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Interleukin-20 Gene Polymorphism and Serum Levels of Interleukin-20 and Interleukin-23 in Rheumatoid Arthritis

Iman Najat Ali [1], Najat Jabbar Ahmed Berwary [1]

[1] Department of Medical Laboratory Technology, Erbil Health and Medical Technical College, Erbil Polytechnic University, Erbil, Iraq.

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Correspondence: Iman Najat Ali
Email iman.ali@epu.edu.iq

Website: https://djm.uodiyala.edu.iq/index.php/djm

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Abstract

Background: Rheumatoid arthritis (RA) is an autoimmune disease marked by complex causes and ongoing joint inflammation. Recently, more efforts have been directed at finding new non-invasive prognostic biomarkers for RA, which can help in disease monitoring.

Objectives: To investigate the circulatory levels of IL-20 and IL-23 in RA patients relative to healthy volunteers and to evaluate their correlation with disease activity. Additionally, to explore the association between IL-20 genetic polymorphism and RA susceptibility.

Patients and Methods: The study comprised a total of 85 individuals with RA and 45 healthy subjects. IL-20 and IL-23 concentrations were measured using ELISA, and disease activity was assessed using the DAS-28 score. The IL-20 rs2981573 polymorphism was genotyped using Amplification Refractory Mutation System-Polymerase Chain Reaction.

Results: The study found that RA participants exhibited significantly elevated serum IL-23 levels compared to controls (P = 0.0347), while IL-20 levels did not differ (P = 0.6354). Correlations between IL-20 and IL-23 with DAS-28 were significant (P = 0.0021 and P = 0.0030, respectively). Regarding the IL-20 gene polymorphism, no significant association was found between the rs2981573 gene polymorphism and RA susceptibility

Conclusion: This study demonstrated that IL-23 levels were significantly higher in RA patients and may have diagnostic value. Although IL-20 levels were not significantly different between groups, both cytokines showed a positive correlation with disease activity and could be valuable markers. Furthermore, the IL-20 gene polymorphism showed no association with rheumatoid arthritis susceptibility.

Keywords: IL-20 Genetic polymorphism, DAS-28, rheumatoid arthritis, IL-20, IL-23.

Introduction

Rheumatoid arthritis (RA) is an autoimmune condition that leads to chronic joint inflammation, progressive joint damage, impairment, and reduced quality of life. The pathogenesis of RA is complicated, including a delicate interplay of environment and genes that eventually result in the dysregulation of the immune system (1). Cytokines, which are small proteins that mediate various cellular functions, have been recognized as significant factors in the development of RA (2).

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Interleukin-20 is a cytokine within the IL-10 family that mediates complex immune and inflammatory responses (3). It binds to the IL-20RA or IL-20RB ligand, triggering intracellular signaling pathways. Primarily produced by activated macrophages and neutrophils infiltrating the synovial tissue (4). IL-20 induces the production of fibroblasts in the synovium, the proliferation of pro-inflammatory cytokines that amplify inflammation, and the recruitment of additional immune cells to the affected tissue, further exacerbating the condition (5). IL-23 also plays a role in the inflammatory response associated with RA. This cytokine consists of two subunits forming a heterodimer, primarily induced through active macrophages and dendritic cells (6, 7). IL-23 stimulates and maintains T helper 17 cells, which induce proinflammatory cytokine IL-17, crucial for RA pathogenesis (7). Additionally, IL-23 can directly stimulate other immunocytes, for example, neutrophils and macrophages, further contributing to the inflammatory process in RA (8). Furthermore, there is a growing trend toward identifying new prognostic biomarkers for RA that could aid in monitoring disease improvement or guiding decisions regarding subsequent treatment. A 2010 study by Lindstrom and Robinson. Found that cytokine biomarkers in RA are more accurate in determining disease activity outcomes than other biomarkers, such as autoantibodies and acute-phase reactants, which may be less reliable (9). High levels of both interleukin-20 and interleukin-23 have been observed within the joint fluid and serum of RA individuals, suggesting they are likely laboratory markers for disease activity and progression (5, 10).

The genes that encode IL-20 are in a designated area on chromosome 1 (11). Previous studies have revealed correlations between specific polymorphisms in the IL-10 group as well as an elevated risk for the development of numerous autoimmune, infectious, and malignant disorders (12, 13). The results presented thus far suggest that IL-20 is a key gene in the understanding of RA. But no research has looked at the function of IL-20 gene polymorphisms in RA till now. The objective of this study was to investigate the association of the IL-20 rs2981573 polymorphism with RA susceptibility, to assess IL-20 and IL-23 serum levels in RA patients and healthy controls, and to elucidate their correlation with disease activity.

Patients and Methods

Study design: This case-control investigation was performed on 45 healthy controls and 85 patients confirmed to have RA using the classification criteria of 2010 set by the ACR/EULAR (14). RA patients were taken from the private clinics and inpatient units of the Rheumatology departments at Rizgary Teaching Hospital and Hawler Teaching Hospital between September 2024 and December 2024. The criteria for excluding patients encompassed individuals under 18 years old, those who were pregnant, and those with any malignancy or other autoimmune diseases. For RA patients and healthy controls, demographic data were collected, including age, sex, and body mass index (BMI). The healthy volunteers ' sex, age, and BMI matched those of RA patients who participated in the research. In RA patients, additional clinical information, such as disease duration and disease activity score (DAS28), was documented.

Data collection and laboratory investigations: After obtaining informed consent, blood was taken from every individual. A sample of blood was deposited in EDTA-coated tubes for genetic investigation. The residual blood samples were subsequently gathered in plain tubes and separated at 3000 rpm for fifteen minutes to get serum, and then frozen at -20°C for future investigations. The disease activity in patients was assessed using the Disease Activity Score (DAS28), calculated using

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the C-reactive protein (CRP) serum concentration. All RA participants underwent comprehensive medical history taking, detailed physical examination, and laboratory tests such as CRP, Anti–Cyclic Citrullinated Peptide (Anti-CCP), rheumatoid factor (RF), Complete Blood Count (CBC), and Erythrocyte Sedimentation Rate (ESR). Serum CRP levels were assessed using the Cobas c 111 (Roche Diagnostics, Germany), Anti-CCP antibodies were quantified using the Alegria® system (ORGENTEC Diagnostika, Germany), and RF levels were determined using the Cobas 6000 analyzer (Roche Diagnostics, Germany). CBC parameters were assessed using the Convergys® X3 NG hematology analyzer (Roche Diagnostics, Switzerland). Platelet-to-Lymphocyte Ratio (PLR) and Neutrophil-to-Lymphocyte Ratio (NLR) were calculated manually. And ESR was determined using the Westergren method. Both IL-20 and IL-23 concentrations were assessed in RA participants and controls using ELISA kits from Sun Long Biotech Co., LTD. IL-20 (catalogue number: SL1907HU) and IL-23 (catalogue number: SL0989Hu) followed the same sandwich ELISA procedure.

DNA extraction: DNA has been isolated from the samples of patients and healthy individuals using the Blood DNA Preparation-Solution Kit (Jenabioscience/Germany), following the manufacturer's instructions. DNA concentrations were measured by Nanodrop. The absorbance ratio at 260 nm to 280 nm was employed to check the purity of the extracted DNA.

Genotyping polymorphism by ARMS-PCR: Genotyping of the IL-20 rs2981573 polymorphism was done for all participants using the Amplification Refractory Mutation System–Polymerase Chain Reaction (ARMS-PCR) technique as designated by Kingo and colleagues (15). The PCR process was performed in a reaction containing 25 microliters, consisting of 12.5 µL of master mix (Go TaqGreen Master Mix/ Promega/ USA), 1 µL of each primer (Table 1), 3 microliters of the DNA, and 5.5 microliters of free DNase water. The cycling conditions included a starting denaturation phase at 95°C for five minutes, then ten cycles of 95 degrees Celsius for thirty seconds, 60°C in thirty seconds, and 72°C for 1 minute. This was succeeded by 30 cycles at 58°C in 30 seconds each, concluding with 72°C for 1 minute. A finalizing extension phase at 72°C in 10 minutes was incorporated. PCR amplicons were viewed, and their sizes were assessed using 2% agarose gel electrophoresis.

Statistical Analysis

The data was examined with the help of GraphPad Prism 8.0.2. Frequencies and percentages were used to represent categorical data, whereas means and medians with ranges were used to represent quantitative data. The Shapiro-Wilk and D'Agostino tests were performed to determine normality. The results showed that IL-20, IL-23, Anti-CCP, RF, CRP, ESR, Hb, PLR, NLR, neutrophil count, and lymphocyte count were not normally distributed, whereas DAS28 and platelet count were normally distributed. Mann-Whitney U-test performed for comparison. For comparisons involving categories, Fisher's exact and chi-square tests were utilized. For correlations, Spearman's correlation is used. When the p-value was less than 0.05, all tests were deemed to have statistical significance. $p < 0.05$ (*): statistically significant, $p < 0.01$ (**): highly significant, $p < 0.001$ (***): very highly significant, $p < 0.0001$ (****): extremely significant, $p \geq 0.05$ (NS): not significant

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Table 1. Primers for amplifying polymorphism.

Genetic polymorphism	Primer sequence	Amplicon size
IL-20 1380 A/G rs2981573	Forward inner primer, (A allele), CCTCTCCTAGCTGATGATGAACTGAA	181 base pairs A allele
	Reverse inner primer, (G allele), CTCTTTCAGACCTCACATTTGGAATAAC	255 base pairs G allele
	Forward outer primer, TCTGAATAGGACCTAGGAATTCAATTCTTT	382 base pairs control

Results

Basic characteristics of study participants:

The age distribution among RA patients and controls was comparable, with the RA cohort averaging 48.92 ± 10.69 years and the control group averaging 47.67 ± 8.555 years, showing no differences ($p = 0.4989$). The percentage of female participants in the RA and control groups was 89.41% and 91.11%, respectively. The body mass index was recorded for each participant, with a mean Body Mass Index (BMI) of 29.85 ± 5.756 kg/m² in rheumatoid arthritis patients and 31.03 ± 4.613 kg/m² in the controls ($p = 0.0782$). The mean duration of disease in patients with rheumatoid arthritis was 8.61 years. Rheumatoid arthritis patients had markedly elevated CRP ($p = 0.0039$), ESR ($p < 0.0001$), and neutrophil counts levels ($p = 0.0009$), and reduced hemoglobin levels ($p < 0.0001$) than healthy subjects. The basic characteristics and lab parameters of the study cohorts are delineated in Tables 2 and 3.

Comparing serum IL-20 and IL-23 levels in RA patients with healthy controls: The healthy group exhibited a mean IL-20 serum level of 80.45 ± 15.11 pg/ml, whereas the RA individuals presented a mean of 97.22 ± 97.07 pg/ml ($p = 0.6354$), indicating a non-significant difference. The two groups demonstrated significant distinction in IL-23 levels ($p = 0.0347$). The mean serum IL-23 level in those with RA was 61.70 ± 51.39 pg/ml, compared to 49.39 ± 10.34 pg/ml in the healthy group. Table 4 summarizes the comparison of serum IL-20 and IL-23 levels between the patient and control subjects.

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Table 2. Demographic and Clinical Data of Patient and Control Group**.**

Variables Mean ± SD (range), n (%)	RA patients (n=85)	Healthy subjects (n=45)	p-value
Age/year	48.92 ± 10.69 (24-74)	47.67 ± 8.555 (30-65)	0.4989 NS
Gender			>0.9999 NS
Female	76 (89.41%)	41 (91.11%)	
Male	9 (10.59%)	4 (8.89%)	
BMI (kg/m²)	29.85 ± 5.756 (18.90-44.80)	31.03 ± 4.613 (19.90-42)	0.0782 NS
Disease duration/Year	8.61 ± 7.81 (0.08 -30)	N/A	
Tender Joints Count	14.98 ± 9.838 (0-28)	N/A	
Swollen Joints Count	2.294 ± 2.979 (0-12)	N/A	
DAS-28	4.651 ± 1.477 (1.19-7.41)	N/A	
Family history		N/A	
No	42 (49.4%)		
Yes	43 (50.6%)		
Hypertension		N/A	
No	64 (75.29%)		
Yes	21 (24.71%)		
Smoking		N/A	
No	77 (90.59%)		
Yes	8 (9.41%)		
Medications		N/A	
csDMARDs	76 (89.4%)		
Biologic agents	37 (43.5%)		
Corticosteroids	58 (68.2%)		
NSAIDs	23 (27.1%)		

Rheumatoid arthritis (RA); body mass index (BMI); disease activity score (DAS28); conventional synthetic disease-modifying anti-rheumatic drugs (csDMARD); nonsteroidal anti-inflammatory drugs (NSAID); SD: standard deviation; not applicable (N/A). A p-value exceeding 0.05 suggests non-significance (NS); $p < 0.05$ is considered significant.

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Table 3. Laboratory Characteristics of Patient and Healthy Subjects.

Variables Mean ± SD (range)	RA patients n=85	Controls n=45	p-value
CRP (mg/L)	12.71 ± 28.38 (0.20-244.5)	3.936 ± 3.549 (0.18-16)	0.0039**
ESR (mm/1h)	31.62 ± 21.33 (2-111)	17.09 ± 10.79 (1-43)	<0.0001****
RF (IU/ml)	119.5 ± 248.8 (0.1-1314)	N/A	
Anti-CCP (U/ml)	248.6 ± 345.7 (0.1-1000)	N/A	
Hb (g/dl)	12.41 ± 1.657 (7-16.30)	13.64 ± 1.368 (9.5-18.20)	<0.0001****
Lymphocytes (10^9/L)	2.209 ± 0.8042 (0.60 - 4.790)	2.009 ± 0.6254 (0.44 - 3.580)	0.2455 NS
Neutrophils (10^9/L)	5.096 ± 2.073 (1.4 - 13)	3.981 ± 1.114 (2.29 - 7.17)	0.0009***
Platelets (10^9/L)	278.2 ± 79.51 (115 - 471)	241.6 ± 49.01 (131 - 361)	0.0057**
NLR	2.632 ± 1.593 (0.5833 - 9.5)	2.204 ± 1.137 (1.081 - 7.886)	0.1985 NS
PLR	148.4 ± 93.06 (55.11 - 555)	132.8 ± 53.69 (62.57 - 361.4)	0.8378 NS

Rheumatoid factor (RF), hemoglobin (Hb), platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), anti-cyclic citrullinated peptide (Anti-CCP), and DAS28: disease activity score, not applicable (N/A). A p-value exceeding 0.05 suggests non-significance (NS); $p < 0.05$ is considered significant.

Table 4. Comparison of IL-20 and IL-23 between the control and study groups.

Variables	mean ± SD median (ranges)	mean ± SD median (ranges)	p-value
	Study (n=85)	Control (n=45)	
IL-20 (pg/ml)	97.22 ± 97.07 80.09 (23.08-900)	80.45 ± 15.11 80.71 (54.26-120.4)	0.6354 NS
IL-23 (pg/ml)	61.70 ± 51.39 52.63 (40.89-500)	49.39 ± 10.34 49.20 (24.09-63.13)	0.0347*

SD: standard deviation; NS: non-significant; $p < 0.05$ considered significant.

Correlation of IL-20 and IL-23 with clinical and laboratory variables: A positive relationship between DAS-28 and IL-20 and IL-23 was found (p = 0.0021, p = 0.0030, respectively), the number of tender joints (p = 0.0061, p = 0.0126, respectively), and the number of swollen joints (p = 0.0238, p = 0.0401, respectively). However, hemoglobin and IL-23 levels were inversely correlated (p = 0.0462). Furthermore, other parameters did not correlate with both IL-20 and IL-23 (Table 5).

Correlation between NLR and PLR with RA disease activity: Platelet-to-Lymphocyte Ratio (PLR) revealed a statistically significant correlation with DAS-28 (r = 0.2158, p = 0.0473), while Neutrophil-to-Lymphocyte Ratio (NLR) did not (r = 0.1751, p = 0.1089) (Figure 1).

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Table 5. Spearman's correlation between serum concentrations of IL-20 and IL-23 and the clinical and laboratory variables in RA participants.

Variables	IL-20 level r	p-value	IL-23 level r	p-value
Age	-0.08295	0.4504 NS	0.05875	0.5933 NS
Disease duration(years)	0.0589	0.5923 NS	-0.04735	0.6669 NS
Tender Joints Count	0.2951	**0.0061****	0.2696	**0.0126***
Swollen Joints Count	0.2450	**0.0238***	0.2231	**0.0401***
CRP (mg/L)	0.1460	0.1825 NS	0.08871	0.4195 NS
ESR (mm/1h)	0.08111	0.4606 NS	0.09735	0.3754 NS
Hb (g/dl)	0.04512	0.6818 NS	-0.2169	**0.0462***
Lymphocytes (10^9/L)	-0.03380	0.7588 NS	0.02525	0.8186 NS
Neutrophils (10^9/L)	-0.1535	0.1606 NS	-0.07112	0.5178 NS
Platelets (10^9/L)	0.1293	0.2382 NS	0.1619	0.1387 NS
NLR	-0.09102	0.4074 NS	-0.08072	0.4627 NS
PLR	0.1067	0.3311 NS	0.08512	0.4386 NS
RF level (IU/ml)	0.02295	0.8349 NS	0.1755	0.1082 NS
Anti-CCP level (U/ml)	0.09672	0.3785 NS	0.03235	0.7688 NS
DAS28-CRP	0.3290	**0.0021****	0.3182	**0.0030** **

C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), hemoglobin (Hb), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), rheumatoid factor (RF), anti-cyclic citrullinated peptide (Anti-CCP), DAS28: disease activity scorer, and r: correlation. Not significant (NS), and $p < 0.05$ is considered significant, and biochemical predictors of bone health metrics**.**

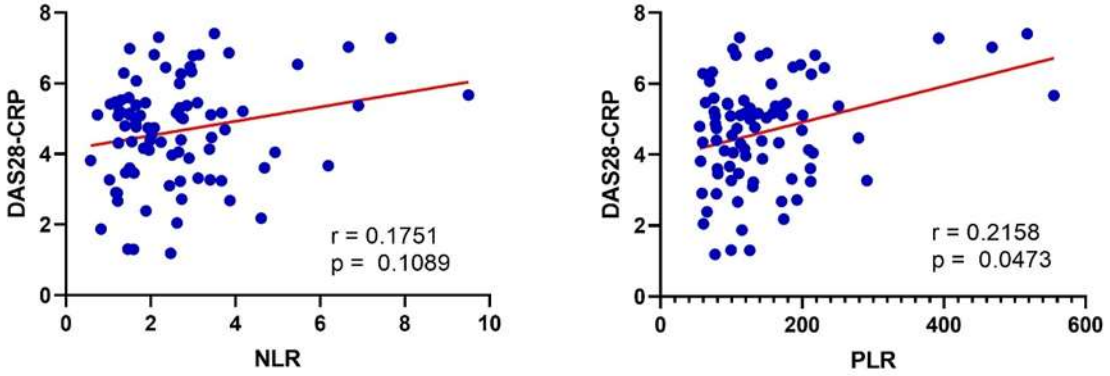

Figure 1. Correlation of NLR and PLR with DAS28.

Genotypic distribution and allele frequency of IL20 rs2981573 polymorphisms in the RA patients and control groups: The genotype distribution of the IL-20 rs2981573 SNP among the control group (n=45) was evaluated for Hardy-Weinberg equilibrium (HWE). The analysis showed a significant deviation from HWE ($\chi^2 = 45.37$, $p < 0.0001$), which may reflect a small sample size, population substructure, or the nature of the studied variant.

The AA genotype exhibited the highest prevalence in both patient and control groups, observed in 94.1% of RA patients and 95.6% of the healthy group. The AG genotype was detected in 1.2% of RA patients, while it was absent in the healthy group. The GG genotype was found in 4.7% of RA patients and 4.4% of the control group. The study could not identify a statistically significant correlation between the IL-20 rs2981573 polymorphism and susceptibility to RA ($p > 0.9999$). Similarly, allele frequency analysis

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revealed no significant differences, with the A allele accounting for 94.7% in RA patients and 95.6% in control group and the G allele representing 5.3% in RA patients and 4.4% in controls (p = 0.7649) as in (Table 6).

Figures 2 and 3 illustrate the agarose gel electrophoresis results for IL-20 1380 A/G (rs2981573) polymorphism analysis. The gel images confirm the successful amplification of the target 181 bp (A allele) and 255 bp (G allele) fragments, along with the 382 bp internal control band.

Receiver operating characteristic (ROC) curve analysis: The ROC analysis for IL-20 revealed a non-significant discriminative value. A serum IL-20 level >79.99 pg/ml resulted in an AUC of 0.5255 (95% CI: 0.4272–0.6238; p = 0.6333), with a sensitivity of 50.59% and a specificity of 48.89%. In contrast, the analysis of the ROC curve revealed that a serum IL-23 level more than 49.27 pg/ml can significantly differentiate rheumatoid arthritis patients from healthy subjects (AUC=0.613; 95% CI: 0.5064–0.7189; p=0.0349), demonstrating a 51.11% specificity and 65.88% sensitivity (Figure 4).

Table 6. Genotypic and allelic frequencies of IL20 rs2981573 in rheumatoid arthritis patients and controls.

Genotype/Allele	Cases (n=85) N (%)	Control (n=45) N (%)	OR	CI (95%)	p-value
Genotype					
AA	80 (94.1%)	43 (95.6%)	Ref	-	-
AG	1 (1.2%)	0 (0%)	-	-	-
GG	4 (4.7%)	2 (4.4%)	0.93	(0.1716 - 4.130)	>0.9999
Allele					
A	161 (94.7%)	86 (95.6%)	Ref	-	-
G	9 (5.3%)	4 (4.4%)	0.83	(0.2767 - 2.729)	0.7649
Confidence interval (CI); odds ratio (OR); A p-value that is lower than 0.05 is considered to be statistically significant.					

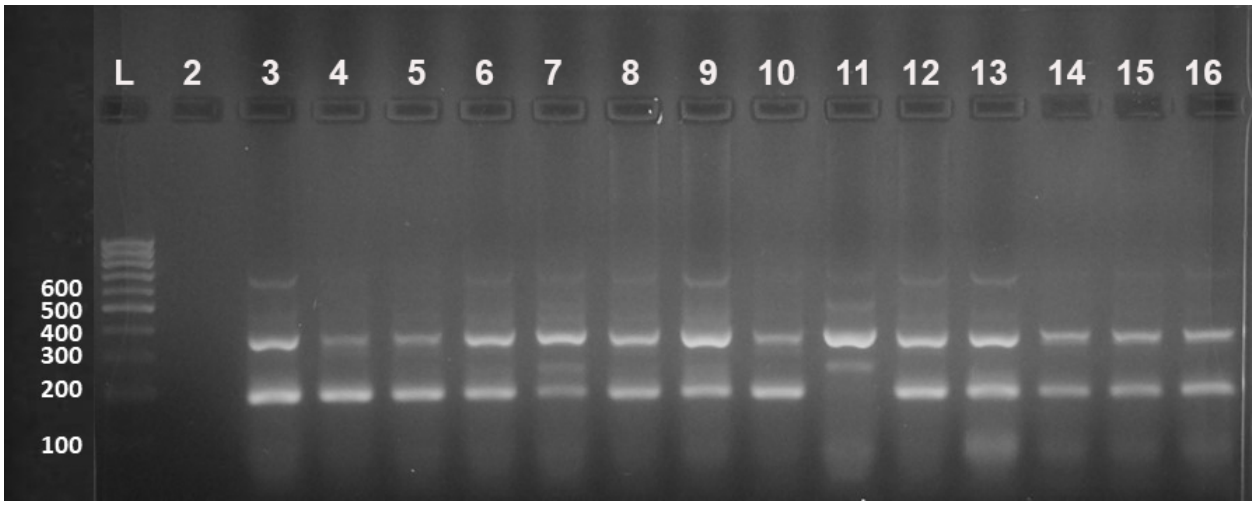

Figure 2. Agarose gel electrophoresis showing IL-20 1380 A/G rs2981573: Lane 1: Ladder of 100bp. Lane 2: Negative control. The 382bp is an internal positive control. Lane 7: Amplified both alleles A/G (181/255) bp, Lane 11: Amplified allele G 255bp. The remaining samples: Allele A target amplification (181bp).

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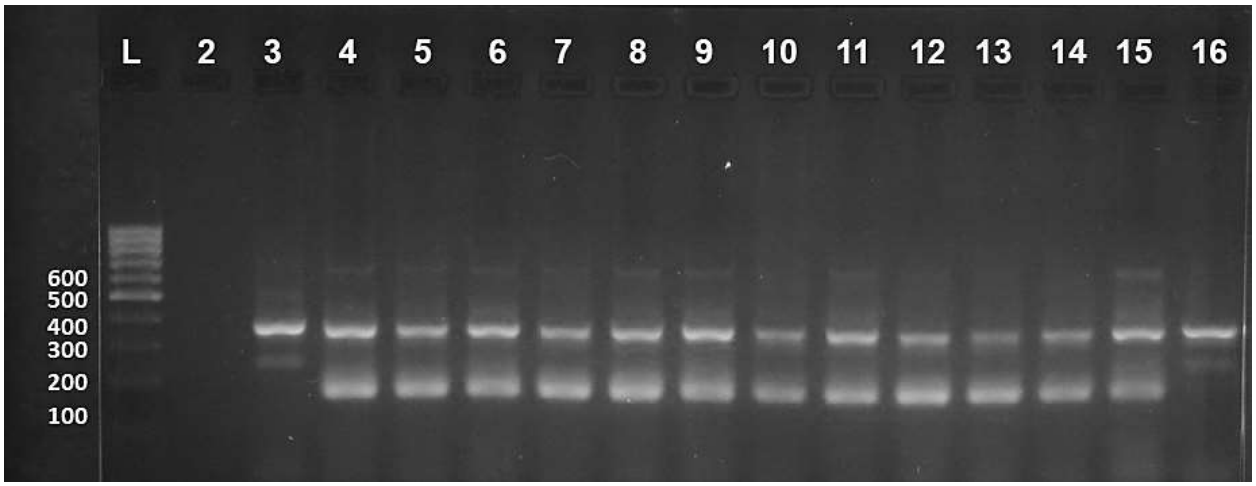

Figure 3. Agarose gel electrophoresed showing IL-20 1380 A/G rs2981573: Lane 1: Ladder of 100bp. Lane 2: Negative control. The 382bp is an internal positive control. Lanes 3 and 16 show the amplified allele G (255) bp. The remaining samples: Allele A target amplification (181bp).

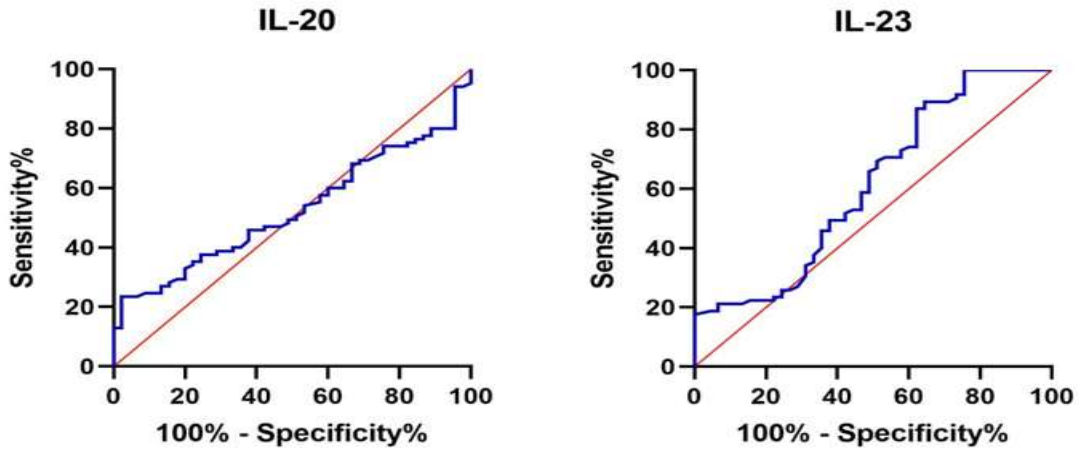

Figure 4. ROC curve of IL-20 and IL-23 levels.

Discussion

This study investigated the levels of IL-20 and IL-23 in rheumatoid arthritis participants, their association with clinical and laboratory assessments, and analyzed the genotypic distribution of the IL-20 rs2981573 polymorphism. Recent study aids in identifying potential indications of disease activity and offers significant insights into the inflammatory profile of rheumatoid arthritis patients. The age, sex distribution, and Body Mass Index (BMI) of the RA and control groups were analogous, with no statistically significant differences detected. As anticipated, RA patients demonstrated markedly elevated levels of C - C-reactive protein (CRP), Erythrocyte Sedimentation Rate (ESR), and neutrophil counts, in addition to reduced hemoglobin (Hb) levels relative to controls. These findings correspond with prior research demonstrating that systemic inflammation and anemia are prevalent characteristics of rheumatoid arthritis resulting from persistent inflammation and bone marrow suppression (16). Serum IL-20 concentrations were not significantly higher in RA patients compared to healthy individuals, according to this study's results. This finding aligns with studies reporting that IL-20 was not increased in the serum of RA patients, suggesting a localized rather than systemic role in RA inflammation (5, 17). In contrast, IL-20 serum levels were found to be

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considerably higher in RA patients compared to control groups in multiple investigations (18-20). Kragstrup et al. (19) specifically observed that IL-20 was elevated in early RA but normalized after six months of treatment. Despite these differences, IL-20's function in neutrophil chemotaxis, endothelial cell proliferation, and synovial fibroblast migration is still crucial to its role in the pathophysiology of RA (17). The present investigation found that serum IL-23 levels were considerably greater in RA patients compared to healthy individuals. This observation aligns with previous research documenting increased IL-23 concentrations in individuals with RA compared to non-affected individuals, highlighting its significance as a key proinflammatory cytokine in disease pathogenesis (21, 22). The growth and proliferation of Th17 cells, which generate IL-17 and other inflammatory mediators that promote chronic inflammation, joint degradation, and the progression of illness, are greatly aided by the cytokine IL-23 (7). The observed elevation of IL-23 in this study reinforces its involvement in the dysregulated immune response characteristic of RA. It validates its prospective utility as an indicator for disease identification and progression.

This study found a favorable relationship between serum IL-20 and IL-23 and disease activity indices such as the Disease Activity Score (DAS28), tender joint count (TJC), and swollen joint count (SJC). Therefore, it can be inferred that elevated levels of these cytokines are linked to more active disease in RA patients. Consistent with the current findings, Hussien et al. (20) identified a high association between blood IL-20 levels and DAS28, SJC, TJC, ESR, and CRP. Similarly, Šenolt et al. (5) demonstrated robust associations between IL-20 levels in the blood and TJC, SJC, CRP, and DAS-28 in RA subjects, thus confirming IL-20's involvement in disease progression. In contrast, Kragstrup et al. (23) documented no significant correlation in IL-20 serum levels with disease activity. The findings of this study support previous research on IL-23, which has shown a strong association between serum IL-23 levels and disease activity. The fact that IL-23 promotes Th17 cell development and the generation of inflammatory cytokines lends credence to the concept that it is crucial in the pathophysiology of RA (21, 22). However, Zaky and El-Nahrery's investigation indicated an insignificant association between IL-23 and DAS28. (10). The disparities in findings between research may be attributed to variances in patient demographics, sample sizes, and other participant factors. The inverse relationship between concentration of IL-23 and hemoglobin levels demonstrated by this study suggests a possible contributory role for IL-23 in the development of anemia related with RA, which may in turn be facilitated by chronic inflammatory pathways that impair erythropoiesis. While Alsheikh et al. (22) did not documented association between hemoglobin and IL-23. This study also investigated the relationship between Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR) and DAS28 in patients with RA. The analysis showed a statistically significant positive relationship between PLR and DAS-28 scores, indicating its potential as an accessible and cost-effective biomarker for evaluating disease activity in RA. NLR, on the other hand, showed no significant correlation. This is in line with the results from Du et al. (24), who also reported a significant relationship between PLR and disease activity score 28 (DAS28), while the association between NLR and DAS28 was not significant. Similarly, Gökmen et al. (25) discovered that NLR was not associated with DAS-28. In contrast, several other studies reported that both NLR & PLR correlated with DAS-28 (16, 26). Taken together, these findings suggest that PLR is

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likely a reliable and informative biomarker in the context of RA. In contrast, the utility of NLR may be more heterogeneous, related to the specific patient cohort, disease duration, and type of treatment (in particular immunotherapy). Meng et al. (26) documented that NLR and PLR were considerably lowered in RA patients after therapy. The use of NLR as an indicator of disease activity may be limited due to methotrexate-induced neutropenia and thrombocytopenia in their patient population (27).

According to the literature review, this is the first study investigating the IL-20 rs2981573 polymorphism in rheumatoid arthritis, which will provide further insights into the potential involvement of IL-20 in the disease's susceptibility. Another way of looking at this is that there were no differences in genotypic distribution. Similarly, the A allele was the most prevalent in both groups, and no statistically significant differences were observed in allele frequency analysis between the control group and RA patients. Although in this study population, there was no relationship established between this genetic variant and RA susceptibility, IL-20 is known to contribute to synovial inflammation, immune cell recruitment, and joint damage (4). This suggests that genetic variations in IL-20 may still influence RA pathogenesis. Since this is the first study examining rs2981573 in RA, direct comparisons with previous research are not available. This emphasizes the novelty of these findings and the need for further investigations in broader and more varied communities to elucidate the potential role of IL20 polymorphisms in RA. In the present study, the ROC curve analysis demonstrated that IL-23 levels above 49.27 pg/ml could distinguish RA patients from controls. Although the AUC value for IL-23 was statistically significant, its diagnostic accuracy (AUC = 0.613) was relatively weak, indicating limited clinical usefulness when used alone as a diagnostic marker, its combination with other inflammatory markers could enhance diagnostic accuracy. A post hoc power analysis indicated that the study may have been underpowered to detect small differences in IL-20 levels due to the sample size, which could explain the non-significant findings for this cytokine. Future studies with larger and more balanced sample sizes are recommended to validate these results.

Conclusion

This study demonstrated that serum IL-23 levels were significantly elevated in RA patients compared to healthy controls, and ROC analysis revealed a modest diagnostic utility for IL-23. While no significant difference was found in IL-20 levels between the groups, correlation analysis revealed positive associations between both IL-20 and IL-23 with DAS-28, tender joint count, and swollen joint count. Notably, IL-23 levels showed an inverse correlation with hemoglobin levels. These findings suggest that IL-23 may play a role in the pathogenesis of RA and could be a useful marker of disease activity. Regarding the IL-20 gene polymorphism, no statistically significant differences in genotype and allele distribution were observed between RA patients and healthy controls, suggesting that this polymorphism is not associated with increased susceptibility to RA. Further research is recommended to examine IL-20 and IL-23 concentrations in the synovial fluid, as well as to evaluate other relevant cytokines. Additional research with larger sample sizes and more thorough analyses is needed to clarify the roles of IL-20 and IL-23 in rheumatoid arthritis.

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في مصل مرضى التهاب المفاصل الروماتويدي IL-23و IL-20 وتركيز IL-20 تعدد أشكال جين

[1] إيمان نجاة علي ،[1] نجاة جبار أحمد برواري

الملخص

الخلفية: : التهاب المفاصل الروماتويدي (**RA**) هو حالة مناعية ذاتية تتميز بتعقيد في المسببات والتهاب مزمن في المفاصل. ومؤخرًا، ظهرت توجهات جديدة تهدف إلى تحديد مؤشرات حيوية تنبؤية غير جراحية يمكن أن تساعد في مراقبة المرض.

الأهداف: التحقيق في مستويات **IL-20** و**IL-23** في الدم لدى مرضى **RA** مقارنة بالأشخاص الأصحاء، وتقييم ارتباطها بنشاط المرض. بالإضافة إلى ذلك، دراسة العلاقة بين تعدد الأشكال الجيني لـ **IL-20** وقابلية الإصابة بـ **RA.**

المواد والطرق: شملت الدراسة ٨٥ مريضًا يعانون من RA و٤٥ شخصًا سليمًا. تم قياس تراكيز IL-20 وIL-23 باستخدام تقنية ELISA، وتم تقييم نشاط المرض باستخدام مقياس DAS-28. كما تم تحديد النمط الجيني لتعدد الأشكال rs2981573 في جين IL-20 باستخدام تقنية ARMS-PCR.

النتائج: أظهرت الدراسة أن المشاركين المصابين بـ **RA** لديهم ارتفاع ملحوظ في مستويات **IL-23** في الدم مقارنةً بالأصحاء (القيمة الاحتمالية **p = 0.0347**)، في حين لم تكن مستويات **IL-20** مختلفة بشكل معنوي (**p = 0.6354**). كما وُجد ارتباط معنوي بين كل من **IL-20** و**IL-23** مع مقياس **DAS-28** (**p = 0.0021** و**p = 0.0030** على التوالي). أما بالنسبة لتعدد الأشكال الجيني لجين **IL-20**، فلم يكن هناك ارتباط معنوي بين تعدد الأشكال **rs2981573** وقابلية الإصابة بـ **RA**.

الاستنتاج: على الرغم من أن تعدد الأشكال الجيني لـ IL-20 قد لا يكون عاملًا مسببًا للإصابة بـ RA، فإن كل من IL-20 وIL-23 قد يكونان مؤشرين مهمين لنشاط المرض.

الكلمات المفتاحية: تعدد الأشكال الجيني لـ **IL-20**، **DAS-28**، التهاب المفاصل الروماتويدي، **IL-20**، **IL-23**.

المؤلف المراسل: إيمان نجاة علي

الايميل: iman.ali@epu.edu.iq

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[1]قسم تكنولوجيا المختبرات الطبية، كلية أربيل الصحية والتقنية الطبية، جامعة أربيل التقنية، أربيل، العراق.