Correlation between Serum Levels of Some Immunological Biomarkers and Severity of Idiopathic Immune Thrombocytopenia

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ABSTRACT

Background: Idiopathic immune thrombocytopenia (ITP) is an autoimmune hematological disorder characterized by a platelet count below 100 × 10⁹/L due to immune-mediated destruction and impaired production of platelets. Immune regulatory molecules, including cytokines and immune checkpoint markers, play a vital role in modulating the inflammatory environment and immune tolerance. Dysregulation in the expression or function of these biomarkers contributes significantly to disease pathogenesis and progression. **Aim of study:** The objective of this study was to evaluate the correlation between serum levels of five immunological markers—TNFAIP3, CD28, CTLA4, FoxO3, and IL-39—and the severity of ITP in Iraqi patients, with an emphasis on diagnostic utility and inter-marker relationships. **Methodology:** This case-control study enrolled 180 participants, divided into three groups: newly diagnosed ITP patients (ND, n = 62), medicated ITP patients (MD, n = 58), and healthy controls (HC, n = 60). Serum concentrations of TNFAIP3, CD28, CTLA4, FoxO3, and IL-39 were quantified using ELISA. Statistical analysis was conducted using GraphPad Prism v9.0. **Results:** The study found significantly elevated levels of TNFAIP3 (928 ± 127.7 ng/mL), CD28 (17.5 ± 1.28 ng/mL), CTLA4 (147.5 ± 29.34 ng/mL), FoxO3 (711.7 ± 45.17 ng/mL), and IL-39 (12.98 ± 2.98 ng/mL) in ND patients compared to MD patients and HC ($p < 0.001$). IL-39 levels were notably reduced in the MD group (4.61 ± 0.392 ng/mL) but remained elevated in ND patients, suggesting its potential as an early-stage biomarker. ROC analysis demonstrated exceptional diagnostic performance for CD28, CTLA4, FoxO3, and IL-39, with AUC values of 1.0 in ND patients, achieving 100% sensitivity and specificity at optimal cut-off points. TNFAIP3 showed excellent discriminatory power in ND patients (AUC = 1.0), but limited diagnostic value in MD cases (AUC = 0.537). Pearson correlation analysis revealed strong positive correlations among most biomarkers, especially between TNFAIP3 and FoxO3 (r = 0.938), CTLA4 (r = 0.934), and CD28 (r = 0.893), indicating coordinated immunoregulatory dysfunction in ITP pathogenesis. **Conclusion:** The findings highlight that TNFAIP3, CD28, CTLA4, FoxO3, and IL-39 are significantly associated with disease onset and severity in ITP. These markers offer strong diagnostic value, especially in distinguishing newly diagnosed cases, and may serve as potential targets for immunomodulatory therapies. The observed inter-marker correlations further suggest a complex but coordinated dysregulation of immune responses in ITP, meriting further mechanistic studies.

Keywords: ITP, Immunological markers, ELISA.

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INTRODUCTION

Blood platelets are tiny, round-to-oval nucleated cell fragments that are produced from multinucleated megakaryocytes massive bone marrow cells. Megakaryocyte progenitor cells, the main drivers of platelet synthesis, can proliferate and expand in response to a range of cytokines, which also increase the size, survival, and proliferation of megakaryocytes (1,2,3).

Immune thrombocytopenic purpura (ITP) is defined by the American Society of Hematology as an acquired autoimmune disorder characterized by a low platelet count of less than 100,000/μL due to an unbalanced interaction between effective and regulatory immune cells, with normal hemoglobin and white blood cell counts (4,5). Two varieties of ITP exist: primary without a secondary cause or underlying illness ITP with an underlying cause or issue, such as one caused by drugs or a systemic ailment is referred to as a secondary. (6,7,8). The main cause of ITP is an autoantibody (typically IgG) against glycoprotein, namely the platelet membrane protein. Tissue macrophages, which are located in the spleen, rapidly remove the platelets coated with antibodies, reducing the platelets' half-life. as well as an alternative strategy that uses T-cell-mediated cytotoxicity to specifically target bone marrow megakaryocytes The dynamic interaction of ligands with membrane-bound receptors is facilitated by immune mediators including cytokines and immunological checkpoints, which help to maintain and restore health following pathological events. Dysregulation of their expression may occasionally result in the pathophysiology of a disease. (9,10). The zinc finger protein known as tumor necrosis factor, alpha-induced protein 3 (TNFAIP3) is quickly expressed in response to TNF (11) Genome-wide association studies have demonstrated that aberrant expression or function plays a role in the development of a number of autoimmune or inflammatory diseases, including Crohn's disease, systemic lupus erythematosus, rheumatoid arthritis, type 1 diabetes mellitus, psoriasis, and atherosclerosis (12).

Among the key systems that control T-cell-mediated immunological responses are immune checkpoints, which include co-stimulation and co-inhibition signal pathways (13). The outcome of adaptive T cell immunity is determined by co-stimulation and co-inhibition signals, commonly referred to as "second signals," which work in concert to modify the "first signal" produced by the T cell receptor (TCR) and MHC recognition. Aberrant expression of costimulatory and co-inhibitory molecules can cause autoimmunity by either boosting the generation of T cells that are reactive to themselves or causing these cells to avoid both central and peripheral tolerance (14). T cells are known to be costimulatory for CD28 and coinhibitory for CTLA4. These molecules interact with two ligands on the surface of antigen-presenting cells (APCs), CD80 and CD86, to introduce a positive stimulatory signal and a negative inhibitory signal, respectively, into T cells (15,16). The surface expression of CD28 on T cells is constitutively crucial for effector function, proliferation, and survival of T cells. On the other hand, CTLA4, which is extensively produced following T cell activation, functions as a CD28 competitor and causes T cells to become inert and unresponsive (17,18). Immune checkpoint gene SNPs affect immune checkpoint serum levels in ITP patients, which are protective or risk factors for ITP and linked to the severity, refractoriness, corticosteroid sensitivity, and susceptibility of ITP (19).

As a member of the O subclass family of transcription factors, FoxO3 shares the ability to be inhibited and translocated out of the nucleus upon phosphorylation by proteins like Akt/PKB in the PI3K signaling pathway (20). These proteins are distinguished by a unique fork-head DNA-binding domain. There are also post-translational changes that can lead to changed or enhanced FoxO3a activity, including acetylation and methylation (21).IL-39 is a novel heterodimer member of the IL-12 family, made up of the Ebi3 and IL-23p19 subunits,. Lipopolysaccharide-stimulated B cells release IL-39. IL-39 mRNA is expressed by other immune cells, including dendritic cells and macrophages (22). Because the subunits of IL-39 share subunits with IL-23, IL-27, and IL-35, it may be assumed that the gp130, IL-23R, and IL-27R that make up the IL-39 receptor are the same as the produced homo- or hetero-dimer. By triggering the STAT1/STAT3 signaling pathway, IL-39 promotes the inflammatory response in mice that resemble humans with lupus (23). A few of the unanswered questions surrounding IL-39 research at this early stage include the biological effects and mechanisms of IL-39 in inflammatory diseases other than systemic lupus and acute coronary syndrome, the relationship between IL-39 and other cytokines, and whether other cells can produce IL-39 in addition to activated B cells. To create novel treatments for a variety of related diseases, further study is needed on all these subjects (24,25).

METHODOLOGY

This study involved 180 individuals of both sexes, 120 of who were patients and 60 were healthy volunteers. Blood samples were collected from 120 patients suffering from ITP attending the Hematology and Bone Marrow Diseases Unit at the Medical City Hospital in Baghdad. The patients were selected according to international diagnostic and examination standards under the supervision of a hematology consultant. Patients underwent comprehensive evaluations, including detailed medical history review, symptom assessment, physical examinations, imaging examinations, and a battery of laboratory tests such as HIV, direct antiglobulin test for hepatitis C virus, antiphospholipid antibody test, and antinuclear antibody test, thyroid antibody testing, and detection of other infections to confirm that they have the first type of disease, and they are classified according to the severity of the disease based on the number of blood platelets. Severe cases of ITP were defined as those experiencing bleeding requiring urgent treatment or new severe bleeding episodes necessitating alternative or intensified therapies due to very low platelet counts. Corticosteroid treatment included either a dexamethasone regimen or a continuous prednisone course. The term 'Sensitive' referred specifically to an increase in platelet count without any treatment bleeding episodes. A 'Refractory' status indicated no improvement following splenectomy along with severe symptoms or a high risk of bleeding requiring medical intervention as recommended by the treating physician. As for the healthy volunteers, they were carefully selected and blood samples were drawn from them after conducting laboratory tests to ensure that they did not have any infection that could affect the components of the blood, especially the platelet count. Information was taken from all people according to a specific format according to the instructions of the Iraqi Ministry of Health According to the sandwich ELISA kit's manufacturer's recommendations by Sun Long Biotech company /China, ELISA kits (Catalogue No., SL3972Hu, SL3331Hu, SL0594Hu, SL1915Hu and SL3537Hu) used to assess the serum levels of human TNFAIP3, CD28, CTLA4, FoxO3 and IL-39, respectively.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 9.0. Continuous variables were presented as mean ± standard deviation (SD) and compared using one-way ANOVA followed by Tukey's post hoc test. Categorical data were analyzed using the chi-square (χ^2) test. Receiver operating characteristic (ROC) curve analysis was used to assess the diagnostic performance of biomarkers, and Pearson correlation was applied to examine associations between variables. A p-value < 0.05 was considered statistically significant (26).

RESULTS

Results summarize the gender distribution across the study groups: newly diagnosed (ND) patients, medication-treated (MD) patients, and healthy controls (HC). In the ND group (n = 62), females were predominant, comprising 74.2% (n = 46), while males accounted for 25.8% (n = 16). The MD group (n = 58) exhibited a relatively balanced gender distribution, with females representing 58.6% (n = 34) and males 41.4% (n = 24). In contrast, the HC group (n = 60) showed a male predominance, with 61.7% (n = 37) males and 38.3% (n = 23) females. Statistical analysis using the chi-square test indicated a significant difference in gender distribution among the three groups ($p < 0.001$), suggesting a potential gender-related variability in group composition, as depicted in Figure (1) and Table (1). The study's findings demonstrated that women are more likely than men to have the illness, and this percentage is undoubtedly significantly impacted by the sample size as well as other variables.

Table (1): Distribution of ITP patients and healthy control according to gender.

Group	Gender				p-value
	Male		Female		
	N	%	N	%	
ND (N = 62)	16	25.8	46	74.2	< 0.001
MD (N = 58)	24	41.4	34	58.6	
HC (N = 60)	37	61.7	23	38.3	

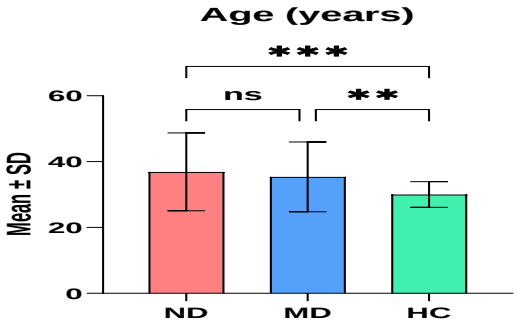

Figure (1): Distribution according to age

The result of Table (2) revealed significant differences in the serum concentrations of immunological biomarkers among healthy controls (HC), medicated ITP patients (MP), and newly diagnosed ITP patients (ND). TNFAIP3 levels were markedly elevated in ND patients (928 ± 127.7 ng/mL) compared to MP (412.4 ± 90.0 ng/mL) and HC (398.2 ± 15.10 ng/mL), indicating its association with active disease. CD28 concentrations increased progressively from HC (8.34 ± 0.88 ng/mL) to MP (10.50 ± 0.70 ng/mL) and ND (17.5 ± 1.28 ng/mL), reflecting heightened T-cell activation. Similarly, CTLA4 levels rose from 46.78 ± 1.82 ng/mL in HC to 63.01 ± 14.12 ng/mL in MP, peaking at 147.5 ± 29.34 ng/mL in ND patients. FoxO3 showed a comparable trend, with levels of 227.3 ± 16.90 ng/mL in HC, 301.6 ± 28.64 ng/mL in MP, and 711.7 ± 45.17 ng/mL in ND, suggesting its role in immune dysregulation. Notably, IL-39 was significantly higher in ND patients (12.98 ± 2.98 ng/mL), whereas levels in MP (4.61 ± 0.392 ng/mL) and HC (5.52 ± 0.406 ng/mL) were comparable, indicating its potential as a marker of disease onset. These findings highlight the dynamic changes in immune biomarkers associated with ITP progression and treatment.

Table (2): Serum levels of immunological markers (TNFAIP3, CD28, CTLA4, FoxO3, IL-39)

Immunological Marker	ND (Mean ±SD) ng/mL	MP (Mean ±SD) ng/mL	HC (Mean ±SD) ng/mL
TNFAIP3	928±127.7[a]	412.4±90.0[a]	398.2±15.10[b]
CD28	17.5 ± 1.28[a]	10.50 ± 0.70[b]	8.34 ± 0.88[c]
CTLA4	147.5 ± 29.34[a]	63.01 ± 14.12[b]	46.78 ± 1.82[c]
FoxO3	711.7 ± 45.17[a]	301.6 ± 28.64[b]	227.3 ± 16.90[c]
IL-39	12.98 ± 2.98[b]	4.61 ± 0.392[a]	5.52 ± 0.406[a]

ND: Newly-diagnosed; MD: Medicated and HC: Healthy Control; (a, b, c) indicate statistically significant differences between groups; values sharing the same letter are **not significantly different**.

Figure (2) illustrate a clear and statistically significant difference in CD28 concentrations among the study groups. The mean concentration was highest in newly diagnosed (ND) patients at 17.5 ± 1.28 ng/mL, followed by treated (MD) patients at 10.50 ± 0.70 ng/mL, and lowest in the healthy control group at 8.34 ± 0.88 ng/mL. ROC curve analysis further confirmed the strong diagnostic potential of the biomarker. In the ND group, the area under the curve (AUC) was 1.0 (95% CI: 1.0–1.0, $p < 0.001$), with a cut-off value of 12.73 ng/mL achieving 100% sensitivity and 100% specificity. For the MD group, the AUC was 0.978 (95% CI: 0.957–0.999, $p < 0.001$); using a cut-off value of 9.44 ng/mL, the sensitivity was 91.4% and specificity was 91.7%. These results underscore the biomarker's high diagnostic accuracy, especially in identifying newly diagnosed cases.

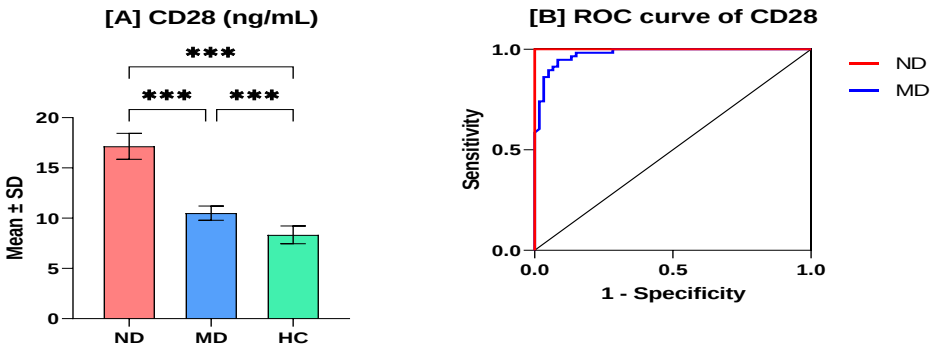

Figure (2): [A] Mean ± SD of CD28 in newly diagnosed (ND) and medicated (MD) patients with ITP and healthy controls (HC). [B] ROC curve analysis of CD28 in ND and MD patients. ***p < 0.001

The serum concentrations of CTLA-4 across the study groups are presented in Figure (3).A statistically significant elevation in CTLA-4 levels was observed among ND patients (147.5 ± 29.34 ng/mL), in comparison to MD patients (63.01 ± 14.12 ng/mL) and healthy individuals (46.78 ± 1.82 ng/mL), indicating substantial variation among the groups. Receiver operating characteristic (ROC) curve analysis further demonstrated the strong diagnostic utility of CTLA-4. In ND patients, the area under the curve (AUC) was 1.0 (95% CI: 1.0–1.0, $p < 0.001$), with a cut-off value of 63.03 ng/mL achieving 100.0% sensitivity and 100.0% specificity. For MD patients, the AUC was 0.931 (95% CI: 0.877–0.985, $p < 0.001$), and a cut-off value of 47.8 ng/mL yielded a sensitivity of 89.7% and specificity of 90.0%. These results support the diagnostic value of CTLA-4, particularly as a highly sensitive and specific marker for identifying newly diagnosed ITP cases.

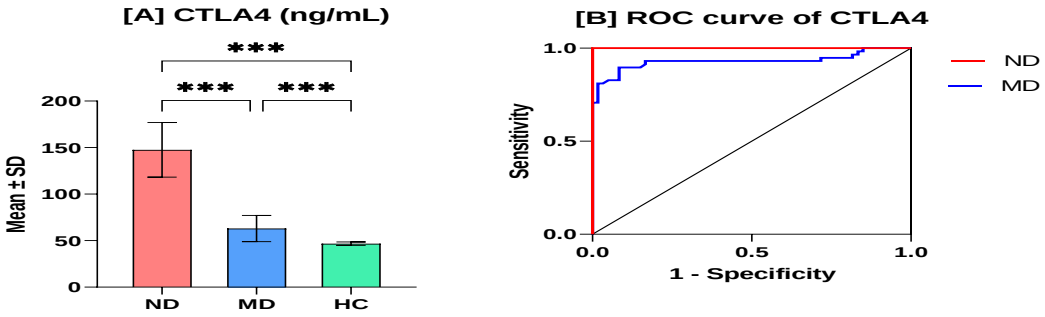

Figure (3): [A] Mean ± SD of CTLA4 in newly diagnosed (ND) and medicated (MD) patients with ITP and healthy controls (HC). [B] ROC curve analysis of CTLA4 in ND and MD patients. ***p < 0.00

According to results of Figure (4), FoxO3 serum levels were significantly higher in newly diagnosed (711.7 ± 45.17 ng/mL) and medicated (301.6 ± 28.64 ng/mL) ITP patients compared to healthy controls (227.3 ± 16.90 ng/mL; $p < 0.001$). ROC curve analysis showed excellent diagnostic performance, with an AUC of 1.0 in newly diagnosed patients (cut-off: 341.0 ng/mL, sensitivity: 100.0%, specificity: 100.0%) and an AUC of 0.988 in medicated patients (cut-off: 253.1 ng/mL, sensitivity: 98.3%, specificity: 98.3%). These findings underscore the high sensitivity and specificity of FoxO3 as a reliable biomarker for early detection and monitoring of ITP.

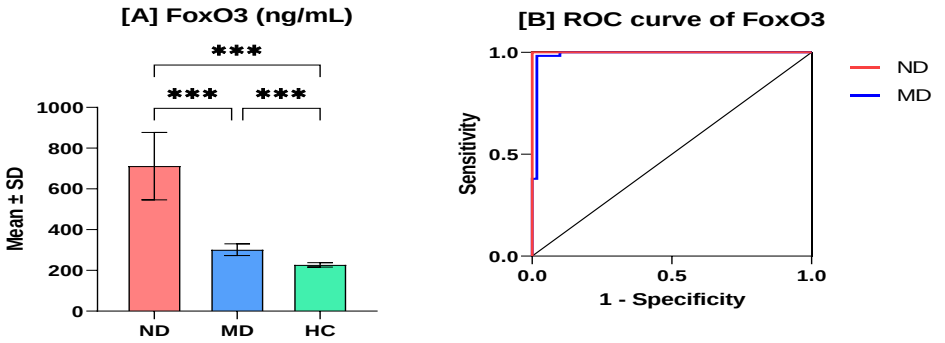

Figure (4): [A] Mean of FoxO3 in newly diagnosed (ND) and medicated (MD) patients with ITP and healthy controls (HC). [B] Receiver operating characteristics (ROC) curve analysis of FoxO3 in ND and MD patients. ***p < 0.001

As shown in Figure (5), IL-39 levels were significantly reduced in medicated ITP patients (4.61 ± 0.392 ng/mL) compared to both newly diagnosed patients (12.98 ± 2.98 ng/mL) and healthy controls (5.52 ± 0.406 ng/mL). ROC curve demonstrated strong diagnostic performance. In newly diagnosed patients, IL-39 showed an AUC of 1.0 (95% CI: 1.0–1.0, $p < 0.001$) with 100.0% sensitivity and specificity at a cut-off value of 7.31 ng/mL. In medicated patients, the AUC was 0.962 (95% CI: 0.92–1.0, $p < 0.001$), with a cut-off value of 5.23 ng/mL yielding 94.8% sensitivity and 95.0% specificity. These findings highlight IL-39 as a highly sensitive and specific biomarker, particularly valuable for identifying newly diagnosed ITP cases and monitoring treatment response.

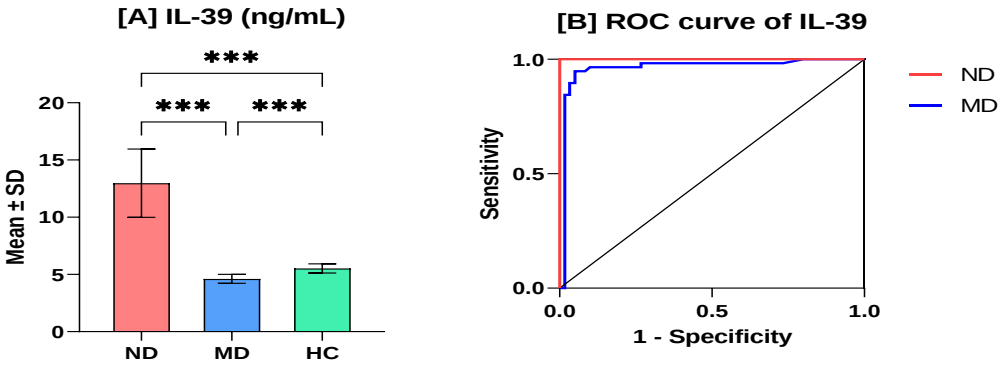

Figure (5): [A] Mean IL-39 in newly diagnosed (ND) and medicated (MD) patients with ITP and healthy controls (HC). [B] Receiver operating characteristics (ROC) curve analysis of IL-39 in ND and MD patients. ***p < 0.001

Significant variations in the mean serum level of TNFAIP3 were seen between newly diagnosed patients (928±127.7) ng/ml, treated patients (412.4±90.0) ng/ml, and healthy individuals (398.2±15.10) ng/ml, according to the results of the classification and comparison of the TNFAIP3 serum levels by groups in Figure (6). Nevertheless, there was no appreciable distinction between the control group and the treated patients. ROC curve analysis demonstrated the acceptable performance of TNFAIP3 in distinguishing between newly and treated patients. For newly diagnosed ITP patients (AUC =1.0; 95% CI 1.0-1.0; p <0.001 cut-off concentration 568.2 ng/ml; sensitivity = 100%; specificity = 100%) while for treated patients (AUC =0.537; 95% CI 0.415-0.659; p< 0.491; cut-off concentration 400.7 ng/ml; sensitivity = 53.3%; specificity = 53.3%).

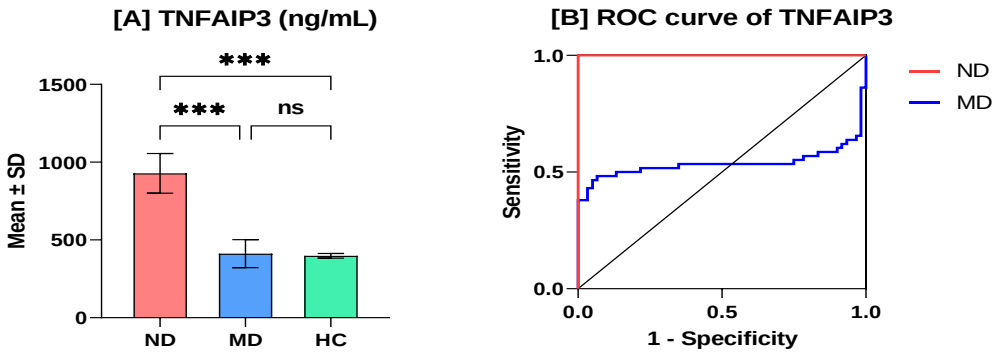

Figure (6): Mean of TNFAIP3 in newly diagnosed (ND) and medicated (MD) patients with ITP and healthy controls (HC). [B] Receiver operating characteristics (ROC) curve analysis of TNFAIP3 in ND and MD patients. ***p < 0.001; ns: not significant

The correlation heatmap in Figure (7) illustrates correlation analysis demonstrated statistically significant and robust positive associations among the evaluated immunological biomarkers—CD28, CTLA4, FoxO3, IL-39, and TNFAIP3—across the study population. TNFAIP3 exhibited the highest degree of correlation with FoxO3 (r = 0.938), CTLA4 (r = 0.934), and CD28 (r = 0.893), indicating a potential regulatory or functional interaction between these molecules in the immunopathogenesis of idiopathic thrombocytopenic purpura (ITP). CTLA4 also showed strong correlations with CD28 (r = 0.912) and FoxO3 (r = 0.903), supporting their established roles in T-cell co-stimulation and immune checkpoint regulation. IL-39 demonstrated moderate-to-strong positive correlations with the remaining markers, including TNFAIP3 (r = 0.849), CD28 (r = 0.873), and CTLA4 (r = 0.848), but a relatively weaker association with FoxO3 (r = 0.720). These findings suggest that while IL-39 may participate in shared immunological pathways, it may also reflect distinct signaling mechanisms. Collectively, the high correlation coefficients among these biomarkers suggest a coordinated immunological response in ITP, potentially reflecting shared upstream regulatory networks or synergistic involvement in disease progression and immune dysregulation.

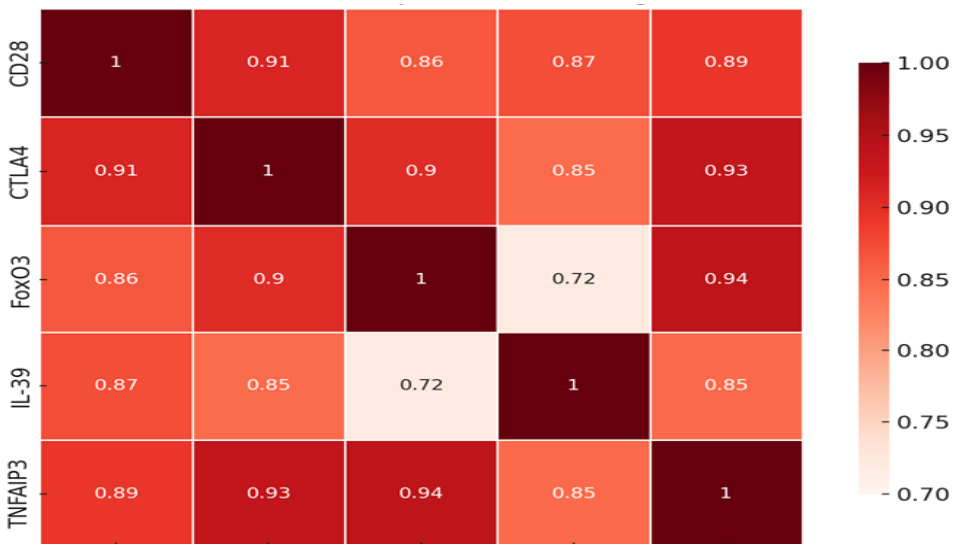

Figure (7): Heatmap of the correlation between TNFAIP3 and other immunological markers (CD28, CTLA4, FoxO3, and IL-39) across different patient groups. The values represent the Pearson correlation coefficients, where:**1** indicates perfect positive correlation, ;**-1** indicates perfect negative correlation and **0** indicates no correlation).

DISSCUSION

In Iraq, immune thrombocytopenia (ITP) represents a significant autoimmune hematological disorder, characterized by isolated thrombocytopenia due to increased platelet destruction and impaired production. Clinical observations suggest variability in presentation and disease course across age groups, with a noticeable female predominance, consistent with global patterns. However, limited regional studies have systematically explored the genetic and immunological factors contributing to ITP in Iraqi populations. This study aims to address that gap by examining key immune-related gene polymorphisms and cytokine profiles relevant to disease pathophysiology. Reports from other countries such as Brazil and Malaysia reinforce the influence of sex and geographic variation on ITP distribution. In Brazil, females accounted for 82.35% of cases, with a female-to-male ratio of 4.7:1 and a median age of 41 years, suggesting that sex hormones and immune differences may modulate susceptibility (27). Similarly, in Malaysia, the incidence of ITP was higher in females, with 73.2% of cases occurring in women between the ages of 19 and 69 (28). These findings align with our observations and highlight the importance of considering demographic factors in ITP epidemiology.

As an autoimmune disorder, ITP results from immune dysregulation, where both genetic predispositions and aberrant immune responses contribute to platelet destruction. The CTLA4 gene, a critical regulator of T-cell-mediated immune responses, plays a central role in maintaining immune homeostasis. CTLA-4 acts by inhibiting T-cell activation through competitive bidding to CD80/CD86, counterbalancing the stimulatory effects of CD28. Our study identified a significant association between ITP and the rs5742909 polymorphism in the CTLA4 promoter region. The T allele of this SNP has been linked to reduced transcriptional activity and, consequently, lower CTLA-4 expression, potentially leading to heightened T-cell activation and loss of peripheral tolerance (29).

In contrast, polymorphisms in the CD28 promoter did not exhibit a significant relationship with ITP in our cohort. While CD28 provides essential co-stimulatory signals for full T-cell activation, its promoter variants may not have a direct functional impact on disease susceptibility in this population. Nevertheless, CD28 remains an important co-regulatory molecule involved in autoimmunity and should not be excluded from future investigations (30,31).

The transcription factor FOXO3a also emerged as a molecule of interest due to its immunoregulatory functions. FOXO3a regulates genes involved in oxidative stress responses, T-cell function, and inflammation, and its altered expression has been linked to various autoimmune disorders (32). Disruption in FOXO3a signaling may contribute to immune imbalance in ITP, and given its ability to regulate NF-κB signaling, it holds promise as a potential therapeutic target (33).

Another molecule examined was IL-39, a relatively novel cytokine belonging to the IL-12 family. Our findings, consistent with prior studies, demonstrated decreased serum levels of IL-39 in autoimmune conditions such as rheumatoid arthritis and autoimmune thyroid disease (24, 25). This reduction could reflect a deficiency in anti-inflammatory signaling or a context-dependent dual role of IL-39 in immune modulation. Although the diagnostic power of IL-39 alone was modest, its combination with other biomarkers could enhance diagnostic accuracy.

Lastly, the TNFAIP3 gene, encoding the anti-inflammatory enzyme A20, has been increasingly recognized for its role in autoimmune disease. TNFAIP3 acts by terminating NF-κB signaling, thereby limiting inflammation. Variants in this gene, particularly within the promoter region, have been associated with increased susceptibility to autoimmune diseases including systemic lupus erythematosus and rheumatoid arthritis (34). In the context of ITP, decreased TNFAIP3 expression was linked to activation of the NF-κB/SMAD7 pathway, leading to dysfunction in mesenchymal stem cells (MSCs) and impaired megakaryopoiesis (35,36). This dysfunction results in reduced platelet production, contributing to disease pathology. Modulating TNFAIP3 activity may offer a therapeutic strategy for restoring immune and hematopoietic balance in ITP (37).

In summary, the study revealed significant correlations between serum levels of specific immunological biomarkers and the severity of idiopathic immune thrombocytopenia (ITP). Notably, TNFAIP3, CTLA-4, and FoxO3 levels were significantly decreased in ITP patients—especially in newly diagnosed cases—compared to healthy controls, indicating impaired immune regulation and increased disease activity. In contrast, CD28 and IL-39 levels were markedly elevated, particularly in newly diagnosed patients, reflecting heightened immune activation.

Statistical analyses showed that low levels of TNFAIP3, CTLA-4, and FoxO3 were negatively correlated with disease severity, as they were associated with lower platelet counts and more severe clinical manifestations. Conversely, higher levels of CD28 and IL-39 were positively correlated with disease severity, suggesting their role in promoting inflammation and autoimmune platelet destruction. These findings highlight the potential of these biomarkers in assessing disease activity and guiding clinical management in ITP.

This study is limited by a relatively small sample size and its single-center design, which may restrict the generalizability of the findings and the cross-sectional nature of the study further limit insights into the dynamic changes in immune markers over time. Future large-scale, multi-center, and longitudinal studies are needed to confirm and extend these results.

CONCLUSION

Significant differences in immunological biomarker levels were observed between ITP patients and healthy controls, as well as between newly diagnosed and treated patient groups. These findings underscore the diagnostic relevance of these immune markers. The biomarkers evaluated in this study play a critical role in accurately assessing disease presence and activity, and they hold promise as potential targets for the development of novel therapeutic strategies aimed at reducing disease severity and improving clinical outcomes.

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العلاقة بين المستوى المصلي لبعض المؤشرات المناعية وشدة نقص الصفيحات المناعي مجهول السبب

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الخلاصة

الخلفية عن الموضوع: يُعد نقص الصفيحات المناعي مجهول السبب أحد اضطرابات الدم المناعية الذاتية، ويتميز بانخفاض عدد الصفائح الدموية إلى أقل من 100×10^9 / لتر نتيجة تدمير الصفائح بوساطة الجهاز المناعي وضعف إنتاجها. تلعب الجزيئات المناعية التنظيمية، بما في ذلك السيتوكينات ونقاط التفتيش المناعية، دورًا أساسيًا في تنظيم البيئة الالتهابية والحفاظ على التوازن المناعي. ويسهم اختلال التعبير أو الوظيفة لهذه العلامات الحيوية بشكل كبير في تطور المرض وشدته. **الهدف من الدراسة:** هدفت هذه الدراسة إلى تقييم العلاقة بين مستويات مصل الدم لخمس علامات مناعية (**TNFAIP3**، **CD28**، **CTLA4**، **FoxO3**، و **IL-39**) وشدة الإصابة بمرض نقص الصفيحات المناعي لدى المرضى العراقيين، مع التركيز على القيمة التشخيصية والارتباطات المتبادلة بينها. **المواد وطرائق العمل:** شملت الدراسة 180 مشاركًا، موزعين على ثلاث مجموعات: مرضى تم تشخيصهم حديثًا(**ND**)، (**n=62**)، مرضى يتلقون العلاج (**MD**، **n=58**)، وأصحاء يمثلون مجموعة الضبط (**HC**، **n=60**). تم قياس تراكيز **TNFAIP3** و**CD28** و**CTLA4** و**FoxO3** و**IL-39** في مصل الدم باستخدام تقنية الاليزا كما أُجريت التحليلات الإحصائية باستخدام برنامج **GraphPad Prism** الإصدار 9.0.. **النتائج:** أظهرت النتائج ارتفاعًا معنويًا في مستويات **TNFAIP3** (928 ± 127.7 نانوغرام/مل)، و**CD28** (17.5 ± 1.28 نانوغرام/مل)، و**CTLA4** (147.5 ± 29.34 نانوغرام/مل)، و**FoxO3** (711.7 ± 45.17 نانوغرام/مل)، و**IL-39** (12.98 ± 2.98 نانوغرام/مل) في مجموعة المرضى المشخّصين حديثًا مقارنة بالمجموعتين الأخريين (**$p < 0.001$**). لوحظ انخفاض واضح في مستوى **IL-39** لدى المرضى المعالجين (4.61 ± 0.392 نانوغرام/مل) مما يدل على أهميته كعلامة تشخيصية في المراحل المبكرة للمرض. أظهر تحليل **ROC** أداءً تشخيصيًا ممتازًا للواسمات **CD28** و**CTLA4** و**FoxO3** و**IL-39** في مجموعة المرضى المشخصين حديثًا (**AUC = 1.0**، الحساسية والنوعية = 100%). أما **TNFAIP3** فقد أظهر قدرة تمييزية عالية في المرضى الجدد، لكنها كانت محدودة في المرضى المعالجين (**AUC = 0.537**). كشفت تحاليل الارتباط بيرسون عن علاقات ارتباط إيجابية قوية بين معظم العلامات المناعية، لاسيما بين **TNFAIP3** و**FoxO3 (r = 0.938)**، و**CTLA4 (r = 0.934)**، و**CD28 (r = 0.893)**، مما يشير إلى خلل مناعي منظم ومترابط في مرضى **ITP**. **الاستنتاج:** تشير النتائج إلى أن **TNFAIP3** و**CD28** و**CTLA4** و**FoxO3** و**IL-39** ترتبط ارتباطًا وثيقًا بشدة الإصابة بـ **ITP**، وتمتلك قدرة تشخيصية عالية، خاصة في تحديد الحالات المشخصة حديثًا. كما تبرز هذه العلامات كأهداف واعدة في تطوير استراتيجيات علاجية مناعية مستقبلية. تؤكد علاقات الارتباط القوية المكتشفة وجود خلل مناعي مشترك يتطلب المزيد من الدراسات التوضيحية.

الكلمات المفتاحية: **ITP** ، المؤشرات المناعية ، **ELISA** .