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Association of Interleukin 10 gene promoter polymorphisms with Rheumatoid Arthritis susceptibility in Iraqi women population

Hiyam Raheem Atiyeh AL-Hasnawi [1], Amir Jalali [2*], Rana Fadhil Mousa [3]

[1] Department of Biology, Faculty of Sciences, Arak University, Arak, Iran. E-mail: hiyamalhasnawi@gmail.com

[2] *Corresponding author*, Department of Biology, Faculty of Sciences, Arak University, Arak, Iran. E-mail: a-jalali@araku.ac.ir

[3] Department of Biochemistry, College of Veterinary Medicine, University of Karbala, Karbala, Iraq. E-mail: rana.f@uokerbala.edu.iq

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ABSTRACT

Background and Aim: Rheumatoid arthritis (RA) is a chronic autoimmune disorder primarily affecting the joints. Interleukin-10 (IL-10) is an important immunoregulatory cytokine involved in regulating the immune response and inflammation. Polymorphism in the promoter region of the *IL-10* can affect the expression of the gene. This study aims to investigate the association of human *IL-10* rs1800896 (-1082 A/G), rs1800871 (-824 C/T), and rs1800872 (-597 C/A) promoter single nucleotide polymorphisms (SNPs) with susceptibility to RA in the Iraqi women population.

Methods: Here, 280 RA female patients who referred to the AL-Hussein Teaching Hospital in Karbala, Iraq between November 2022 to April 2023, and 120 healthy age-matched subjects were examined. The two groups were genotyped for rs1800896, rs1800871, and rs1800872 by tetra-primer ARMS–PCR. Statistical analyses were performed using IBM SPSS windows version 25

Results: All the genotypes did not deviate from Hardy-Weinberg Equilibrium (HWE) expectations in both groups. No positive association was found between allele and genotype frequencies of three SNPs and the incidence of rheumatoid arthritis (all p>0.05). Only the ACC haplotype showed an association with disease susceptibility (p-value=0.0004).

Conclusion: Based on the results, promoter SNPs of the *IL-10* gene cannot be used as a suitable marker to determine the susceptibility to rheumatoid arthritis in the Iraqi women population and to prevent its progression

Camera Ready

Introduction

Rheumatoid arthritis (RA) is a systemic auto-immune disease. RA is primarily caused by a person`s immune system mistakenly attacking the joints' synovium, causing chronic swelling, pain, and damage to the joints, which in severe cases can lead to permanent disability. The prevalence of the disease is 460 people per 100,000 people (Almutairi et al., 2021). The incidence in women is about two to five times higher than in men. Hormones may play a role in preventing or inducing these symptoms in both men and women. RA usually begins in women at the age of 30 to 60, and a little later in men. However, RA can develop at any age, even in young children (Goodson et al.,

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2002; Alamanos et al., 2006; Azer et al., 2015; Radu et al., 2021). RA damage can also affect the skin, lungs, eyes, heart, vascular system, and blood infections, and can be responsible for a quarter of deaths in people with rheumatoid arthritis. There is no definitive cure for RA, but there are various treatments such as anti-rheumatic drugs (DMARDs), glucocorticoids (GCs), and non-steroidal anti-inflammatory drugs (NSAID), aimed at relieving symptoms, lowering inflammation and pain, and preventing long-term joint damage (Conforti et al., 2021; Ding et al., 2023). Without proper treatment, a large number of affected individuals will be unable to work after 10 years of the disease.

Although the exact cause of rheumatoid arthritis is not fully understood, both genetic and environmental factors are believed to contribute to its development. Various studies have shown that the *PTPN22*, *TNFRSF14*, and *HLA-DRB1* genes are closely related to the development of RA (Perdigones et al., 2010; Abbasifard et al., 2020; Wysocki et al., 2020). It appears that RA susceptibility and outcome may be associated with specific alleles of HLA-DR, part of the human version of the major histocompatibility complex (MHC) (Auger et al., 1997; Weyand et al., 2000; Kerlan-Candon et al., 2001; Becart et al., 2021). Environmental factors such as dietary habits, personnel hygiene, and smoking, are the main factors in the development of RA. These factors can change the relative expression of proteins directly through the effect on the post-transcriptional modification of specific genes or indirectly through the effect on the epigenetic mechanisms of susceptibility genes (Klein et al., 2015; Smolen et al., 2018).

One of the characteristics of rheumatoid arthritis patients is the abundant expression of pro- and anti-inflammatory cytokines in serum and affected tissues (Kirkham et al., 2006; Paramalingam et al., 2007). Disturbance of the balance between pro-inflammatory and anti-inflammatory effects may contribute to the development of chronic RA. Interleukin-10 (IL-10) is a cytokine with anti-inflammatory and immunoregulatory properties. The human *IL-10* gene is located on chromosome 1q31–32 and encodes a 160 amino acid 18.5 KD molecule. IL-10 is a homodimer primarily synthesized by monocyte lineage in response to several activation stimuli. A key feature of IL-10 is the inhibition of proinflammatory cytokine and chemokine production by monocytes, and polymorphonuclear leukocytes (PMNs) (Spits et al., 1997; Moore et al., 2001). The increased levels of IL-10 have been reported in inflamed joints (Kirkham et al., 2006).

The *IL-10* gene has several important polymorphic sites, including single nucleotide polymorphisms (SNP) in its promoter region. In addition to endogenous and exogenous factors that stimulate cells to produce IL-10, SNPs in the promoter region can affect the transcription of IL-10 messenger RNA (Crawley et al., 1999; Kingo et al., 2005). The effect of three single nucleotide polymorphisms rs1800896 (-1082 A/G), rs1800872 (-597 C/A), and rs1800871 (-824 C/T) in putative regulatory regions of human *IL-10* gene promoter on gene expression and susceptibility to rheumatoid arthritis in some population has been studied. The rs1800896 is located within a presumed Ets transcription factor binding site, the rs1800872 exists in a putative STAT-3 binding site and a negative regulatory region and the rs1800871 lies inside a putative positive regulatory region (Kube et al., 1995). Some previous *in vitro* studies have shown that the ACC and ATA haplotypes are related to decreased levels of IL-10 production, whereas the GCC haplotype of peripheral blood cells with a single round nucleus is associated with abundant IL-10 output (Song et al., 2013; Salim et al., 2013; Palafox-Sánchez et al., 2014; Vázquez-Villamar et al., 2015).

Hee et al. (2007) reported that *IL-10* SNP rs1800896/1800871/1800872 ATA haplotypes were associated with lower IL-10 production and higher susceptibility to RA compared to other haplotypes in the Malaysian population. Alleles and genotypes with low IL-10 production in ARD patients may be involved in creating a pro-inflammatory environment that ultimately increases the risk of developing LPD. According to Hernández-Bello et al. (2017), such association was not observed in the Western Mexican population and *IL-10* (ATA, GCC, and ACC) haplotypes may not be a susceptibility marker for RA in this population. This study aimed to investigate the association between rs1800871, rs1800872, and rs1800896 polymorphisms in the human *IL-10* gene promoter region and susceptibility to rheumatoid arthritis in Iraqi women patients.

Materials and methods

Patients and controls

This case-control study was conducted using DNA samples of 120 normal healthy women with no history of RA and 280 RA patients who were referred to the AL-Hussein Teaching Hospital in Karbala, Iraq between November 2022 to April 2023. All patients met the 1987 American College of Rheumatology classification criteria for rheumatoid arthritis and both control and patient groups were matched for age (45-77 years), sex, and ethnicity. Based on self-reporting, patients with a history of the following medical conditions were excluded from the study as they may affect the results: diabetes, malignant diseases, overlapping connective tissue diseases, bone fractures, patients with vitamin D deficiency, thyroid and parathyroid disease, cardiovascular disease, renal and hepatobiliary diseases, chronic liver disease, other autoimmune diseases, pregnancy, and patients taking calcium and vitamin D supplements, bisphosphonates, diuretics. This study was approved by the Ethics Committee of the Arak University of Iran (IR.ARAKU.REC.1401.123). Informed written consent was directly obtained from all participants according to the 1975 Declaration of Helsinki principles and relevant local rules.

Laboratory assessments

The following laboratory parameters were measured in the collected sera of two groups: IL-10 (pg/ml), fasting blood glucose (FBG) (mg/dl), creatine kinase (U/L), total cholesterol (mg/dl), triglycerides (mg/dl), low-density lipoprotein cholesterol (LDLc) (mg/dl), high-density lipoprotein cholesterol (HDLc) (mg/dl), atherogenic coefficient (AC), atherogenic index of plasma (AIP) (Log(TG/HDLc)), and Glycated hemoglobin (HbA1c) (%). Triglyceride concentrations and total cholesterol in serum was measured quantitatively using an enzyme colorimetric assay. The Friedewald empirical relationship determined the serum LDLc. The HDLc was measured using a liquid high-density lipoprotein cholesterol precipitant. A Stanbio Kit (Texas, USA) used for measuring HbA1c. The level of interleukin 10 was measured using human ELISA Kit (Elabscience, USA).

DNA extraction and genotyping

Genomic DNA was extracted from the peripheral venous blood sample using the AddPrep Genomic DNA Extraction Kit (Add Bio, Korea) according to the manufacturer's protocol. Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) was performed for genotyping of -1082 A/G, -824 C/T, and -597 C/A SNPs in the promoter region of the human *IL-10* gene. The primer sequences used for SNP genotyping are represented in Table 1. Primer sequences used for rs1800871 were described previously by Perrey et al. (1999) and Primer 1 online software was used for designing sequence-specific primers for rs1800896 and rs1800872. PCR was carried out for rs1800896 and rs1800872 in a total volume of 25 µl with PCR PreMix (Promega-Madison/USA), approximately100 ng genomic DNA, 0.5 µM of each primer, and DNase-free water. For rs1800871, PCR was performed in a total of 10 µl volume containing one of the wild-type or mutant allele-specific primers, which separately mixed with 1 µL (10 mM) generic primer, 0.5 µL of forward and reverse 10 mM internal control primers (human growth hormone), 12.5 µL quick load Taq 2x master mix and 4.5 µL of sterile, DNase-free water. Approximately 5 µL of template DNA was added to the master mix before loading on the thermal cycler. Cycling was carried out in Biometra PCR thermal cycler (Analytik, Germany) with the following cycling profile for -1082 and -597 SNPs: an initial denaturation of 5 min at 94°C, followed by 35 cycles of 60 sec denaturation at 94°C, 45 sec annealing at 64°C (-1082 A/G) and 58°C (-592 A/C); 45 sec extension at 72°C; and final elongation at 72°C for 10 min. PCR amplification protocol for -824 was as follows: 95°C for 15 sec, 65°C for 50 sec, and 72°C for 50 sec with a final extension at 72°C for 5 min. PCR products were stained with ethidium bromide (Sigma, USA) and visualized by UV transilluminator after 2% agarose gel electrophoresis.

Table 1 - Primer sequences used for genotyping single nucleotide polymorphisms of *IL-10* gene promoter region (Reference sequence ID: X78437).

SNPs	Allele specific	Primer sequence (5′→3′)	Reference sequence location	Product size (bp)
rs1800896 (-1082 A/G)	Outer Forward	CTCCCAGTTACAGTCTAAACTGGAATG	7949-7975	433
	Outer Reverse	GGATTAAATTGGCCTTAGAGTTTCTTTT	8378-8351	
	Inner Forward G allele	AAGACAACACTACTAAGGCTTCTTTGGTAG	8188-8207	199
	Inner Reverse A allele	TTTCCTCTTACCTATCCCTACTTCCACT	8237-8213	292
rs1800871 (-824 C/T)	Generic primer (antisense)	AGGATGTGTTCCAGGCTCCT	8685-8666	
	Primer C allele (sense)	CCCTTGTACAGGTGATGTAAC	8453-8473	230
	Primer T allele (sense)	ACCCTTGTACAGGTGATGTAAT	8452-8472	230
rs1800872 (-597 C/A)	Outer Forward	CTCAGTTGGCACTGGTGTACCCTTGTAC	8432-8461	425
	Outer Reverse	TGGGATGAATACCCAAGACTTCTCCTTG	8858-8831	
	Inner Forward C allele	TAATGAAATCGGGGTAAAGGAGCCTAGC	8649-8673	211
	Inner Reverse A allele	GTACAGGCGGGGTCACAGGATGTATT	8699-8679	268

Statistical Analysis

All statistical analyses were performed using IBM SPSS Windows version 25 (SPSS, Chicago, IL, USA (2017). The Kolmogorov-Smirnov test was used as a numerical means to assess normality. Analysis of Variance (ANOVA) test was employed to assess differences in scale variables between diagnostic groups. Allele, genotype, and haplotype frequencies of *IL-10* gene promoter SNPs were compared between control and RA patient groups using the chi-square test. Statistical significance was considered significant at p-values less than 0.05. Odds ratios (OR) and 95% confidence intervals (CI) are calculated using the Odds Ratio Confidence Interval Calculator (http://www.medcalc.org/calc/odds_ratio.php) to evaluate the related risk posed by specific alleles, genotypes, and haplotypes.

Results

Patient characteristics

Demographics and clinical characteristics of RA patients and control groups are presented in Table 2. All individuals were females in both groups. Results showed no significant differences in age, Body Mass Index (BMI), and HDLc levels between patient and control groups. Significant increases in glucose, HbA1c, CK, TG, TC, VLDLc, LDLc, and atherogenic index (Chol/HDLc, LDLc/HDLc, Ath.Co., and AIP) were observed in the patient group compared with the control group. On the other hand, IL-10 showed a significant decrease in the patient group compared with the control group.

Table 2 - Demographic and clinical data of rheumatoid arthritis patients and healthy control.

Parameter	Control n=120	Patients n=280	p-value
Age (years)	62.50±10.481	61.14±9.720	0.533
BMI (kg/m^2)	28.712±5.449	29.877±4.723	0.283
Residency (Urban/Rural)	52/68	108/172	0.656 #
FBG (mg/dl)	96.078±12.579	116.028±17.909	<0.001
HbA1c (%)	5.548±0.711	6.703±1.253	<0.001
IL-10 (pg/ml)	29.575 (15.925-41.405)	13.650 (10.920-17.290)	<0.001*
CK (U/L)	29.000 (23.500-42.000)	39.070 (25.000-54.640)	0.037 *
TG (mg/dl)	107.771±20.052	154.001±37.461	<0.001
TC (mg/dl)	167.467±26.879	206.061±42.944	<0.001
HDLc (mg/dl)	45.032±7.990	45.119±13.787	0.974

VLDLc (mg/dl)	21.554±4.011	30.800±7.492	<0.001
LDLc (mg/dl)	102.881±26.477	130.142±36.377	<0.001
Chol/HDLc	3.745 (3.339-4.344)	4.607 (3.805-5.973)	<0.001*
LDLc/HDLc	2.144 (1.851-2.819)	2.986 (2.008-3.893)	0.002 *
Ath.Co	2.745 (2.339-3.344)	3.607 (2.805-4.973)	<0.001*
AIP	0.373 (0.279-0.484)	0.550 (0.447-0.624)	<0.001*

Chi-square test, *: Mann-Whitney U test for nonparametric variables expressed as median (25%-75%), BMI: body mass index, FBG: fasting blood glucose, HbA1c: Glycated hemoglobin, IL-10: Interleukin-10, CK: creatine kinase, TG: triglycerides, TC: total cholesterol, HDLc: high-density lipoprotein cholesterol, VLDLc: very-low density lipoprotein cholesterol, LDL: low-density lipoprotein cholesterol, Ath.Co: atherogenic coefficient ((Chol-HDLc)/HDLc), AIP: atherogenic index of plasma (Log(TG/HDLc)).

Allele, genotype, and haplotype frequencies

The expected genotype pattern for each SNP was generated by ARMS PCR. The 230 bp specific fragment for C and T alleles of the -824 C/T SNP is shown in Figure 1. Wild type genotype for rs1800896 (-1082 A/G) showed 433 and 292 bp amplicons while the heterozygote genotype displayed an additional band of 199 bp (Figure 2). The mutant allele (G) showed an expected genotyping pattern with 433 and 199 bp amplicons. For rs1800872 (-597 C/A), wild type genotype revealed 425 and 211 bp amplicons whereas the heterozygote genotype indicated an extra band of 268 bp (Figure 3). The mutant allele (A) showed a predictable genotyping pattern with 425 and 268 bp amplicons.

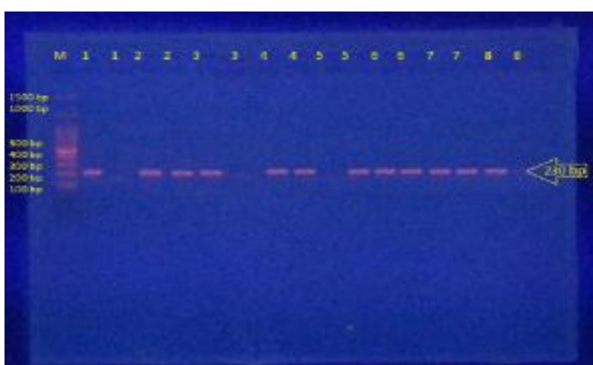

Fig. 1 - Amplicons for interleukin-10 -824 C/T single nucleotide polymorphism.

M: molecular ladder of 1500 bp; lanes 2, 6, and 7 represented heterozygote CT genotypes; lanes 1, 3, and 8 represented homozygous TT genotype, and lane 5 represented homozygous CC genotype.

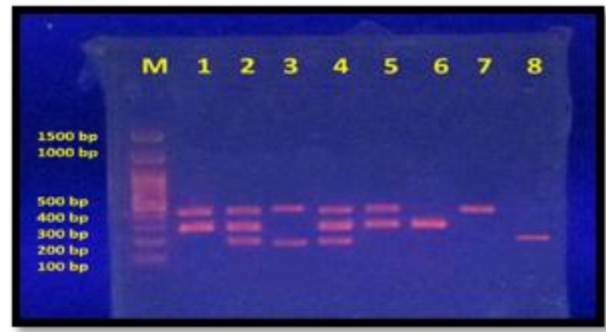

Fig. 2 - Tetra-primer amplification refractory mutation system-polymerase chain (ARMS-PCR) reaction for rs1800896 (-1082 A/G).

M: molecular ladder of 1500 bp; 2 and 4: heterozygote genotype; 1 and 5: A allele; 3: G allele; 6: inner reverse with outer forward with 292 bp; 7: outer forward and outer reverse primers with 433 bp; 8: inner forward with outer reverse primers with 199 bp.

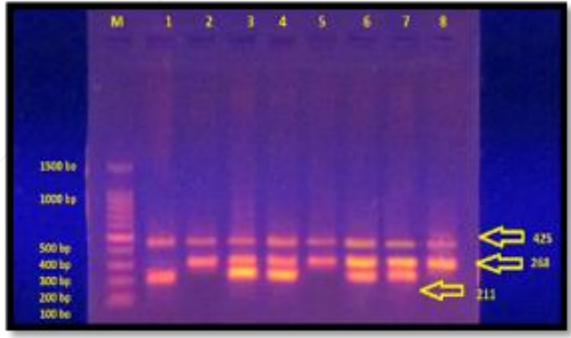

Fig. 3 - Tetra-primer amplification refractory mutation system-polymerase chain (ARMS-PCR) reaction for rs1800872 (-597 C/A).

M: molecular ladder of 1500 bp; 1: C allele; 2, 5, and 8: A allele; 3, 4, 6, and 7: heterozygote genotype.

Table 3 shows the genotype, allele, and haplotype frequencies of the *IL-10* promoter SNPs in RA patients and healthy controls. All the genotypes did not deviate from Hardy-Weinberg Equilibrium (HWE) expectations in both groups. No positive associations were found between allele and genotype frequencies and the risk of developing RA. GTA, ACC, and GCA were the three major haplotypes, and ATA was a minor haplotype in the patients. In the control group, ATA, GTA, and

ACC were the most frequent haplotypes and ATC was an infrequent haplotype. Only the ACC haplotype showed an association with disease susceptibility (p-value=0.0004). There is a significant reduction (p=0.002) in serum IL-10 of the -1082 AG genotype compared with AA and GG genotypes in the patient group. This difference is not present in the control group. Other blood biochemical parameters showed no significant differences between the three SNPs in patients or control groups.

Table 3 - Distribution of genotype, allele, and haplotype frequencies of the *IL-10* promoter single nucleotide polymorphisms in rheumatoid arthritis patients and healthy controls.

Variables		**RA Patients No. (%)**	**Controls No. (%)**	**OR (CI 95%)**	**p-value ***
Genotype frequency					
rs1800896 (-1082 A/G)					
AA		56 (20.00)	28 (23.33)	0.821 (0.294-2.299)	0.708
GA		108 (38.57)	40 (33.33)	1.256 (0.511-3.085)	0.619
GG		116 (41.43)	52 (43.33)	0.925 (0.390-2.196)	0.860
Recessive Model	AA+GA	164 (58.57)	68 (56.67)	1.081 (0.455-2.567)	0.860
	GG	166 (41.43)	52 (43.33)		
Dominant Model	AA	56 (20)	28 (23.33)	0.821 (0.294-2.299)	0.708
	AG+GG	224 (80)	92 (76.67)		
rs1800871 (-824 C/T)					
CC		64 (22.86)	24 (20.00)	1.185 (0.413-3.402)	0.752
CT		116 (41.43)	28 (23.33)	2.324 (0.881-6.134)	0.088
TT		100 (35.71)	68 (56.67)	0.546 (0.225-1.325)	0.181
Recessive Model	CC+TC	180 (64.29)	52 (43.33)	2.354 (0.984-5.630)	0.045
	TT	100 (35.71)	68 (56.67)		
Dominant Model	CC	64 (22.86)	24 (20.00)	1.185 (0.413-3.402)	0.752
	TC+TT	216 (77.14)	96 (80.00)		
rs1800872 (-597 C/A)					
CC		72 (25.71)	20 (16.67)	1.731 (0.576-5.198)	0.328
CA		124 (44.29)	44 (36.67)	1.373 (0.570-3.309)	0.480
AA		84 (30.00)	56 (46.67)	0.490 (0.203-1.182)	0.112
Recessive Model	CC+AC	196 (70.00)	64 (53.33)	2.042 (0.846-4.926)	0.112
	AA	84 (30.00)	56 (46.67)		
Dominant Model	CC	72 (25.71)	20 (16.67)	1.731 (0.576-5.198)	0.328
	AC+AA	208 (74.29)	100 (83.33)		
Allele frequency					
rs1800896					
A		220 (39.29)	96 (40.00)	1.237 (0.684-2.235)	0.482
G		340 (60.71)	144 (60.00)		
Minor allele frequency		0.392	0.400		
rs1800871					
C		244 (43.57)	76 (31.67)	1.666 (0.880-3.155)	0.117
T		316 (56.43)	164 (68.33)		
Minor allele frequency		0.435	0.316		
rs1800872					
C		268 (47.86)	84 (35.00)	1.705 (0.912-3.186)	0.048
A		292 (52.14)	156 (65.00)		
Minor allele frequency		0.478	0.350		
Haplotype frequency (-1082/-824/-597)					
GTA		18.87%	16.58%	2.25 (0.879-5.758)	0.084
ATA		9.51%	17.03%	0.575 (0.249-1.326)	0.19
ACC		17.47%	14.17%	16.091 (2.14-120.977)	0.0004
GCA		11.98%	10.86%	2.452 (0.687-8.751)	0.155
ACA		11.76%	11.52%	-	-
GTC		9.64%	12.61%	2.111 (0.584-7.636)	0.245
GCC		10.91%	9.44%	6.556 (0.842-51.036)	0.079
ATC		9.81%	7.77%	-	0.102

*: p-value was calculated by chi-square test and statistically significant at ≤ 0.05, RA: rheumatoid arthritis, OR: Odds ratio, CI: Confidence interval.

4. Discussion

The promoter region of the *IL-10* gene is highly polymorphic and has been associated with altered expression levels of the cytokine as well as susceptibility and severity of joint inflammation in different populations. However, the results of studies on the association between these SNPs and

susceptibility to RA onset and/or progression in different regions and ethnic groups are inconsistent, furthermore, there is a lack of reliable studies in this regard in Iraq. Therefore, this study aimed to investigate the association between different genotypes of rs1800871, rs1800872, and rs1800896 with susceptibility to RA in a population of Iraqi women.

The genotyping results showed there were no significant differences in allele, genotype, and haplotype frequencies between RA patients and healthy controls who participated in this study, suggesting that the three SNPs of the *IL-10* gene promoter did not appear to affect overall susceptibility to RA in Iraqi Arab women.

The results differ from those of Hassanzadeh et al. (2022), which was conducted in Iraq's neighboring country, Iran. They showed that SNP rs1800872 with mutant T allele increases the risk of RA in the population of southwestern Iran. A significant relationship between -1082 G/A, -824 C/T, and -1082 A/G alleles and genotypes with increased risk of RA in a Malaysian population was displayed by Hee et al. (2007). They observed a significantly lower IL-10 production in RA patients carrying the −824TT, −824CT, -597AA, and −597CA genotypes compared to the control subjects. In addition, a significantly more frequent -1082 G allele and GCC haplotype in RA patients compared with healthy controls in a Poland population was reported by Pawlik et al. (2005). In another study conducted in 2010 by Paradowska-Gorycka et al. (2010) on Polish rheumatoid arthritis patients, it was found that the frequency of the -1082GA genotype was more ($P = 0.009$), while the frequency of the -1082GG genotype was lower ($P = 0.0001$) in 244 RA patients compared to the control group ($N = 106$). The results of the study by Ge et al. (2015) on East Chinese Han patients suggest that the *IL-10* rs1800872 A/C allele might increase the risk of RA. The higher frequency of -592AA and -592CA genotypes, as well as the -592A allele compared to controls in Polish (Paradowska-Gorycka et al., 2010) and Chinese (Ying et al., 2011) patients with RA, suggesting the possible association of allele A with the lower expression of the IL-10 protein. Low production of IL-10 in patients carrying the -1082A allele and AA genotype was reported by Tayel et al. (2020). In this study, we also observed a significant decrease ($p=0.002$) in serum IL-10 of the -1082AG genotype compared to AA and GG genotypes in the patient group. A correlation between -1082 (G/A) genetic variants and RA susceptibility was shown by Martinez et al. (2003) in Spanish patients. According to de Paz et al. (2010) the frequency of the -1082AA genotype was lower in RA Spanish patients compared with the control group.

In contrast with these reports, Yucel et al. (2020) reported a possible protective role for the -1082 AA genotype against rheumatoid arthritis in the Turkish population. Furthermore, consistent with the results of this study, a few other reports did not observe an association between rs1800871, rs1800872, and rs1800896 with rheumatoid arthritis susceptibility. In the Caucasoid RA patients included in the Hajeer et al. (1998) study, *IL-10* gene promoter polymorphism does not appear to affect the overall RA susceptibility. In another study in India, Gambhir et al. (2010) showed that -1082G/A, and -592C/A, SNPs did not affect the susceptibility to rheumatoid arthritis.

The differences in the distribution of SNP genotypes in the *IL-10* promoter gene may account for ethnic differences in RA susceptibility and/or severity. However, due to the lack of studies on the association of the *IL-10* promoter gene polymorphisms and susceptibility to RA in populations of Arab ethnicity, it is not possible to compare the Iraqi group with others.

Conclusions

In general, we have demonstrated that the *IL-10* promoter SNPs at -1082, −824, and −597 are not significantly associated with RA patients. However, this study had several limitations that should be considered. The main limitation is the small sample size which could influence the results by random error. Besides, complete information on possible risk factors for patients was not available. Furthermore, the results of this study do not cover the entire Iraqi population. Therefore, to use these polymorphisms as a marker to determine the susceptibility to rheumatoid arthritis in the Iraqi women population, it is necessary to examine larger samples.

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