Sawa Medical Journal
Reprint from
Vol.2 Issue.2 2026
ISSN : 3007-4738

Polymorphisms of *MTHFR*, *PTPN22* and *STAT4* Genes and Association to Inflammatory Markers in Rheumatoid Arthritis Patients

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Article Information
Received: 6-5-2026
Accepted: 28-5-2026
Published: 1-7-2026

Abstract

Background: Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder characterized by persistent synovial inflammation, progressive joint destruction, and significant extra-articular manifestation.

Aim: Investigation the association between genetic polymorphisms in *MTHFR*, *PTPN22* and *STAT4* genes and inflammatory markers including rheumatoid factor (RF), C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) in patients with RA in order to elucidate their role in disease susceptibility, pathogenesis, and clinical expression.

Materials and methods: A total of 50 RA patients and 40 healthy control (HC) individuals were selected, and subjected to sampling of venous blood that used molecularly to indicate genetic polymorphisms restriction fragment length polymorphism analysis (RFLP-PCR) for *MTHFR* gene (*rs1801131*T>G) and genotyping of the allele-specific tetra primers amplification refractory mutation system (T-ARMS-PCR) for *PTPN22* (*rs2476601*G>A) and *STAT4* (*rs7574865*G>T) genes. Serologically, the obtained sera were served for measurement rheumatoid factor (RF) by qualitative ELIZA, as well as C reactive protein (CRP), interleukine-6 (IL-6), and tumor-necrosis factor-alpha (TNF-α) throughout the quantitative ELISA.

Results: Findings of RFLP-PCR revealed genetic polymorphism of *MTHFR* gene in RA patients through increasing the prevalence and risk of TG genotype and G allele. On other hand, results of T-ARMS-PCR determined the marked frequency and risk of GA genotype in *PTPN22* gene of RA patients, with significant elevation in prevalence of G allele and risk of A allele. For *STAT4* gene, results of genotyping frequency reported a significant increase in prevalence and risk of GT genotype, with marled prevalence of G allele and risk of T allele. Among RA patients, serological results of RF (0.35864±0.0186), CRP (330.36±12.99pg/ml), IL-6(35.64±0.94ng/L), and TNF-α (173.16±4.22pg/ml) were significantly higher than the values of HC individuals (0.1811±0.008, 101.13±3.1599pg/ml, 8.58±0.32ng/L, and 49.33±2.39pg/ml, respectively).

Conclusion: This study provides compelling evidence that genetic polymorphisms in key regulatory genes are significantly associated with susceptibility to RA, and are closely related to inflammatory responses. This suggests that integration of genetic and serologic techniques can enhance the understanding pathogenesis and elucidates the mechanistic pathways of RA.

Keywords: Folate metabolism, RFLP-PCR, *rs1801131, rs2476601, rs7574865*, T-ARMS-PCR, Iraq

Introduction

Worldwide, RA represents one of the most prevalent inflammatory rheumatic diseases which affecting approximately 0.5-1% of the global population with a higher incidence in women and peak onset between the fourth and seventh decades of life (Nandakumar et al., 2023). Clinically, RA manifests as symmetrical polyarthritis involving small joints, accompanied by pain, stiffness, and swelling which may ultimately lead to joint deformity, disability, and reduced quality of life if not adequately treated (Almaliky et al., 2024; Perera et al., 2024). Beyond joint involvement, RA is increasingly recognized as a systemic inflammatory disease, contributing to comorbidities such as cardiovascular disease, pulmonary complications, and metabolic disturbances, thereby increasing morbidity and mortality (Mitrović et al., 2023). The etiology of RA is complex and multifactorial involving interplay between genetic susceptibility, environmental exposure, and immunological dysregulation (Arleevskaya et al., 2022). Although the precise cause remains incompletely understood, genetic factors play a central a role in conferring susceptibility to the disease (Wysocki et al., 2020; Chen et al., 2025). Environmental triggers like cigarette smoking, infections, and alterations in the microbiome have been implicated in initiating autoimmune responses in genetically predisposed individuals (Klareskog et al., 2020; Arleevskaya et al., 2022). These factors contribute to a breakdown of immune tolerance and the generation of autoantibodies notably RF and anti-citrullinated protein antibodies which are considered the hallmark features of RA and may precede clinical onset by several years (Romão and Fonseca, 2022).

The pathophysiology of RA is driven by a complex network of innate and adaptive immune mechanisms that sustain chronic inflammation within the synovial membrane, and the disease process begins with immune activation and infiltration of synovial tissue by T cells, B cells, macrophages, and dendritic cells, leading to the production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-17 (Wu et al., 2021; Alivernini et al., 2022; Salnikova et al., 2024). These mediators promote synovial hyperplasia and the formation of pannus, an invasive granulation tissue that erodes cartilage and bone, in addition to fibroblast-like synoviocytes acquire an aggressive phenotype to further perpetuating tissue destruction and joint damage (Papadogianni and Lambrou, 2023; Yue et al., 2025). Osteoclast activation *via* receptor activator of nuclear factor kappa-B ligand signaling leads to bone resorption, while sustained inflammatory signaling contributes to systemic complications (Orsini et al., 2023). Recent advances in molecular immunology have revealed importantly that RA pathogenesis involves not only classical immune pathways but also epigenetic modifications and metabolic reprogramming, highlighting the disease's heterogeneity and complexity (Cai and Yao, 2025; Mołoń et al., 2026). Therefore, the current study was aimed to determine the frequency and distribution of genetic polymorphisms in *MTHFR*, *PTPN22* and *STAT4* genes among RA patients compared to healthy control, evaluation the relationship between identified gene polymorphisms and susceptibility to RA including allele and genotype risks, and

evaluation of relationship between gene polymorphisms and key inflammatory markers including RF, CRP, IL-6, and TNF-α in patients with RA in order to elucidate their role in disease susceptibility, pathogenesis.

Materials and methods

Ethical approval

This study was licensed by the Scientific Committee in the Department of Biology (Collage of Science, University of Wasit).

Samples

A total of 50 RA patients in addition to 40 HC individuals of various ages and sexes were selected for the current study during clinical and laboratory examination in the private orthopedic clinics at Wasit province during December (2025). Approximately, 5ml of venous blood were collected from each study participant and divided equally into free-anticoagulant glass gel tube for serology and an EDTA-tube (whole blood) for molecular testing. After centrifugation, the obtained sera were pipetted and both the serum and whole blood samples were kept frozen until be tested.

Molecular examination

After preparation at room temperature, DNAs were extracted from the whole blood samples using the gSYNC^{TM} DNA Extraction Kit (Genaid, Taiwan), and subjected to further concentration and purity evaluation. Then, primers of studied genes were selected based on recent references through targeting the *rs1801131*, *rs2476601*, and *rs7574865* variants for *MTHFR*, *PTPN22* and *STAT4* genes, respectively. Then, the DNAs and primers were utilized to preparation Mastermix tubes at 20µL to be served for RFLP-PCR for *MTHFR* gene, and genotyping of the allele-specific tetra primers amplification refractory mutation technique for *PTPN22* and *STAT4* genes (T-ARMS-PCR), (Table 1). The conditions of Thermocycler for DNAs amplification were outlined in Table 2, and electrophoresis was done in 1.5% Agarose-gel at 100V and 80mA for 1 hour. Visualization was done under the UV transilluminator, and frequency of potential genotypes and alleles for *MTHFR*, *PTPN22* and *STAT4* genes was TG, GA, and GT, respectively.

Table (1): Primers utilized for identification of genotypes and alleles frequency in *MTHFR*, *PTPN22* and *STAT4* genes

Gene	Primer (5′--3′)		Product size	Reference
MTHFR	F	TCCCGAGAGGTAAAGAACGTAGAC	90bp	Mutlak and Kasim (2024a)
	R	TCCCCCAAGGAGGAGCTGCTGTAGA		
PTPN22	FO	CAATGAACTCCTCAAACTCAAGG	330bp	Taher and Nassir (2024a)
	RO	ACTGATAATGTTGCTTCAACGGA		
	FI	CCACAATAAATGATTCAGGTGTACA	203bp	
	RI	CCCCTCCACTTCCTGGAC	170bp	
STAT4	FO	ACACTTGACTGTTAATACGGATG	401bp	
	RO	CTTGCTTTAGGAGTTCTAATTTTC		
	FI	AAAAGTTGGTGACCAAAATCTA	243bp	
	RI	CCACTGAAATAAGATAACCACTAATA	2026bp	

Table (2): Thermocycler conditions for amplification of DNAs in MasterMix tubes of *MTHFR*, *PTPN22* and *STAT4* genes

Step	Temperature (°C)	Time (Minute)	No. of cycle(s)
Initial denaturation	95	5	1
Denaturation	95	0.5	30
Annealing	58		
Extension	72	1	
Final extension	72	7	1

Serological examination

Qualitative and quantitative enzyme-linked immunosorbent assay kits (ELISA) provided by the SunLong Biotech Company (China) were utilized to determine the levels of RF (Cat.No.SL1538Hu) as well as CRP (Cat.No.SL0535Hu), IL-6 (Cat.No.SL1001Hu), and TNF-α (Cat.No.SL1761Hu) in serum samples of EA patients and healthy control. Post preparation of serum samples and the contents of each kit separately, processing steps were initiated, and the optical density (OD) was measured at 450nm. The ODs of RF in serum samples were considered qualitatively as positives or negatives based on CUT-OFF value; whereas, the concentrations of CRP, IL-6 and TNF-α were measured quantitatively based on ODs and their respective concentrations in diluents of Standard Solution and ODs of serum samples which plotted on the Standard Curve in the Microsoft Office Excel (Gharban et al., 2023).

Statistical analysis

One-way ANOVA, t-test and 95% confidence intervals (95%CI) were estimated in GraphPad Prism software to indicate significant differences between values of RA patients and healthy control groups. Odds ratio (OR) and relative risk (RR) were calculated using the MedCalc Statistical Software. Variation in large horizontal and small vertical letters refers to significant differences at $p<0.05$ (Hussen et al., 2024; Wahab et al., 2024).

Results

Genetic polymorphisms

The findings of genotyping frequency and risk of *MTHFR* gene polymorphism were revealed a significant variation ($p<0.05$) in their values (Tables 3, 4). In comparison to HC, the results of RF patients were shown a marked decrease ($p<0.0169$) in TT genotype (30%), and a significant increase ($p<0.0144$) in TG genotype (60%) but insignificant differences ($p<0.1$) in GG genotype (10%). Concerning the risk (OR, RR) of *MTHFR* gene polymorphism, the results of TG genotype (2.5, 1.3) were elevated significantly ($p<0.0001$) when compared to TT (0.3878, 0.7479) and GG (1, 1) genotypes.

Subsequently, the results of allele frequency *MTHFR* gene in RF patients were recorded a significant reduction ($p<0.0466$) in T allele (60%) but a significant increase ($p<0.0403$) in G allele (40%). Additionally, the risk (OR, RR) of allele frequency in *MTHFR* gene were demonstrated an observable elevation ($p<0.0001$) in G allele (1.6522, 1.1456) in comparison with the T allele (0.6053, 0.8729), (Tables 5, 6).

Table (3): Genotyping frequency of *MTHFR* gene among the RF and HC individuals

Gene	HC (No.40)	RF (No.50)	p-value	95%CI
TT	21 (52.5%) Aa	15 (30%) Bb	0.0169	101.7 to 184.2
TG	15 (37.5%) Bb	30 (60%) Aa	0.0144	94.19 to 191.7
GG	4 (10%) Bc	5 (10%) Bc	0.1	0
p-value	0.0116	0.0149	-	-
95%CI	20.21 to 86.88	29.18 to 95.85	-	-

Table (4): Risk of genetic polymorphism in *MTHFR* gene among the RF and HC individuals

Gene	OR	RR	NNT	95%CI
TT	0.3878	0.7479	10.087 (Benefit)	15.390 (Harm) to ∞ to 3.799 (Benefit)
TG	2.5	1.3	10.833 (Harm)	3.988 (Harm) to ∞ to 15.127 (Benefit)
GG	1	1	-	-
p-value	0.0001	0.0001	-	-
95%CI	1.404 to 3.996	0.3294 to 1.703	-	-

Table (5): Allele frequency of *MTHFR* gene among the RF and HC individuals

Allele	HC	RF	p-value	95%CI
T	57 (71.25%) Aa	60 (60%) Ba	0.0466	5.847 to 137.1
G	23 (28.75%) Bb	40 (40%) Ab	0.0403	37.10 to 105.8
p-value	0.0256	0.0385	-	-
95%CI	220.0 to 320.0	77.06 to 177.1	-	-

Table (6): Risk of genetic polymorphism in alleles of *MTHFR* gene among the RF and HC individuals

Allele	OR	RR	NNT	95%CI
T	0.6053	0.8729	20.257 (Benefit)	14.953 (Harm) to ∞ to 6.038 (Benefit)
G	1.6522	1.1456	20.257 (Harm)	6.038 (Harm) to ∞ to 14.953 (Benefit)
p-value	0.0001	0.0001	-	-
95%CI	5.522 to 7.78	0.7232 to 2.742	-	-

For *PTPN22* gene, the results of RA patients have revealed a significant increase ($p<0.0234$, $p<0.0001$) in genotyping frequency and risk (OR, RR) of GA genotype (62%, 4.3014, 1.0728) but a marked decrease ($p<0.05$, $p<0.0001$) was seen among the values of genotyping frequency and risk of GG (32%, 0.3478, 0.6281) and AA (6%, 0.3617, 0.6809) genotypes (Tables 7, 8). Regarding allele frequency, though the findings of G allele were reduced apparently ($p<0.0391$) in RF patients (63%) when compared to HC (71.25%), they remain significantly ($p<0.0162$) higher than the values of A allele (37%). However,

frequency of A allele in RF patients (37%) was markedly ($p<0.0479$) higher than recorded in HC (28.75%). Additionally, the risk (OR, RR) of A allele in RF patients (1.4555, 1.1080) was significantly ($p<0.0001$) higher than observed in HC individuals (0.6871, 0.9025), (Tables 9, 10).

Table (7): Genotyping frequency of *PTPN22* gene among the RF and HC individuals

Gene	HC (No.40)	RF (No.50)	p-value	95%CI
GG	23 (57.5%) Aa	16 (32%) Bb	0.0177	117.3 to 206.8
GA	11 (27.5%) Bb	31 (62%) Aa	0.0234	174.4 to 263.9
AA	6 (15%) Ac	3 (6%) Bc	0.0405	46.68 to 67.68
p-value	0.0118	0.0176	-	-
95%CI	20.93 to 87.59	36.28 to 102.9	-	-

Table (8): Risk of genetic polymorphism in *PTPN22* gene among the RF and HC individuals

Gene	OR	RR	NNT	95%CI
GG	0.3478	0.6281	5.806 (Benefit)	3.007 (Benefit) to ∞ to 83.964 (Benefit)
GA	4.3014	1.0728	34.693 (Harm)	4.805 (Harm) to ∞ to 6.647 (Benefit)
AA	0.3617	0.6809	8.533 (Benefit)	6.036 (Harm) to ∞ to 2.5 (Benefit)
p-value	0.0001	0.0001	-	-
95%CI	3.99 to 7.331	0.1904 to 1.397	-	-

Table (9): Allele frequency of *PTPN22* gene among the RF and HC individuals

Allele	HC	RF	p-value	95%CI
G	57 (71.25%) Aa	63 (63%) Ba	0.0391	14.71 to 119.5
A	23 (28.75%) Bb	37 (37%) Ab	0.0479	19.54 to 85.29
p-value	0.0026	0.0162	-	-
95%CI	220.0 to 320.0	115.2 to 215.2	-	-

Table (10): Risk of genetic polymorphism in alleles of *PTPN22* gene among the RF and HC individuals

Allele	OR	RR	NNT	95%CI
G	0.6871	0.9025	26.895 (Benefit)	12.393 (Harm) to ∞ to 6.449 (Benefit)
A	1.4555	1.1080	26.895 (Harm)	6.449 (Harm) to ∞ to 12.393 (Benefit)
p-value	0.0001	0.0001	-	-
95%CI	3.81 to 5.953	0.3003 to 2.311	-	-

The results of genotyping frequency in *STAT4* gene determined the significant prevalence ($p<0.0258$) of GT and TT genotypes in RF patients (50% and 16%, respectively) and low incidence of GG genotype (34%) when compared to HC (20%, 7.5% and 72.5%, respectively). In addition, though GG was the almost prevalent genotype in HC individuals, GT incidence was increased markedly in RF patients (Table 11).

Subsequently, the risk (OR, RR) of GT genotype (4, 1.4138) was significantly ($p<0.0001$) higher than identified in GG (0.1954, 0.6296) and TT (2.3492, 1.213) genotypes (Table 12).

Table (11): Genotyping frequency of *STAT4* gene among the RF and HC individuals

Gene	HC (No.40)	RF (No.50)	p-value	95%CI
GG	29 (72.5%) Aa	17 (34%) Bb	0.022	191.3 to 297.8
GT	8 (20%) Bb	25 (50%) Aa	0.0258	155.6 to 225.6
TT	3 (7.5%) Bc	8 (16%) Ac	0.0429	42.25 to 65.75
p-value	0.0024	0.0341	-	-
95%CI	52.35 to 119	8.921 to 75.59	-	-

Table (12): Risk of genetic polymorphism in *STAT4* gene among the RF and HC individuals

Gene	OR	RR	NNT	95%CI
GG	0.1954	0.6296	6.3 (Benefit)	3.164 (Benefit) to ∞ to 731.57 (Benefit)
GT	4	1.4138	7.927 (Harm)	3.497 (Harm) to ∞ to 29.75 (Benefit)
TT	2.3492	1.213	13.524 (Harm)	3.275 (Harm) to ∞ to 6.35 (Benefit)
p-value	0.0001	0.0001	-	-
95%CI	2.558 to 6.921	0.07353 to 2.097	-	-

Significantly, though the frequency of G allele was higher ($p<0.0105$ and $p<0.0243$, respectively) in both HC (82.5%) and RF (59%) individuals than T allele (17.5% and 41%, respectively), the findings of T allele in RF patients (41%) were higher than detected in HC (17.5%), (Table 13). Additionally, the risk (OR, RR) of T allele frequency (3.2760, 1.3319) was significantly ($p<0.0001$) higher than the values of G allele (0.3052, 0.7508), (Table 13).

Table (13): Allele frequency of *STAT4* gene among the RF and HC individuals

Allele	HC	RF	p-value	95%CI
G	66 (82.5%) Aa	59 (59%) Ba	0.0105	78.55 to 220
T	14 (17.5%) Bb	41 (41%) Ab	0.0243	120 to 178.5
p-value	0.0037	0.0397	-	-
95%CI	363 to 463	64.36 to 164.4	-	-

Table (14): Risk of genetic polymorphism in alleles of *STAT4* gene among the RF and HC individuals

Allele	OR	RR	NNT	95%CI
G	0.3052	0.7508	9.396 (Benefit)	89.678 (Harm) to ∞ to 4.464 (Benefit)
T	3.2760	1.3319	9.396 (Harm)	4.464 (Harm) to ∞ to 89.678 (Benefit)
p-value	0.0001	0.0001	-	-
95%CI	17.08 to 20.66	2.65 to 4.733	-	-

Inflammatory markers

The findings of qualitative measurement of RF detected that the CUTOFF value for positive reactivity was ≥0.258. Among the study individuals, values (M±SE) and range of RF were significantly (p<0.0023; 95%CI: 0.8581 to 1.398) higher in RA patients (0.35864±0.0186, 0.175-0.779) than HC individuals (0.1811±0.008, 0.094-0.342), (Figure 1).

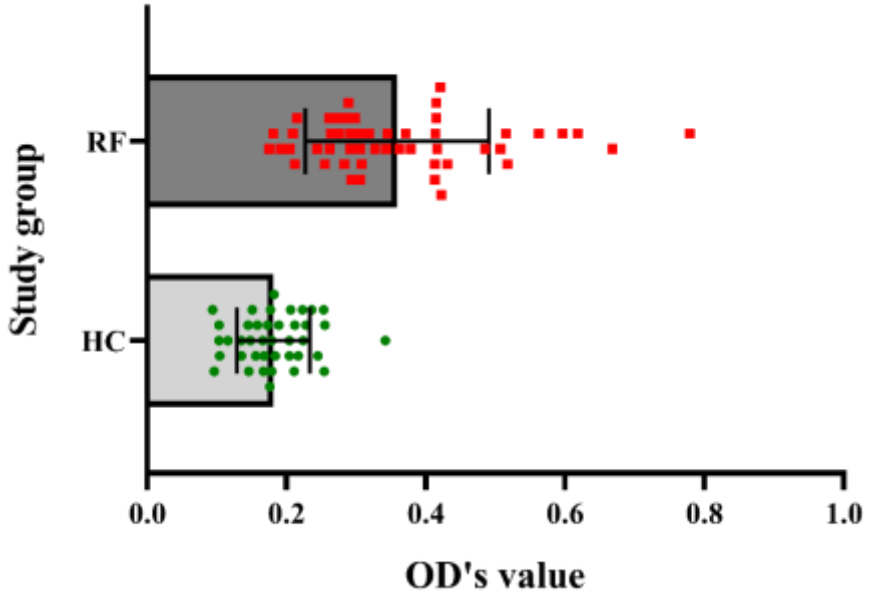

Figure (1): Levels of serum RF among the study population, HC and RF

Quantitative measurement of serum CRP detected that the findings of RA patients (330.36±12.99pg/ml) were elevated apparently (p<0.0003; 95%CI: 1241 to 1672) in comparison with the values of HC individuals (101.13±3.1599pg/ml), (Figure 2). For IL-6, significant increases (p<0.0001; 95%CI: 149.8 to 194.0) were identified in RA patients (35.64±0.94ng/L) when compared to HC individuals (8.58±0.32ng/L), (Figure 3).

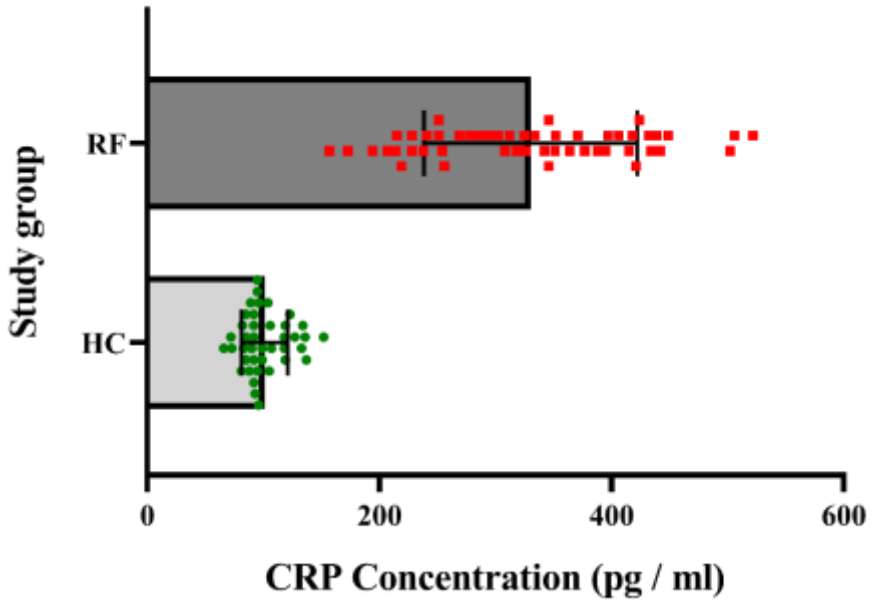

Figure (2): Levels of serum CRP among the study population, HC and RF

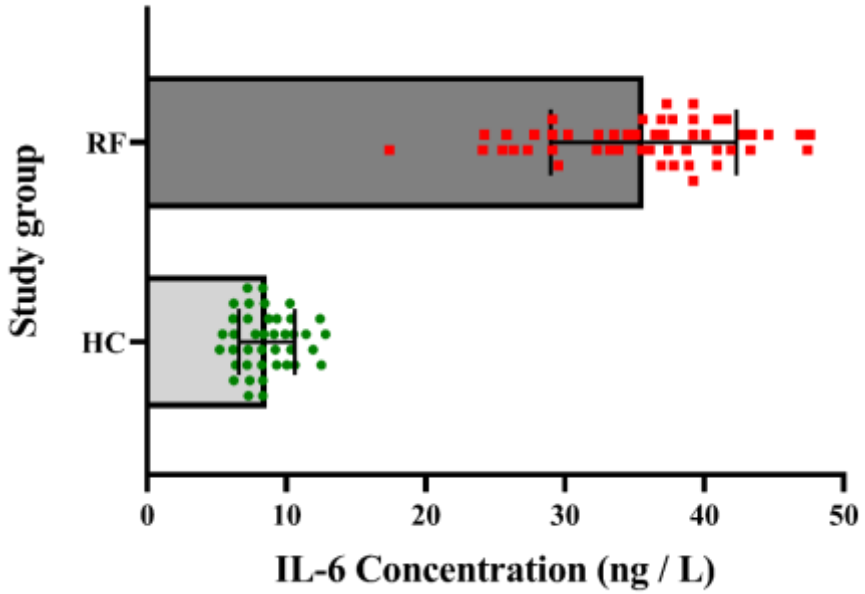

Figure (3): Levels of serum IL-6 among the study population, HC and RF

Relation to serum TNF-α, the findings of RA patients (173.16±4.22pg/ml) were significantly ($p<0.0001$; 95%CI: 675.5 to 898) higher than reported in HC individuals (49.33±2.39pg/ml), (Figure 4).

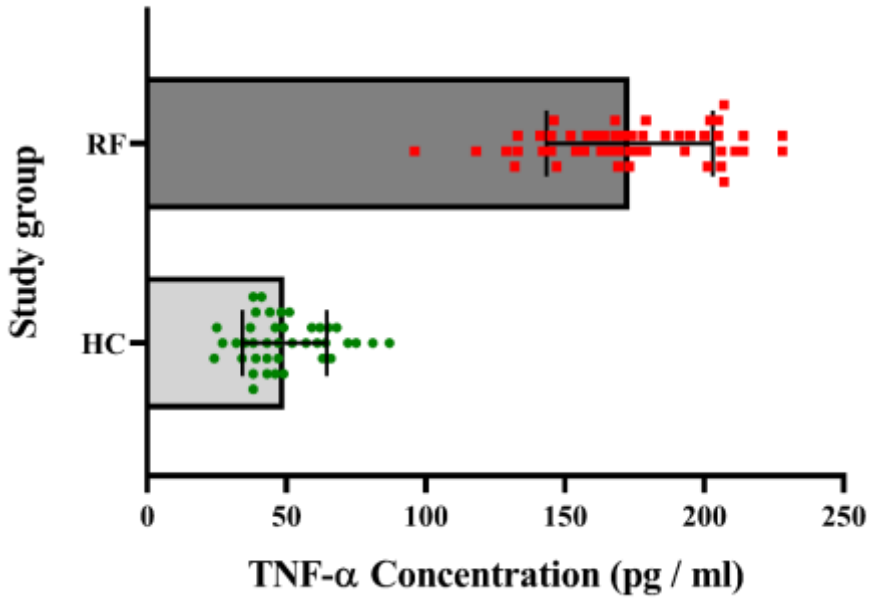

Figure (4): Levels of serum TNF-α among the study population, HC and RF

Discussion

Findings of RFLP-PCR revealed genetic polymorphism of *MTHFR* gene in RA patients through increasing the prevalence and risk of TG genotype and G allele. Comparatively, various studies have been conducted in RA patients and demonstrated the role of *MTHFR* gene polymorphisms in increasing of susceptibility (Al-Akhras and Al-Nahi, 2009), relation to methotrexate response and toxicity (Younis et al., 2022), outcome of methotrexate treatment and adverse drug reaction (Mutlak and Kasim, 2024a, b), and treatment response (Marjoon and Abdulkareem, 2025). Globally, one of the most comprehensive evaluations of the relationships between *MTHFR* polymorphisms and RA risk is meta-analysis study involved 2000 RA patients and 2000 controls; in which, the findings have been confirmed the significant association between gene polymorphism and increased susceptibility to RA under multiple genetic models including dominant, recessive and allelic comparisons (Bagheri-Hosseinabadi et al., 2020). Ravaei et al. (2023) showed that the genetic polymorphisms in the *MTHFR* gene were related with a response to anti-TNF-α therapy, with a potential significance to anti-TNF-α drug type. In a recent systematic review, Araszkiewicz et al. (2025) mentioned that polymorphisms in *MTHFR* gene have a broad spectrum of medical conditions such as oncology, metabolic disorders, neonatal complications, depressive and psychiatric disorders, and cardiovascular diseases.

Concerning the *PTPN22* gene, the findings of this study have shown a significant relevance of genetic polymorphism to RA patients as confirmed by various national (Abdul-Jabbar, 2021; Jasiem, 2022; Sanguor et al., 2026) and international (Tang et al.,2016; Mustelin et al., 2019; Schulz et al., 2020; Pasha et al., 2023) studies. In contrast, low number of reports found no marked association between *PTPN22* gene and RA (Hinks et al., 2006; Sahin et al., 2009; Taher and Nassir, 2024). However, Taher and Nassir (2024b) reported that *PTPN22* gene act as master regulation in autoimmune diseases and susceptibility to RA. Importantly, primary mechanism by which *PTPN22* polymorphisms contribute to RA is through

dysregulation of T-cell signaling which collectively promote chronic inflammation and autoimmunity (Brownlie and Salmond, 2024). In addition to T-cell effects, *PTPN22* polymorphisms influence B-cell receptor signaling to result in increasing production of autoantibodies such as RF leading to immune complex formation and joint damage (Chen et al., 2025).

For *STAT4* gene, our results reported a significant increase in prevalence and risk of GT genotype, with marled prevalence of G allele and risk of T allele. These findings were agreed those identified by various authors in Iraq (Abdalhussein and Zayed, 2025; Dawood et al., 2025; Kadhim et al., 2026) and other countries (Bravo-Villagra et al., 2025; De Araujo et al., 2025; Louahchi et al., 2025). One of the primary mechanisms by which *STAT4* gene polymorphisms contribute to RA is through enhancing differentiation of Th1 and Th17 cells, and the role of this gene as a key mediator of IL-12 and IL-23 signaling, with driving the development of these pro-inflammatory T-cell subsets (Buzzelli et al., 2023; Tuleuov et al., 2025; Deng et al., 2026). *STAT4* also influences innate immune responses by modulating the activity of macrophages and dendritic cells which increasing the antigen presentation, up-regulating of co-stimulatory molecules, and sustaining activation of inflammatory pathways (Wang et al., 2023; Sohrabi et al., 2024).

Among the RA patients, this study demonstrated a significant elevation in levels of serum RF, CRP, IL-6, and TNF-α as recorded by several studies locally (Abdulridha et al., 2024; Al-Yasiri, 2025; Fadhil et al., 2025) and globally (Rajaei et al., 2020; Alturaiki et al., 2022; Saleh et al., 2026). One of the principle pathogenic role of RF is the participation of this marker in immune complex formation and activation the complement system leading to recruitment of neutrophils and macrophages, releasing of lysosomal enzymes, production of ROS, increasing the production of inflammatory cytokines such as IL-6 and TNF-α, and amplification of inflammatory cascades (Wright et al., 2021; Łączna et al., 2022; Rodríguez-González et al., 2024). Additionally, the major mechanism responsible for CRP elevation in RA is IL-6-induced hepatic synthesis, activation of macrophages and synovial fibroblasts release IL-6 into circulation (Favalli, 2020; Alhilali et al., 2025). Synovial inflammation, joint destruction, disease activity and systemic symptoms correlate strongly with high levels of TNF-α (Poznyak et al., 2024). This suggests that the close-relationship between these biomarkers and disease progression can encourage the widespread use in diagnosis, monitoring therapeutic response and predicting clinical outcomes.

Conclusion

This study provides compelling evidence that genetic polymorphisms in key regulatory genes are significantly associated with susceptibility to RA, and are closely related to inflammatory responses that strongly supported by serological data that demonstrated the substantial increases in biomarkers reflects an amplified systemic inflammatory state and corroborates the functional impact of the identified genetic variants on immune activation and disease activity. Our results were not only enhanced the understanding of RA pathogenesis but also emphasize the potential utility of *MTHFR*, *PTPN22*, and *STAT4* polymorphisms as molecular markers for early diagnosis, risk stratification, and personalized therapeutic approaches. This suggests that integration of genetic and serologic techniques underscores the multifactorial nature of RA, where specific gene polymorphisms contribute to both disease susceptibility and magnitude of inflammatory responses.

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