a multifaceted interplay of both humoral and cellular immune mechanisms. Historically, ITP was primarily attributed to autoantibody-mediated opsonization of platelets, resulting in their accelerated clearance by Fc receptor-bearing phagocytes [5, 6]. However, emerging evidence indicates that this mechanism alone does not fully account for the observed thrombocytopenia. Recent studies have elucidated the pivotal role of cytotoxic $CD8^+$ T lymphocytes in mediating direct lysis of platelets and megakaryocytes. Moreover, aberrant T-cell responses and pro-inflammatory cytokine profiles contribute to impaired megakaryopoiesis within the bone marrow microenvironment [7, 8]. Collectively, these findings underscore that both immune-mediated platelet destruction and defective platelet production are central to the pathophysiology of ITP. [9, 10]. Based on the presence or absence of an identifiable underlying cause, immune

thrombocytopenia (ITP) is classified as either primary or secondary. Primary ITP, defined by isolated thrombocytopenia without an apparent triggering factor, accounts for approximately 80% of newly diagnosed adult cases [11]. In contrast, secondary ITP arises in association with predisposing conditions or external triggers. These may include hematological disorders such as autoimmune lymphoproliferative syndrome, large granular lymphocytic leukemia, lymphoma, or chronic lymphocytic leukemia; chronic infections such as Helicobacter pylori, HIV, hepatitis C virus, or cytomegalovirus (CMV); or exposure to certain medications, including quinidine and heparin [12,13]. Immune thrombocytopenia (ITP) is a complex autoimmune disorder resulting from dysregulated immune responses that promote platelet destruction and impair platelet production. Several immunological abnormalities have been implicated in its pathogenesis, including an imbalance between T helper cell subsets (Th1/Th2), reduced number and functional impairment of regulatory T cells (Tregs), increased cytotoxic T-cell activity, and expansion of Th17 cells, all of which contribute to the breakdown of immune tolerance against platelet antigens [14]. In addition to immune dysregulation, genetic factors are increasingly recognized as key contributors to the susceptibility and development of ITP. Polymorphisms in genes involved in immune regulation and cytokine signaling, including TNF-α, TNFAIP3, IL-17, and IFN-γ, have been reported to influence autoimmune disease risk [15, 16]. Among these genes, TNFAIP3 encodes tumor necrosis factor alpha-induced protein 3 (A20), a ubiquitin-editing enzyme that functions as a critical negative regulator of the nuclear factor-κB (NF-κB) signaling pathway, thereby maintaining immune homeostasis and preventing excessive inflammatory responses [17]. Genetic alterations or reduced activity of TNFAIP3 have been implicated in multiple autoimmune and inflammatory disorders, including autoimmune cytopenia, which are characterized by immune-mediated destruction of blood cells. Immune thrombocytopenia represents one of the most common forms of autoimmune cytopenia, in which immune mechanisms specifically target platelet antigens, leading to accelerated platelet clearance and impaired megakaryocyte-mediated platelet production. Experimental studies have demonstrated that TNFAIP3 deficiency results in uncontrolled NF-κB activation and systemic autoimmune inflammation, including lupus-like disease in animal models. Furthermore, altered TNFAIP3 expression has been associated with impaired mesenchymal stem cell function and defective megakaryopoiesis in patients with ITP [18,19].

The single nucleotide polymorphism rs10499194 (C>T), located in the regulatory region of the TNFAIP3 gene, has been suggested to influence gene expression and immune regulatory mechanisms. Given the central role of TNFAIP3 in suppressing NF-κB–mediated inflammatory signaling and maintaining immune tolerance, genetic variants such as rs10499194 may contribute to the development of autoimmune diseases, including ITP, by altering the regulation of inflammatory and immune pathways [20–22].

2. Methods

This case–control study enrolled 180 participants, including 120 patients diagnosed with primary immune thrombocytopenia (ITP) and 60 age-comparable healthy controls. Patients were recruited from the Haematology and Bone Marrow Diseases Unit at Medical City Hospital, Baghdad, Iraq, between October 2023 and January 2024. Peripheral venous blood samples were collected from all participants using standard venipuncture procedures. The diagnosis of ITP was established according to internationally accepted diagnostic criteria and confirmed by hematological evaluation under the supervision of a consultant hematologist. Individuals with secondary thrombocytopenia, other autoimmune diseases, malignancies, or cancer-related conditions were excluded to minimize potential confounding factors. Ethical approval was obtained from the Ethics Committee of the Iraqi Ministry of Health (Approval No. 37972; October 8, 2023), and written informed consent was obtained from all participants before enrolment and sample collection. Because a significant difference in sex distribution was observed between patients and controls, sex was included as a covariate in the multivariate logistic regression analysis to adjust for its potential confounding effect. All patients underwent a comprehensive clinical and laboratory evaluation, including detailed medical history assessment, clinical examination, imaging investigations, and extensive diagnostic testing. Laboratory investigations included serological screening for human immunodeficiency virus (HIV), hepatitis C virus (HCV) infection, antiphospholipid antibodies, antinuclear antibodies, thyroid autoantibodies, and other relevant infectious and immunological markers to confirm the diagnosis of primary ITP and exclude secondary etiologist. Disease severity was stratified according to platelet count and clinical manifestations. Peripheral blood

samples were collected from all patients at the time of diagnosis and prior to the initiation of any disease-specific treatment, including corticosteroids, intravenous immunoglobulin (IVIG), rituximab, or thrombopoietin receptor agonists. Accordingly, all samples used for baseline molecular and biochemical analyses were obtained from treatment-naïve patients. This pretreatment sampling strategy was adopted to minimize potential treatment-related effects on TNFAIP3 concentration and circulating TNFAIP3 levels and to ensure that these measurements reflected the patients' baseline disease status. Genomic DNA was used for determination of the TNFAIP3 rs10499194 genotype, which is not affected by treatment. Patients were diagnosed with ITP according to internationally accepted diagnostic criteria and underwent comprehensive clinical and hematological evaluation under the supervision of a consultant hematologist. Following baseline sample collection, patients received treatment according to their clinical condition, and treatment response was subsequently assessed using predefined clinical and hematological criteria. Thereby reducing treatment-related heterogeneity and minimizing its potential influence on the investigated immunological and molecular parameters. Healthy control subjects were carefully selected following laboratory screening to exclude infectious diseases, haematological abnormalities, and any underlying conditions that could affect haematological indices, particularly platelet count.

2.1. DNA Extraction and Electrophoresis

Genomic DNA was isolated from peripheral blood samples obtained from all study participants using the Easy Pure® Blood Genomic DNA Kit (Transgene Biotech, China; Catalog No. EE121-01), following the manufacturer's instructions. The concentration and purity of the extracted genomic DNA were evaluated using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). DNA concentration was determined from the absorbance at 260 nm, while purity was assessed using the A260/A280 absorbance ratio. Samples with A260/A280 ratios between 1.8 and 2.0 were considered sufficiently pure and were used for subsequent molecular analyses. The purified DNA samples were then stored at −20 °C until further use for TaqMan real-time PCR genotyping of the **TNFAIP3 rs10499194** polymorphism [23].

2.2. TaqMan Real-Time PCR

Genotyping of the TNFAIP3 rs10499194 polymorphism was performed using TaqMan real-time PCR based on allele-specific fluorescent probes for SNP discrimination. The reaction was carried out using the TaqMan Genotyping Master Mix (Transgene Biotech, China; Catalog No. AQ712) according to the manufacturer's protocol. The assay employed sequence-specific primers and fluorescently labelled probes designed for allelic discrimination of the target SNP. For custom-designed assays, primer and probe sequences were designed and validated prior to analysis to ensure specificity and amplification efficiency. Allelic discrimination was performed by real-time fluorescence detection, allowing identification of homozygous and heterozygous genotypes [24]. To ensure the accuracy and reproducibility of genotyping, rigorous quality control procedures were applied throughout the analysis. Positive and no-template controls were included in each real-time PCR run to verify assay performance and exclude contamination. In addition, approximately 10% of the samples were randomly selected for repeat genotyping. The repeated analyses demonstrated 100% concordance with the initial genotyping results, confirming the robustness and reproducibility of the assay.

2.3. rs10499194 genotyping

The rs10499194 (C>T) single nucleotide polymorphism (SNP) is located in the upstream regulatory region of the human TNFAIP3 gene on chromosome 6q23.3, within a regulatory region that may influence gene transcription. Genotyping of rs10499194 was performed using the TaqMan allelic discrimination assay based on real-time PCR technology. The reaction was carried out using a TaqMan PCR Master Mix (Catalog No. AQ712, Transgene Biotech, China) according to the manufacturer's instructions. The PCR reaction mixture consisted of 10 µL of 2× TaqMan PCR Master Mix, 1 µL of genomic DNA (50–100 ng), allele-specific probes and primers, and nuclease-free water to a final reaction volume of 20 µL. Amplification was performed using a real-time PCR system under the following conditions: an initial denaturation step at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60°C for 1 min. Genotypes were determined using allelic discrimination analysis generated by the real-time PCR system based on the fluorescence signals emitted from allele-specific probes labeled with different reporter dyes.

2.4. Estimation Serum Levels of TNFAIP3

Serum TNFAIP3 concentrations were quantified using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit (Catalogue No. SL3972Hu, Sunlong Biotech, China) according to the manufacturer's instructions. Serum samples were diluted 1:5 with the supplied sample diluent before analysis to ensure that the measured concentrations were within the assay's validated analytical range. Subsequently, 10 µL of the diluted serum was added to each well, and absorbance was measured at 450 nm using a microplate reader. TNFAIP3 concentrations were calculated from a standard calibration curve generated using the supplied standards. The measured concentrations were expressed in pg/mL and multiplied by the dilution factor (×5) to obtain the final serum TNFAIP3 concentrations. According to the manufacturer's specifications, the assay had a detection range of 30–2000 pg/mL with a sensitivity of 5.6 pg/mL. The reported intra-assay and inter-assay coefficients of variation were <10% and <12%, respectively. All samples were analyzed in accordance with the manufacturer's protocol, and only measurements falling within the assay's validated analytical range were accepted for analysis.

Table.1 Custom primers and allele-specific TaqMan probes used for real-time PCR genotyping of TNFAIP3 rs10499194 (C>T). The polymorphic nucleotide is shown in bold. The expected amplicon size was 74 bp.

Component	Sequence (5′–3′)	Reporter Dye
Forward primer	CTTCAAAAGCTATCAGTTTCATTACC	–
Reverse primer	CACAGTTTCTAGGATTTCTCTTTCA	–
Probe (C allele)	CTTCAAATGTGTTCAACCCTTTTGTT	FAM
Probe (T allele)	CTTCAAATGTGTTTAACCCTTTTGTT	HEX

Table 2. Reaction mixture used for TaqMan real-time PCR genotyping of TNFAIP3 rs10499194

Component	Volume (µL)
2× TaqMan PCR Master Mix	10
Genomic DNA (50–100 ng)	1
Forward primer (10pmol/µL)	0.4
Reverse primer (10 pmol/µL)	0.4
Probe (Allele C)	0.2
Probe (Allele T)	0.2
Nuclease-free water	7.8
Total volume	20 µL

Table 3. Thermal cycling conditions used for TaqMan real-time PCR

Step	Temperature	Time	Cycles
Initial denaturation	95°C	10 min	1
Denaturation	95°C	15 s	40
Annealing/Extension	60°C	1 min	40

2.5. Statistical Analysis

Statistical analyses were performed using SPSS software version 27 (IBM Corp., Armonk, NY, USA). Continuous variables were initially assessed for normality using the Shapiro–Wilk test. Data with normal distribution were expressed as mean ± standard deviation (SD) and analyzed using the independent samples t-test, Categorical variables were expressed as frequencies and percentages. Comparisons of genotype and allele frequencies between groups were performed using the Chi-square (χ^2) test, while Fisher's exact test was applied when expected cell counts were <5. The Hardy–Weinberg equilibrium (HWE) was evaluated for genotype distribution in the control group using the Chi-square test. The association between the rs10499194 polymorphism and susceptibility to ITP was estimated by calculating odds ratios (ORs) with

95% confidence intervals (CIs). A two-tailed p-value <0.05 was considered statistically significant.

3. Results

This case–control study included 180 Iraqi participants, comprising 120 patients with ITP and 60 age-comparable healthy controls. The patient group consisted of 40 males (33.3%) and 80 females (66.7%), with a mean age of 36.21 ± 11.25 years, whereas the control group included 37 males (61.7%) and 23 females (38.3%), with a mean age of 34.03 ± 3.89 years. The baseline clinical and haematological characteristics of the study population are summarized in Table 4. The mean age was slightly higher in patients than in controls (36.21 ± 11.25 vs. 34.03 ± 3.89 years, respectively), although no statistically significant difference was observed (p = 0.18). Gender distribution differed between groups, with a predominance of females among patients (66.7%) and males among controls (61.7%). Regarding disease severity, 56.7% of ITP patients were classified as severe, 20.8% as moderate, and 22.5% as mild. Splenectomy had been performed in 16.7% of patients. Platelet counts were significantly lower in the ITP group compared with controls (61.43 ± 45.98 vs. 268.26 ± 76.83 $\times 10^9$/L, p < 0.001), consistent with the clinical characteristics of the disease. Haemoglobin levels were also lower in patients than in healthy controls (12.24 ± 2.33 vs. 13.67 ± 1.50 g/dL). In contrast, white blood cell (WBC) counts were comparable between groups (7.79 ± 2.01 vs. 7.87 ± 1.96 $\times 10^9$/L), indicating no statistically significant difference.

Table 4. Baseline clinical characteristics of ITP patients and healthy controls

Variable	ITP Patients (n = 120)	Controls (n = 60)	p-value
Age (years, Mean ± SD)	36.21 ± 11.25	34.03 ± 3.89	0.18
Sex (M/F)	40 / 80	37 / 23	<0.01
ITP severity (No., %)			—
Severe	68 (56.7%)	—	—
Moderate	25 (20.8%)	—	—
Mild	27 (22.5%)	—	—
Splenectomy (No., %)	20 (16.7%)	—	—
Platelet count ($\times 10^9$/L, Mean ± SD)	61.43 ± 45.98	268.26 ± 76.83	<0.001
Hemoglobin (g/dL, Mean ± SD)	12.24 ± 2.33	13.67 ± 1.50	<0.01
WBC ($\times 10^9$/L, Mean ± SD)	7.79 ± 2.01	7.87 ± 1.96*	0.76

The distribution of TNFAIP3 rs10499194 genotypes among patients with immune thrombocytopenia (ITP) and healthy controls is presented in Table 5. Among ITP patients, the frequencies of the CC, CT, and TT genotypes were 30.0% (36/120), 64.2% (77/120), and 5.8% (7/120), respectively, whereas the corresponding frequencies in healthy controls were 66.7% (40/60), 26.7% (16/60), and 6.6% (4/60). The genotype distribution in the control group was consistent with Hardy–Weinberg equilibrium (HWE) (p = 0.197), whereas a significant deviation from HWE was observed in the patient group (p = 0.001) The deviation from Hardy–Weinberg equilibrium (HWE) observed in the patient group is consistent with previous genetic association studies, in which enrichment of disease-associated alleles among affected individuals has resulted in departures from HWE. Nevertheless, the contribution of other factors, including sampling variation and technical artifacts, cannot be entirely excluded, and the findings should therefore be interpreted with appropriate caution. In the present study, the maintenance of HWE in healthy controls reduces the likelihood of population stratification or selection bias. Furthermore, the possibility of genotyping artifacts was minimized through quality control procedures, including the use of positive and negative controls and repeat genotyping of approximately 10% of randomly selected samples, which demonstrated complete concordance.

Table 5. Genotype distribution and Hardy–Weinberg equilibrium analysis of TNFAIP3 rs10499194 in ITP patients and healthy controls

Genotypes	Patients (n = 120)		Controls (n = 60)	
	Observed N (%)	Expected N (%)	Observed N (%)	Expected N (%)
CC	36 (30.0)	46.25 (38.54)	40 (66.7)	38.40 (64.00)
CT	77 (64.2)	56.50 (47.08)	16 (26.7)	19.20 (32.00)
TT	7 (5.8)	17.25 (14.38)	4 (6.7)	2.40 (4.00)
C allele	149 (62.1)	—	96 (80.0)	—
T allele	91 (37.9)	—	24 (20.0)	—
HWE χ^2	15.806	—	1.667	—
HWE P-value	<0.001	—	0.197 (NS)	—
HWE: Hardy–Weinberg equilibrium; NS: not significant; p-values indicate deviation between observed and expected genotype frequencies.				

Allelic and genotypic association analyses revealed significant differences between ITP patients and healthy controls, as summarized in Table 6. The frequency of the T allele was significantly higher in patients (37.9%) than in controls (20.0%), conferring a 2.44-fold increased risk of ITP (OR = 2.44, 95% CI: 1.46–4.09; p = 0.001). The CT genotype was significantly more frequent among patients (64.2%) than controls (26.7%) and was associated with increased odds of ITP (OR = 5.35, 95% CI: 2.66–10.73; p < 0.001). These findings indicate a significant association between the TNFAIP3 rs10499194 polymorphism and ITP in this study population but do not establish a causal relationship. In contrast, although the TT genotype was slightly more frequent in patients (5.8%) than in controls (6.7%), the difference did not reach statistical significance (OR = 1.94, 95% CI: 0.53–7.09; p = 0.352). The CC genotype, which predominated among healthy controls, was used as the reference genotype. Collectively, these findings support a significant association between the T allele, particularly in the CT genotype, and increased odd of ITP.

Table 6. Association analysis of rs10499194 genotypes and alleles in ITP patients and healthy controls.

Variable	Category	Patients (n = 120)	Controls (n = 60)	OR	95% CI	p-value
Allele	C	149 (62.1%)	96 (80.0%)	Reference	–	–
	T	91 (37.9%)	24 (20.0%)	2.44	1.46–4.09	0.001
Genotype	CC	36 (30.0%)	40 (66.7%)	Reference	–	–
	CT	77 (64.2%)	16 (26.7%)	5.35	2.66–10.73	<0.001
	TT	7 (5.8%)	4 (6.7%)	1.94	0.53–7.09	0.352
Note: OR = odds ratio; CI = confidence interval. The C allele and CC genotype were used as reference categories. Statistical significance was considered at p < 0.05.						

Among patients with immune thrombocytopenia (ITP), whereas the CC genotype was more frequently observed in healthy controls. A statistically significant difference in genotype distribution was identified between the two groups (p < 0.001). Similarly, allele frequency analysis demonstrated that the T allele was more prevalent in ITP patients (37.9%) than in healthy controls (20.0%), while the C allele predominated in controls (80.0%) compared with patients (62.1%). The observed differences in allele distribution between patients and controls were also statistically significant (p < 0.001). Serum TNFAIP3 concentrations, determined using ELISA, were significantly elevated in patients with ITP compared with healthy controls (638.63 ± 62.13 pg/mL vs. 401.94 ± 45.32 pg/mL, respectively; p < 0.001), indicating a potential association between increased TNFAIP3 concentration and disease status.

Table 7. Serum levels of TNFAIP3 in ITP patients and healthy controls

Group	TNFAIP3 level (pg/mL) Mean ± SD	p-value
Patients (n = 120)	638.63 ± 62.13	<0.001
Healthy controls (n = 60)	401.94 ± 45.32	

Data are presented as mean ± standard deviation (SD). Comparisons between groups were performed using the independent samples t-test. Figure 1 illustrates serum TNFAIP3 levels according to rs10499194 genotypes. Individuals carrying the CT and TT genotypes exhibited higher serum TNFAIP3 concentrations compared with carriers of the CC genotype. The CC genotype demonstrated the lowest mean TNFAIP3 level (522.0 ± 251.7 pg/mL), whereas progressively higher concentrations were observed in the CT (623.3 ± 268.4 pg/mL) and TT (700.1 ± 249.5 pg/mL) genotypes. Statistical analysis revealed significantly elevated TNFAIP3 levels in carriers of the CT and TT genotypes relative to the CC genotype ($p < 0.05$), whereas no statistically significant difference was detected between the CT and TT groups. Given that TNFAIP3 (A20) functions as a critical negative regulator of the NF-κB signalling pathway, the elevated TNFAIP3 levels observed in T-allele carriers may represent a compensatory regulatory response to persistent immune activation rather than a direct pro-inflammatory effect. This finding suggests that the rs10499194 polymorphism may influence TNFAIP3 concentration and contribute to immune dysregulation associated with ITP pathogenesis, potentially through modulation of inflammatory signalling pathways and feedback regulatory mechanisms.

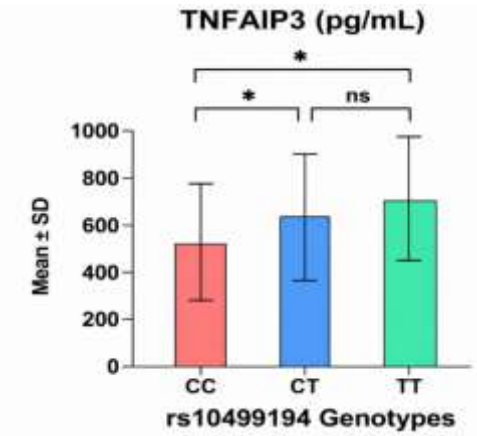


Figure 1. . Distribution of serum TNFAIP3 levels among different TNFAIP3 rs10499194 genotypes.

4. Discussion

The present case–control study provides novel evidence regarding the demographic, hematological, genetic, and immunological characteristics of Iraqi patients with primary immune thrombocytopenia (ITP). Consistent with previous epidemiological studies, females constituted the majority of the patient group, reflecting the well-recognized female predominance observed in many autoimmune diseases. This sex disparity has been attributed to complex interactions among sex hormones, genetic factors, and immune regulatory mechanisms that enhance humoral and cellular immune responses in females [25,26]. Although the distribution of males and females differed between patients and controls, subsequent multivariable logistic regression analysis adjusted for age and sex demonstrated that the association between the TNFAIP3 rs10499194 polymorphism and ITP remained statistically significant, indicating that the observed genetic association was independent of these potential confounding variables. The mean age of patients was comparable to that of healthy controls, minimizing the likelihood of age-related bias. The relatively young age of disease onset observed in this cohort is consistent with previous reports indicating that adult ITP commonly affects individuals during early and middle adulthood, although the disease may occur across all age groups [27]. More than half of the patients were classified as having severe thrombocytopenia, highlighting the substantial clinical burden of the disease. Likewise, the proportion of patients who had undergone splenectomy reflects the continued clinical role of this procedure in selected patients with persistent or refractory disease despite the availability of thrombopoietin receptor agonists and other second-line therapies [28]. As expected, platelet counts were markedly reduced in patients with ITP compared with healthy controls, confirming thrombocytopenia as the principal hematological feature of the disease. Platelet destruction in ITP results from a combination of autoantibody-mediated clearance by splenic macrophages and impaired platelet production secondary to immune-mediated megakaryocyte dysfunction. In addition to pathogenic IgG antibodies directed against platelet glycoproteins, cytotoxic T lymphocytes and dysregulated cellular immunity contribute substantially to platelet

destruction and defective thrombopoiesis [29,30]. These mechanisms collectively explain the profound thrombocytopenia observed in the present study. Patients also exhibited significantly lower hemoglobin concentrations than healthy controls. Although anemia in ITP is frequently attributed to chronic or recurrent bleeding resulting from severe thrombocytopenia, immune-mediated hemolysis should also be considered, particularly in patients with overlapping autoimmune disorders such as Evans syndrome. Consequently, mild reductions in hemoglobin may reflect the combined effects of hemorrhage, chronic inflammation, or concomitant autoimmune cytopenia rather than isolated platelet destruction alone [31].In contrast, total leukocyte counts did not differ significantly between patients and controls. This finding is consistent with the pathophysiology of primary ITP, in which the autoimmune response is predominantly directed against platelets and megakaryocytes while leukocyte production remains largely preserved. Unlike bone marrow failure syndromes or systemic inflammatory disorders that profoundly alter leukocyte homeostasis, uncomplicated ITP generally maintains normal white blood cell counts, making routine leukocyte evaluation useful for distinguishing ITP from other hematological disorders presenting with pancytopenia or leukopenia [32].Collectively, these hematological findings are consistent with the established clinical characteristics of primary ITP and support the continued value of routine complete blood count parameters as essential components of disease diagnosis and clinical evaluation. However, conventional hematological indices alone cannot fully explain the complex immunopathogenesis of ITP. Therefore, investigation of genetic susceptibility factors together with molecular biomarkers may provide additional insights into disease mechanisms and facilitate the development of more individualized diagnostic and therapeutic strategies. The present study investigated the association between the TNFAIP3 rs10499194 (C>T) polymorphism and primary ITP, together with circulating TNFAIP3 protein concentrations. TNFAIP3 encodes **A20**, a ubiquitin-editing enzyme that serves as a critical negative regulator of the NF-κB signaling pathway, thereby limiting excessive inflammatory responses and maintaining immune homeostasis. Dysregulation of TNFAIP3 concenteration or function has been implicated in numerous autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, psoriasis, inflammatory bowel disease, and systemic sclerosis, highlighting its central role in immune tolerance and inflammation [33,34].In the present study, the **T allele** was significantly more frequent among patients than healthy controls and was associated with increased odds of ITP (OR = 2.44, 95% CI: 1.46–4.09; p = 0.001). Similarly, the CT genotype was significantly more prevalent in patients and was associated with a 5.35-fold increase in the odds of ITP (OR = 5.35, 95% CI: 2.66–10.73; p < 0.001), whereas the TT genotype showed no statistically significant association with disease. Furthermore, the association between the CT genotype and ITP remained significant after adjustment for age and sex in multivariable logistic regression analysis, indicating that the observed association was independent of these potential confounding variables. These findings suggest that the TNFAIP3 rs10499194 polymorphism is associated with increased odds of ITP in the studied population rather than establishing a causal relationship. The biological mechanisms underlying this association remain incompletely understood. The rs10499194 variant is located within an upstream regulatory region of **TNFAIP3** and has been proposed to influence transcriptional regulation through modification of enhancer activity, transcription factor binding, or chromatin accessibility. Consequently, altered TNFAIP3 expression may impair the negative regulation of NF-κB signaling, resulting in prolonged activation of inflammatory pathways and loss of immune tolerance. Nevertheless, these mechanisms remain speculative because functional analyses were not performed in the present study. Additional molecular studies are therefore required to determine whether rs10499194 directly influences TNFAIP3 transcription, protein expression, or downstream immune signaling pathways [35,36,37]. The deviation from Hardy–Weinberg equilibrium observed among patients may reflect enrichment of disease-associated alleles, a phenomenon that has been reported in case–control genetic association studies involving susceptibility loci. However, alternative explanations, including sampling variation and technical factors, cannot be completely excluded. The absence of HWE deviation among healthy controls supports the overall reliability of the genotyping assay and suggests that the observed deviation is unlikely to be attributable solely to methodological error. Our findings are consistent with previous studies demonstrating that TNFAIP3 polymorphisms contribute to susceptibility to autoimmune diseases, although the magnitude and direction of association vary among different ethnic populations. Several investigations have reported significant associations between rs10499194 or

closely linked TNFAIP3 variants and autoimmune disorders such as systemic lupus erythematosus, rheumatoid arthritis, psoriasis, and inflammatory bowel disease. These differences among populations may reflect variation in allele frequencies, linkage disequilibrium patterns, environmental exposures, and gene–environment interactions. To date, however, studies evaluating rs10499194 specifically in patients with ITP remain limited. Therefore, the present findings provide additional evidence supporting a potential role of **TNFAIP3** in the genetic architecture of ITP while emphasizing the need for validation in larger and ethnically diverse populations [38,39]. In addition to the genetic findings, circulating TNFAIP3 protein concentrations (pg/mL) were significantly higher in patients than in healthy controls. Because **A20 is** predominantly an intracellular ubiquitin-editing protein rather than a classically secreted cytokine**,** the presence of detectable circulating TNFAIP3 should not be interpreted as active physiological secretion. Instead, elevated serum concentrations may reflect increased intracellular expression accompanied by passive release from activated or apoptotic immune cells, enhanced cellular turnover, extracellular vesicle-associated protein, or tissue injury resulting from persistent immune activation. Consequently, circulating TNFAIP3 is more likely to represent a biomarker of immune activation and dysregulated inflammatory responses than a direct pathogenic mediator [40,41]. Interestingly, circulating TNFAIP3 concentrations differed significantly among rs10499194 genotypes, with individuals carrying the CT and TT genotypes exhibiting higher serum concentrations than those with the CC genotype. This observation suggests that rs10499194 may influence TNFAIP3 expression through regulatory mechanisms affecting gene transcription or chromatin organization. However, because the present study measured circulating protein concentrations without evaluating mRNA expression or functional activity, the precise molecular relationship between genotype and protein concentration remains uncertain. Future investigations integrating genotyping with transcriptomic, epigenetic, and functional analyses will be necessary to elucidate the biological consequences of this variant.

Overall, the combined genetic and protein expression findings support the hypothesis that TNFAIP3 contributes to immune dysregulation in ITP**.** Nevertheless, the observational nature of this case–control study precludes causal inference, and further mechanistic investigations are required to establish whether the rs10499194 polymorphism directly influences TNFAIP3 concentration, NF-κB signalling, and the pathogenesis The present study possesses several strengths that enhance the significance and reliability of its findings. To our knowledge, this is the first study in an Iraqi population to investigate the combined relationship between the TNFAIP3 rs10499194 polymorphism, circulating TNFAIP3 protein concentrations, and susceptibility to primary immune thrombocytopenia (ITP). By integrating genetic analysis using TaqMan real-time PCR with quantitative measurement of serum TNFAIP3 concentrations by ELISA, this study provides a comprehensive evaluation of both genetic variation and its potential phenotypic consequences. Moreover, the inclusion of clinically well-characterized patients, rigorous genotyping quality-control procedures, and multivariable logistic regression adjusted for age and sex strengthens the validity of the observed associations and minimizes the influence of potential confounding factors. Nevertheless, several limitations should be acknowledged. First, the study was conducted at a single tertiary referral centre with a relatively modest sample size, which may limit the statistical power and generalizability of the findings to other ethnic populations. Second, the genetic investigation was restricted to a single polymorphism within the TNFAIP3 gene; therefore, the contribution of additional variants, haplotypes, or gene–gene interactions involved in immune regulation could not be evaluated. Third, although circulating TNFAIP3 concentrations differed significantly according to rs10499194 genotype, the present study did not include functional experiments to determine whether this polymorphism directly influences TNFAIP3 transcription, protein expression, ubiquitin-editing activity, or downstream NF-κB signalling. Consequently, the proposed biological mechanisms remain hypothetical and require experimental validation. Furthermore, the cross-sectional case–control design precludes causal inference and does not permit evaluation of temporal changes in circulating TNFAIP3 concentrations during disease progression or treatment. Finally, although deviation from Hardy–Weinberg equilibrium was observed in the patient group, this finding may reflect enrichment of disease-associated alleles; however, the potential contribution of sampling variation or technical factors cannot be completely excluded of immune thrombocytopenia.

5. Conclusions and Recommendations

This study demonstrated a significant association between the TNFAIP3 rs10499194 polymorphism and susceptibility to ITP in the Iraqi population. The CT genotype was significantly more frequent among ITP patients than among healthy controls and was associated with higher circulating TNFAIP3 levels. These findings support a possible relationship between the rs10499194 variant, circulating TNFAIP3 concentrations, and ITP susceptibility; however, further studies are required to clarify the functional significance of this polymorphism and its potential contribution to ITP pathogenesis. Based on the findings of this study, it is recommended that future research investigates the functional role of the rs10499194 polymorphism in the TNFAIP3 gene, with a focus on its potential impact on gene transcription, mRNA splicing, and regulation of the NF-κB signaling pathway. Broader genetic studies involving additional SNPs within TNFAIP3 and related regulatory regions should be conducted in larger and more diverse populations to confirm and extend the current results. Additionally, the potential use of TNFAIP3 variants, particularly rs10499194, as prognostic biomarkers or predictors of treatment response—such as sensitivity to corticosteroids or thrombopoietin receptor agonists—should be explored to support the development of personalized therapeutic strategies for ITP.

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