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The Influence of TNFRSF1B, PADI4, and miRNA 499 Gene Polymorphisms on Susceptibility and Responsiveness to TNF Inhibitors in Patients with Rheumatoid Arthritis

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ABSTRACT

Background & Objective: Rheumatoid arthritis (RA) is a systemic autoimmune disease that causes joint deterioration. Over the past decade, the primary approach to treat RA has relied on biological medications. Despite confirming the effectiveness of this therapy, patients have shown significant diversity in their clinical responses to treatment. This variability can be attributed to various genetic polymorphisms that influence the response to biological drugs. This study was conducted to investigate whether TNFRSF1B (rs1061622), PADI4 (rs1748033), and miRNA 499 (rs3746444) gene polymorphisms are associated with susceptibility and responsiveness to TNF-α inhibitors in RA patients.

Materials & Methods: 100 RA patients (50 responders and 50 non-responders) and 100 apparently healthy subjects as the control group were studied. Genotyping of the polymorphisms was carried out using real-time polymerase chain reaction (PCR) with the TaqMan allelic discrimination assay.

Results: The frequency of TG (*P*0.039) and GG genotypes of TNFRSF1B (rs1061622) were higher in RA patients than in the control group. At the alleles level the mutant G allele was significantly more frequent among patients than control group (*P*=0.018). For PADI4 (rs1748033), the mutant C allele was more frequent among patients than controls (*P*=0.041). Sub-dividing of patients into responders and non-responders revealed that the mutant homozygous CC genotype of PADI4 (rs1748033) was significantly more frequent in non-responders than responders patients (*P*=0.046). AG genotype (*P*=0.016) and G allele (*P*=0.036) of miRNA 499a (rs3746444) were more frequent in non-responders than responders.

Conclusion: Variant genotypes of TNFRII (Rs1061622) and PADI4 (rs1748033), may be associated with an increased risk of RA while PADI4 (rs1748033) and miRNA-499a (rs3746444) polymorphism may be associated with non-response to infliximab.

Keywords: miRNA499, PADI4, Rheumatoid Arthritis, TNF inhibitors, TNFRSFIB

Introduction

Rheumatoid arthritis, an autoimmune inflammatory disease, displays first in synovial tissues. Afterwards, it is mediated by an interaction between T lymphocytes, B lymphocytes, and synovial fibroblasts, which leads to the dysregulation of the inflammatory cascade (1). RA patients suffer from major consequences, such as loss of function and work disability, which given its economic consequences, cause significant complications to society (2).

During the inflammatory response in RA, Tumor necrosis factor alpha (TNFa), as one of the prime proinflammatory cytokines, plays a crucial role in initiating and regulating the cytokine cascade. Binding to its 2 main receptors TNFRI and TNFRII, the biological effects of TNFa are mediated. Both TNFRI and TNF-RII are expressed in the synovial tissue and cartilage-pannus junction in patients with RA. The modulation of TNF-alpha activity in the joints of patients with RA leads to the local production of biologically active soluble TNFRs and their up-regulation (3). Numerous non-HLA genes have been recognized for their association with rheumatoid arthritis susceptibility. Among these, a significant focus has been on a particular class of genetic variants known as DNA polymorphisms (4). Through biological and molecular investigations, the TNF-RII gene has emerged as one of the most promising candidates among the genes associated with susceptibility to RA (5). Polymorphisms in the TNFR are also associated with responsiveness (6). The Peptidyl arginine deiminase type IV (PADI4) gene,

situated on chromosome 1, plays a vital role in catalyzing the conversion of the amino acid arginine to citrulline through post-translational deamination (7). In the context of rheumatoid arthritis, the presence of autoantibodies targeting citrullinated proteins (known as anti-cyclic citrullinated peptides) is highly specific and suggests the potential involvement of PADIs in the development of RA (8).

MicroRNAs (miRNAs) are a class of small endogenous non-coding single-stranded RNAs, approximately 22 nucleotides long, which play a crucial role in the post-transcriptional regulation of gene expression (9). Polymorphisms of miRNA impact the binding of microRNAs to their target messenger RNA (mRNA), resulting in translational repression or mRNA degradation (10). Such variations in miRNA-mRNA interactions have been implicated in the pathogenesis of RA. Hence, since gene polymorphisms associated with rheumatoid arthritis can influence common inflammatory pathways, they might also impact the therapeutic response to TNF inhibitors (TNFi). As far as we know, this is the first study on gene polymorphisms and anti-TNF alpha infliximab treatment response in rheumatoid arthritis patients in Iraq which holds a significant promise in advancing personalized medicine, improving treatment outcomes, reducing healthcare costs, and contributing to the overall understanding of RA management in the country.

Materials and Methods

A group of 100 patients with Rheumatoid Arthritis under the treatment with TNF alpha inhibitor (infliximab) after ≤ 6 months duration were enrolled in this case-control study.

Inclusion criteria: -

1. Patients with rheumatoid arthritis under treatment with TNF alpha inhibitors after ≤ 6 months duration.
2. Adults

Exclusion criteria: -

1. Early-diagnosed RA patients and
2. patients with other chronic or auto-immune diseases

They were collected from the unit of Rheumatology in Baghdad Teaching Hospital and Al-Yarmouk Teaching Hospital from January 2022 to September 2022. The diagnosis of each case was done by a rheumatologist confirmed by laboratory investigations. They were diagnosed according to the criteria of EULAR/ACR (2010) (11). The patients were subjected to a questionnaire about the name, age, weight, disease duration, smoking, and dose of biological drug. A control group consisting of 100 individuals who were matched for age and sex and appeared to be healthy was included in the study.

Clinical disease activity was assessed using CDAI. EULAR criteria were used to categorize patients into different clinical subgroups, based on their CDAI (12).

Treatment response evaluated after ≤ 6 months duration (CDAI < 10 = responders, CDAI >10 = non-responders)

In this study, all enrolled patients had initially undergone treatment with corticosteroids and non-biological DMARDs as part of standard therapeutic protocols for rheumatoid arthritis. However, due to the inadequate response observed with these conventional treatments, a critical therapeutic shift was implemented. Subsequently, patients were transitioned to receive biological drugs, specifically infliximab, which was chosen based on its mechanism of action and potential to address the underlying disease pathways that had proven refractory to previous therapies. This therapeutic transition was guided by the principle of optimizing patient outcomes by tailoring treatments to their specific response profiles, reflecting a clinical approach that prioritizes precision and efficacy.

Sample Collection

From patients and control groups 2 ml of blood sample was collected in an EDTA tube for whole blood DNA extraction.

Molecular Technique for Detection of TNFRSF1B, PADI4 and miRNA 499a SNPs

Single Nucleotides Polymorphism Genotyping

DNA was extracted from whole blood samples using a ready kit from Promega Company, USA, according to the manufacturer's instructions. The Quantus fluorometer was utilized to measure the concentration of extracted DNA, enabling the assessment of sample quality for subsequent downstream applications. This process helps ensure that the DNA samples are of sufficient quantity and purity for further analysis. The computer programs were used for primers/probes sequencing which were NCBI Blast, NCBI gene, and ApE. As shown in Table 1. Real-time PCR employs specific probes to identify and differentiate genotypes by their ability to bind to target DNA sequences with high precision.

Table 1. Primers sequence of TNFRSF1B, PADI4 and miRNA 499a

Gene	Primer/probe Name	Sequence 5`-3`
TNFRSF1B	rs1061622-F	GGACGTGGACGTGCAGA
	rs1061622-R	CCTCCTCCTCCAGCTGTAAC
	rs1061622-T	FAM-CTGCATCCATGCTTG

Gene	Primer/probe Name	Sequence 5`-3`
	rs1061622-G	HEX-TGCATCCCTGCTTG
PADI4	rs1748033-F	CTCACCAACCTCTCCTCTTACTTG
	rs1748033-R	ACAGCTCTGGTTGGCTTCACTT
	rs1748033-C	FAM-CAGAAATCTCCCTGTGC
	rs1748033-T	HEX-AGAAATCTCCTTGTGCGC
miRNA-499a	rs3746444-F	GGACGGGAAGCAGCACA
	rs3746444-R	CGGCTGTTAAGACTTGCAGTGAT
	rs3746444-C	Fam-ACTTGCTGTGAGGTTCAC
	rs3746444-A	HEX-TTGCTGTGATGTTCACG

Statistical Analysis

Utilizing SPSS software version 25.0 (SPSS Inc., Chicago, Ill., USA), statistical analyses were conducted. The categorical variables were presented as numbers and percentages, and their analysis and examination of genotype deviations from Hardy-Weinberg equilibrium (HWE) were conducted using the Chi-square test. Statistical significance was defined as p-value < 0.05.

Results

The mean age of the patients was 46.22±11.33 years which was nearly comparable with controls (45.1±11.95 years) with no significant difference. However, the categorization of the study population into age groups revealed that the age group (31-45 and 61-75 years) was more frequent among patients than controls with a significant difference. Also, the mean age for females was (46.19±11.52 years) while the mean age for males was (44.16±10.69 years). Sex frequency was comparable between the two groups with no significant difference.

Regarding the patient group, the mean number of infliximab doses and treatment duration was 4.76±1.23 doses and 4.64±1.69 months for responders and 4.48±1.54 doses and 4.68±1.21 months for non-responders as shown in Table 2.

Table 2. Characteristics of the study population

Variables	Patients (n=100)	Controls (n=100)	P-value
Age, years			
Mean ±SD	46.22±11.33	4٥.1±11.95	
Range	20-75	18-75	0.059
16-30	9(9%)	15(15%)	
31-45	42(42%)	31(31%)	
46-60	35(35%)	48(48%)	0.039
61-75	14(14%)	6(6%)	
Age, years **Mean ±SD** **Female-male**	46.19±11.52- 44.16±10.69	41.91±11.52- 46.06±11.88	0.296 0.095
Sex			
Male	25(25%)	31(31%)	0.345
Female	75(75%)	69(69%)	
Variables	**Responders (n=50)**	**Non-responders (N=50)**	***P-value***
Number of doses of infliximab			

Variables	Patients (n=100)	Controls (n=100)	P-value
Mean ±SD			
Range	4.76±1.23	4.48±1.54	0.746
	2.0-7.0	2.0-6.0	
Duration of treatment, months			
Mean ±SD	4.64±1.69	4.68±1.21	0.622
Range	3.0-6.0	2.0-6.0	

For TNFRII rs1061622 the frequency of the (TG and GG) genotypes were higher in patients compared to the control group. This polymorphism seems to act in a recessive mode of inheritance, as the combination of the heterozygous and mutant homozygous genotypes (TG+GG) was more frequent among patients compared to the control group. At the allele level, the mutant allele G allele was more frequent among patients than in the control group.

Regarding PADI4 rs1748033m the frequency of the (TC and CC) genotypes were higher in patients than in the control group (37% vs. 29%) and (12% vs. 7%), respectively. Although this polymorphism seems to act in recessive mode, the frequency of (TC+CC) genotypes was more common among patients than the control group (49% vs. 36%). At the allele level, the mutant allele C allele was more frequent among patients than the control group (30.5% vs. 21.5%) with a significant difference.

Regarding miR-499a-5p SNP (rs3746444) appeared in only 2 genotypes: wild-type homozygous AA, and wild-type heterozygous AG/GA in both patients and the control group. The frequency of the two genotypes and alleles were almost the same in patients and control groups and without a significant difference as shown in Table 3.

Table 3. The frequency of different genotypes and alleles of the polymorphisms rs1061622, rs1748033, and rs3746444 in patients and control group

rs1061622	patients (100)	Controls (100)	P-value	OR (95%CI)
Genotypes				
TT	48(48%)	62(62%)	0.067	1.0
TG	41(41%)	34(34%)	0.039	3.55(1.06-11.85)
GG	11(11%)	4(4%)	0.189	2.28(0.67-7.81)
HWE	0.618	0.466		
Dominant model				
TT+TG	89(89%)	96(96%)	0.071	1.0
GG	11(11%)	4(7%)		2.97(0.91-9.65)
Recessive model				
TT	48(48%)	62(62%)	0.047	1.0
TG+GG	52(52%)	38(38%)		1.77(1.0-3.1)
Alleles				
T	137(68.5%)	158(79%)	0.018	1.0
G	63(31.5%)	42 (21%)		1.73(1.1-2.72)
rs1748033	**Patients (100)**	**Controls (100)**	**P-value**	**OR (95%CI)**
Genotypes				
TT	51(51%)	64(64%)	0.156	1.0
TC	37(37%)	29(29%)	0.134	2.15(0.79-5.86)

rs1061622	patients (100)	Controls (100)	P-value	OR (95%CI)
CC	12(12%)	7(7%)	0.582	1.34(0.47-3.84)
HWE	0.203	0.159		
Dominant model				
TT+TC	88(88%)	93(93%)	0.223	1.0
CC	12(12%)	7(7%)		1.81(0.68-4.81)
Recessive model				
TT	51(51%)	64(64%)	0.064	1.0
TC+CC	49(49%)	36(36%)		1.71(0.97-3.1)
Alleles				
T	139(69.5%)	157(78.5%)	0.041	1.0
C	61(30.5%)	43 (21.5%)		1.6(1.02-2.52)
rs3746444	**patients (100)**	**Controls (100)**	***P*-value**	**OR (95%CI)**
Genotypes				
AA	60(60%)	59(59%)	0.885	1.0
AG	40(40%)	41(41%)		0.96 (0.55-1.89)
Alleles				
A	120(60%)	118(59%)	0.839	1.0
G	80(40%)	82(41%)		0.96(0.64-1.43)

HWE: Hardy Weinberg equilibrium

Association of Gene Polymorphisms with Early Clinical Responsiveness:

The distribution of genotypes and alleles for the TNFRII rs1061622 variant did not show any notable distinctions between non-responder and responder patients in this study. The homozygous mutant genotype (GG) was more prevalent among non-responder patients, but no statistically significant variations in genotype frequency were observed between the two groups under both dominant and recessive inheritance models.

Regarding PADI4 rs1748033, the distribution of (CC) genotype was more frequent in non-responder-responder patients (18% versus 4%) with a significant difference. As a risk for non-responsiveness, a single allele seems sufficient for this trait as the frequency of genotypes that involve the non-harmful allele (TT+TC) were more frequent among responders (96%) than non-responder patients (82%) with a significant difference (OR= 5.27, 95%CI= 1.08-25.78, p= 0.040). At the allele level, the mutant allele (C allele) was more frequent among non-responders than responders (35% vs. 24%).

For miR-499a-5p SNP (rs3746444) the distribution of both heterozygous mutant genotype (AG) and mutant allele (G) was more frequent in non-responder (52% and 26% respectively) than responder patients with a significant difference as shown in Table 4.

Table 4. The frequency of different genotypes and alleles of the polymorphism rs1061622, rs1748033, and rs3746444 in responder and non-responder patients

rs1061622	Responders (n=50)	Non-responders (n=50)	*P*-value	OR (95%CI)
Genotypes				
TT	25(50%)	23(46%)	0.302	1.0
TG	22(44%)	19(38%)	0.148	2.9(0.68-12.27)
GG	3(6%)	8(16%)	0.131	3.1(0.72-13.32)

rs1061622	Responders (n=50)	Non-responders (n=50)	*P*-value	OR (95%CI)
Dominant model				
TT+TG	47(94%)	42(84%)	0.123	1.0
GG	3(6%)	8(16%)		2.98(0.74-12.0)
Recessive model				
TT	25(50%)	23(46%)	0.689	1.0
TG+GG	25(50%)	27(54%)		1.17(0.53-2.57)
Alleles				
T	72(72%)	65(65%)	0.287	1.0
G	28(28%)	35(35%)		1.36(0.76-2.52)
rs1748033	**Responders (n=50)**	**Non-responders (n=50)**	***P*-value**	**OR (95%CI)**
Genotypes				
TT	28(56%)	24(48%)	0.122	1.0
TC	20(40%)	17(34%)	0.985	1.0(0.43-2.31)
CC	2(4%)	9(18%)	0.046	5.25(1.03-26.7)
Dominant model				
TT+TC	48(96%)	41(82%)	0.040	1.0
CC	2 (4%)	9(18%)		5.27(1.08-25.78)
Recessive model				
TT	28(56%)	24(48%)	0.424	1.0
TC+CC	22(44%)	26(52%)		1.34(0.63-3.03)
Alleles				
T	76(76%)	65(65%)		1.0
C	24(24%)	35(35%)	0.090	1.71(0.921-3.16)
rs3746444	**Responders (n=50)**	**Non-responders (n=50)**	***P*-value**	**OR (95%CI)**
Genotypes				
AA	36(72%)	24(48%)	0.016	1.0
AG	14(28%)	26(52%)		0.36 (0.16-0.82)
Alleles				
A	86(86%)	74(74%)	0.036	1.0
G	14(14%)	26(26%)		0.46(0.23-0.95)

Discussion

Through multiple genome screens, the genomic region 1p36 has been identified as a significant locus of interest in rheumatoid arthritis (RA). This region encompasses the TNFR2 gene, which encodes the TNFα receptor 2 (TNFR2). Given the essential role of TNFα in the pathophysiology of RA and the fact that the TNFR2 gene lies within this locus, it emerges as a prominent candidate gene for RA (13). In the TNFRSF1b gene, Polymorphisms can have different forms or versions, and they can potentially affect how genes function or influence the risk of certain diseases. Many studies have analyzed a SNP located in exon 6 (rs1061622, 676T.G). Our work showed that the TNFR2 (T/G, G/G) genotypes and G allele increased the risk of RA and this result suggests an association of the polymorphism with RA development, this disagreed with Canet, Filipescu (14) and Xie, Li (15) study which reported that TNFRSF1B+676 gene polymorphism was not associated with the genetic risk of RA (14, 15).

Also, regarding the inheritance model, the (TG+GG) in the recessive model was more frequent among patients than controls. This suggests that (TG+GG) genotypes may be associated with an increased risk of developing RA or may play a role in disease development. TNFR2 has been considered to be a candidate gene for RA because of its chromosomal location and functional significance. Positioned on chromosome 1p36, TNFR2 is a known susceptibility locus for rheumatoid arthritis (RA) **(16)**, the involvement of which in autoimmune disease was observed in animal models. The (G) allele has been linked to susceptibility to various autoimmune diseases, including RA. TNFR2 exhibits prevalent expression in myeloid-origin cells, particularly in stimulated T and B cells **(17)**, and possesses a higher affinity for TNF-α. Notably, TNFR2 is the primary TNF receptor present in T cells, and it plays a role in inducing apoptosis in CD8+ T cells **(18)**. So, TNFR2 plays a crucial role in regulating TNF-α signaling and immune responses. Changes in the amino acid sequence resulting from the rs1061622 SNP may affect the functional properties of TNFR2, including its ability to transmit signals and modulate immune responses upon TNF-α binding.

PADI4 is placed at the same locus of interest at status 1p36 as TNFR2. This locus includes the cluster that contains genes for peptidyl arginine deiminases (PADs) that catalyze the citrullination of arginine remainders. The Citrullinated epitopes induce the generation of RA-specific autoantibodies, containing anti-Sa, anti-filaggrin, and anti-cyclic citrullinated peptide (anti-CCP). The specificity of these autoantibodies for RA indicates that citrullination and PADs can play an important role in the pathophysiology. Therefore, PAD is the momentous candidate **(13)**. For this SNP the current results found that (TC, CC) genotypes and C allele were more frequent in RA patients than controls and may have a role in the susceptibility and disease development, although the frequency of (TC+CC) in the recessive model was more common among patients than controls so may be considered as a risk factor in disease development. Shaker, El Boghdady **(19)** provided evidence that the frequency of PADI4 rs1748033 (CC) genotype and C alleles are significantly higher in rheumatoid arthritis (RA) patients, indicating their association with increased risk in codominant and recessive models **(19)**. Other studies, such as Suzuki, Yamada **(20)**, Hashemi, Zakeri **(21)**, and studies on the Iranian and Korean populations, have also shown an increased risk of RA associated with the rs1748033 variant **(20-22)**. However, the association was not observed in studies conducted on the UK and German populations **(23)**. The divergent findings across these studies can be ascribed to the heterogeneous nature of populations and the impact of diverse environmental factors. These variations in genetic correlations underscore the intricate nature of the disease. The examined SNP might induce alterations in enzymatic activity concerning the PADI4 enzyme's role in the citrullination process, potentially influencing protein structure and function. The presence of the (C) allele could potentially correlate with elevated PADI4 enzyme activity, potentially resulting in excessive citrullination, which might perturb immune responses. Alternatively, heightened PADI4 activity linked to the (C) allele could lead to increased production of citrullinated proteins, possibly triggering immune responses and fostering autoantibody generation in individuals predisposed to rheumatoid arthritis. On the other hand, our work found that miR-499a-5p SNP (rs3746444) has no effect on susceptibility or disease development. Our study yielded concordant results with Yang, Zhang **(24)** demonstrating that the miR-499a-5p SNP (rs3746444) does not influence susceptibility or disease development in rheumatoid arthritis RA **(24)**. However, our findings differ from those of Hashemi, Eskandari-Nasab **(25)** who reported an association of hsa-mir-499 rs3746444 with an increased risk of RA in various inheritance models **(25)**. Additionally, another study indicated a significant association between miRNA-499 (rs3746444) polymorphism, particularly the C allele, and RA susceptibility **(26)**. Also, a Pakistani study revealed that this SNP is likely to have a role in the pathogenesis of RA in the Pakistani population **(27)**. The observed discrepancy in the results may be attributed to variations in the racial and ethnic backgrounds of the patients.

The main genetic effects on the response to anti-TNF treatment are likely to originate from variations in genes involved in the TNF-pathway. For rs1061622 the current work was shown a possible trend towards the GG genotype being associated with non-responsiveness to infliximab in which the individuals carrying the TNFRSF1Brs1061622G/G genotype and G allele had worse response to anti-TNF drugs and this agrees with Canet, Filipescu **(14)** study which found the same results. Also, Vasilopoulos, Bagiatis **(28)** mentioned that carriage of TNFRII G allele was associated with poorer response to drug treatment in anti-CCP positive patients (P=0.03), after 6 months of anti-TNF therapy **(28)**. Moreover, Fabris, Tolusso **(29)** reported a three-fold higher chance of responding to anti-TNF therapy in the TT group than in the TG/GG group using a cohort of 66 patients receiving infliximab (n=47) or etanercept (n=19) and lower degree of response to anti-TNF-α treatments in patients carrying the G allele **(29)**.

For PADI4 rs1748033 the present study revealed that the (CC) genotype was associated with an increased risk of non-responsiveness to infliximab in RA patients. Also, an individual that has a single allele of the mutant genotype (C) may be sufficient to confer a risk for non-responsiveness to infliximab and having at least one copy of the (T) allele may increase the likelihood of a positive treatment response. Interestingly, Bagheri-Hosseinabadi, Mirzaei **(30)** reported that anti-CCP and DAS28 were higher

significantly in (TT) carriers for rs1748033 polymorphism in an Iranian study (30). Additionally, this result showed that having at least one copy of the non-harmful allele (T) appears to be protective against non-responsiveness, with the TT+TC genotypes being more frequent among responder patients. These findings highlight the importance of this specific polymorphism in predicting the response to infliximab treatment in RA patients.

In this study, we observed that the presence of the AG genotype of rs3746444 might be linked to an elevated risk of non-responsiveness to infliximab among rheumatoid arthritis patients. Furthermore, the presence of the G allele could potentially heighten the likelihood of non-responsiveness to infliximab in RA patients. These findings strongly suggest that the miR-499a-5p SNP (rs3746444) exerts an influence on how RA patients respond to infliximab treatment. The increased occurrence of both the heterozygous mutant genotype (AG) and the mutant allele (G) in non-responder patients underscores a potential connection between this genetic variation and the reduced effectiveness of infliximab treatment.

Conclusion

This study explored the potential influence of genetic polymorphisms on the susceptibility and responsiveness to TNF-α inhibitors in patients with rheumatoid arthritis (RA). The investigated polymorphisms - TNFRSF1B (rs1061622), PADI4 (rs1748033), and miRNA 499 (rs3746444) - demonstrated associations with RA susceptibility and treatment response. The TNFRSF1B (rs1061622) polymorphism showed higher frequencies of specific genotypes and alleles among RA patients compared to the control group. Similarly, the PADI4 (rs1748033) polymorphism revealed an increased occurrence of the mutant allele among patients. Notably, within the RA patient cohort, the PADI4 (rs1748033) mutant homozygous CC genotype was more prevalent in non-responders, indicating a potential link between this genotype and poor response to TNF-α inhibitors. Moreover, the miRNA 499a (rs3746444) polymorphism exhibited an association with non-responsiveness to treatment, as the AG genotype and G allele were more frequent in non-responders compared to responders. These findings collectively underscore the importance of genetic factors in modulating both the susceptibility to RA and the responsiveness to TNF-α inhibitor therapy. Further studies are warranted to elucidate the underlying mechanisms and to validate these associations in larger and more diverse patient populations. Such insights could contribute to personalized treatment strategies for RA patients based on their genetic profiles. So, further studies with larger sample size to study the association between serum miRNA and susceptibility to RA is recommended. Also, further identification of genetic variations in patients with RA for personalized medicine is essential for disease course prediction and treatment strategy selection.

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Ethics approval

To ensure ethical standards were met, the study obtained ethical approval and informed consent from each participant in accordance with the guidelines outlined in the declaration of Helsinki. The Institutional Review Board (IRB) provided the necessary ethical agreement for the study in Al-Nahrain University-college of Medicine under the number 20211045 in 30/12/ 2021.

Conflict of Interest

No conflict of interest.

Author contribution

Zainab J. Fadhil (PhD in Microbiology): Help in project design, performing, doing the tests of the research, interpretation of the results, writing and manuscript preparation; Dr. Ahmed Abdul-Hassan Abbas (PhD in Immunology): Help in project design, reviewing the thesis, and interpretation of the results done under his supervision; Dr. Mohammad Hadi Al-Osami (FIBM “med”, FIBM” rheum”, CABM): facilitated patient recruitment and aided in the collection of samples.

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