ORIGINAL STUDY

Janus Kinase 2 and Protein Tyrosine Phosphatase Receptor Type C mRNA Expression Levels in Ankylosing Spondylitis and Systemic Lupus Erythematosus Patients

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Abstract

Objectives: **To evaluate RNA expression levels of Janus kinase 2 (*JAK 2)* and protein tyrosine phosphatase receptor type C *(PTPRC)* genes in Systemic lupus erythematosus (SLE) and Ankylosing spondylitis (AS) and correlate them with the severity of the two diseases.**

Background: **SLE is an autoimmune disorder with a wide range of clinical symptoms. AS is an autoimmune inflammatory disease of young adults, affecting the axial skeleton and articular joints.**

Methods: **A case−control study included 96 participants: (32 SLE patients, 32 AS patients, and 32 healthy control participants). Differences in the expression levels of *JAK2* and *PTPRC* in the blood of AS and SLE patients were tested using quantitative real-time PCR (qRT-PCR).**

Results: **Expression levels of *JAK2* mRNA increased significantly ($P < 0.001$), and the expression levels of *PTPRC* mRNA decreased significantly ($P < 0.001$) in AS and SLE patients as compared with healthy participants. *JAK2* was positively correlated in SLE with systemic lupus erythematosus disease activity index (SLEDAI) ($r = 0.517$, $P = 0.002$), and in AS with Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) ($r = 0.378$, $P = 0.033$). PTPRC was negatively correlated in SLE with SLEDAI ($r = -0.621$, $P < 0.001$) and in AS with BASDAI ($r = -0.459$, $P = 0.008$).**

Conclusions: **Altered expression levels of *JAK2* and *PTPRC* genes in both AS and SLE suggest their potential role in disease pathogenesis. Also, their correlation with the severity of the two diseases provides a promising molecular diagnostic tool for them.**

Keywords: Ankylosing spondylitis, Janus kinase 2, Protein tyrosine phosphatase receptor type C, Systemic lupus erythematosus

1. Introduction

Ankylosing spondylitis (AS) and systemic lupus erythematosus (SLE) are two different autoimmune diseases with distinct etiologic and pathogenic patterns [1]. SLE is an immunological disorder with a female predominance of unclear cause characterized by dysregulated interferon response and loss of cellular antigens' self-tolerance causing damage to many organs in the body with alternating activity and remission [2]. The production of antinuclear antibodies (ANA) is the most prominent immunologic abnormality of this disease [3]. Clinical symptoms of SLE are variable ranging from mild arthritis and skin affection to life-threatening kidney, blood, or brain and spinal cord involvement. Due to clinical heterogeneity and the lack of pathognomonic features or tests, SLE

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diagnosis is considered a difficult challenge [3]. SLE diagnosis depends generally on clinical manifestations and laboratory data. The possibility of SLE is suggested by various serologic findings including some antibodies like anti-Smith (anti-Sm) and anti-double-stranded deoxyribonucleic acid (anti-dsDNA) [4]. Several studies have shown that SLE susceptibility is associated with a strong genetic predisposition [5]. One member of the protein tyrosine phosphatase (PTP) family, named CD45, is encoded by the protein tyrosine phosphatase receptor type C *(PTPRC)* gene. It has an important role in the regulation of the signaling of antigen receptors in B and T cells, causing negative regulation of cytokine receptor signaling, and inhibition of Janus kinases. One of the members of the Janus kinase (*JAK*) family is the *JAK2* gene, which has a pivotal role in immune cell differentiation, proliferation, and survival. *JAK1, JAK3*, and *Tyk2* are further members of this family that make up non-receptor protein tyrosine kinases (PTKs). Also, PTKs are the most crucial component of the JAK/STAT signaling pathway and dysregulation is thought to be involved in SLE progression [6]. Ankylosing spondylitis is the one type of seronegative spondyloarthritis (SpA) that affects young adults and involves the articular joints and the axial skeleton. The inflammatory process also affects tendons, cartilaginous tissue, and peripheral joints and lasts for many years before causing inevitable damage. Also, uveitis and psoriasis are examples of extra-articular manifestations that could occur in this disease [7]. Although the precise pathophysiology of AS is unknown, inflammation is thought to be the first stage of the pathogenic process, which then advances to new bone development, which causes erosion of the cartilage, local osteitis, bone degradation, and ultimately ankylosis. Plain radiography can reveal sacroiliitis and syndesmophytes (radiographic AS), but magnetic resonance imaging (MRI) can find these conditions earlier. The best biomarkers for diagnosing AS are still human leukocyte antigen (HLAB-27) and C-reactive protein (CRP), which measure the activity of the disease, assess therapy effectiveness, and track structural development. There may be additional genetic abnormalities that contribute to the pathogenesis of AS, as HLAB-27 only accounts for around 30% of the genetic causes of this disease [8]. Finding novel biomarkers that could serve as prognostic or diagnostic indicators for AS are needed urgently. The identification of such biomarkers will probably be crucial for the diagnosis, treatment, and management of this illness. The mRNA expression profiling has been used in previous studies to know molecular pathways and new biomarkers linked to cancer, stroke, and diabetes [9]. As shown by knockout mice for certain *JAKs, JAK*-mediated signaling transduction is related to surface receptors for a variety of cytokines and is crucial for bone formation and metabolism. Small medicines that target *JAK*, such as tofacitinib, have been utilized to treat AS as the condition is marked by severe inflammation and abnormal osteoblastic differentiation. In this context, clinical trials are currently evaluating the idea of targeting *JAK* in AS. Because of this, cytokine-mediated *JAK* stimulation is essential for osteoblast activation, differentiation, and function; inhibiting *JAK* signaling may therefore improve the ankylosis microenvironment [10]. Each pair of *JAK* molecules is responsible for the regulation of distinct biological processes. *JAK1*, *JAK2*, and/or *TYK* regulate important cytokines such as IFN-c and IL-6. *JAK2* and *TYK2* control the signaling of IL-23 and IL-12; these cytokines play an important role in the CD4þ, Th1, and Th17 cell differentiation [11]. Finally, downstream signaling of erythropoietin and thrombopoietin is regulated by *JAK2* homodimers, so they are believed to play an important role in erythropoiesis, and regulating myelopoiesis. Also, the pathogenesis of SpA is linked by the granulocyte-macrophage colony-stimulating factor (GM-CSF), which is downstream signaled by *JAK2* homodimers [12].

This study aimed to evaluate RNA expression levels of Janus kinase 2 (*JAK* 2) and protein tyrosine phosphatase receptor type C (*PTPRC*) genes in SLE and AS and correlate them with the severity of the two diseases.

2. Methods

2.1. Study design and patient groups

This present hospital-based case–control study was conducted on 96 participant separated into three groups. Group I included 32 Egyptian SLE patients, and Group II with 32 Egyptian AS patients These two groups were recruited from the rheumatology and clinical immunology outpatient clinic, the outpatient clinic of the Rheumatology, Physical Medicine, and Rehabilitation Department, in collaboration with the Biochemistry and Molecular Biology Department, between January 2021 and July 2021 with Group III of 32 healthy persons matched in age and sex, considered as a control group. The study has been conducted after ethical approval from the institute's ethics and research committee [under code No: 2/2021BIO22]. Informed consent has been obtained from all the patients to be included in the study. SLE patients were fulfilling

four or more criteria of the updated American College of Rheumatology (ACR) criteria [13]. The patients of AS were diagnosed using the modified New York Criteria 1985 [14].

2.2. Exclusion criteria

Patients with various autoimmune illnesses, active infection, lymphoproliferative disorders, cancer, myocardial infarction, heart failure, ischemic injury, and thrombus formation, as well as patients with diabetic nephropathy, were not included in this study.

2.3. Clinical assessment

Patients underwent a thorough clinical examination, a full history review, and the collection of demographic information. For AS patients, BASDAI with CRP (mg/l) and ESR (mm/h) were used to assess disease activity [15]. Bath AS metrology index (BASMI) and Bath AS functional index (BASFI) were used to assess mobility and functional limitations [16,17]. In the SLE group of patients, the disease activity was assessed by SLEDAI [18]; active SLE patients have an SLEDAI score greater than or equal to 10 and inactive SLE patients have an SLEDAI score less than 10.

2.3.1. Laboratory investigations

A measure of 6 ml of blood samples were taken under strict aseptic conditions from each person using clean venipuncture. Each sample was divided into three tubes as follows: Tube 1: EDTA-contained sterile tube for complete blood count (CBC assay and RNA extraction. Tube 2: Sodium citrate sterile tube presents at room temperature for the measurement of ESR. Tube 3: sterile plain tube that allowed to clot at 37 °C, and used for the measurement of kidney function, CRP, vitamin D, C3, C4, ANA, and anti-double stranded DNA after serum separation by centrifugation.

Complete blood count (CBC) was measured by Sysmex1 XN-1000 Automated Hematology Analyzer (Sysmex Corporation, Japan). Kidney function tests (creatinine, urea, and protein creatinine ratio) were assessed by auto analyzer AU 680 (Beckman Colter, AU chemical analyzer, USA). ESR was assessed by the Westergren method, and CRP was assessed by turbidimetric immunoassay using Mispa-i2 (Agappe Diagnostics, Switzerland). 25-Hydroxyvitamin D [25(OH) D] levels were measured by the enzyme immunoassay method [19]. Genetic factor (HLA-B27) antigen was assessed by PCR (Cobas fully automated AmpliPrep TaqMan Roche). The quantification of Complement 3 (C3) and Complement 4 (C4) was determined by the nephelometric method using Mispa-i2 (Agape Diagnostics, Switzerland). Anti-ds-DNA and ANA were assayed by iFlash immunoassay analyzer (Shenzhen YHL, Biotech Corporation, China). Detection of mRNA expression levels of JAK2 and PTPRC was by real-time PCR.

2.3.2. mRNA analysis of JAK2 and PTPRC

Real-time PCR for *JAK2* and *PTPRC* mRNA expression. Total RNA was prepared from whole blood using QIAamp RNA Blood Mini kit (Qiagen, USA, 2016). Thermo Scientific (Thermo Fisher Scientific, Inc. EU/Lithuania) was used to synthesize complementary DNA. Real-time PCRs were performed using Quanti Tect SYBR Green PCR Kit with readymade quantiTect Primer Assay, Qiagen. For measurement of *JAK2* and *PTPRC* mRNA expression levels, we use the following primers: forward and reverse primers of *JAK2*, 5′TCTGGGGAGTATGTTGCAGAA3′ and 5′AGACA TGGTTGGGTGGATACC3 forward and reverse primers for *PTPRC*, 5′CTTCAGTGGTCCC ATTGTGGTG3′ and 5′CCACTTTGTTCTCGG CTTCCAG3′ forward and reverse primers for human *GAPDH* 5-CCACTCCTCCACCTTTGAC-3, and 5-ACCCTGTTGCTGTAGCCA-3. PCR was conducted under the following circumstances: Each reaction for each gene was performed in a final volume of 20 μL, 10 μL SYBR Green 2x QuantiTect PCR Master Mix 3 μL cDNA, 1 μL forward primer, 1 μL reverse primer, and 5 μL RNase-free H2O. The mix was incubated at 94 °C for 3 min, followed by 60 cycles; denaturation at 94 °C for 30 s, annealing at 55 °C for 40 s, and extension at 72 °C for 31 s, 45 cycles. Data analysis was conducted in Applied Biosystems 7500 software version 2.0.1. The mRNA expression levels were assessed by the relative quantification (RQ) utilizing the ΔΔCt method as the measurement of the definite gene, which is standardized to an endogenous reference gene (*GAPDH*) and relative to a control. A melting curve was performed to confirm the specificity of the amplification and absence of primer dimers.

2.4. Statistical analysis

Data were input into the computer and assessed using the IBM SPSS software program version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described in terms of numbers and percentages. The Kolmogorov–Smirnov test was used to determine whether the distribution was normal. Quantitative data were described using the range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR) [20]. The

following tests were applied: Chi-square test, Monte Carlo correction, Student's t-test, F-test (ANOVA), Mann–Whitney test, Kruskal–Wallis test, Spearman's coefficient, receiver-operating characteristic curve (ROC), and regression analysis tests.

3. Results

3.1. Demographic and clinical data of the studied groups

SLE group of patients was formed of 8 males (25%) and 24 females (75%) with a mean age of 38.53 ± 8.44 years, while the AS group of patients was formed of 14 males (43.8%) and 18 females (56.3%) with a mean age of 37.16 ± 7.89 years and controls were 13 males (40.6%) and 22 females (59.4%) with a mean age of 35.50 ± 9.23 years. Age and gender disparities between the groups under study were not significant. There were significant disparities between both diseased groups in terms of disease duration ($P < 0.001$). Patients with SLE and AS had considerably greater levels of inflammatory markers (ESR and CRP) than the control group. SLEDAI was (8.06 ± 4.20), BASDAI was (4.76 ± 1.25), BASFI was (4.66 ± 1.49), and BASMI was (4.13 ± 1.02). Regarding *JAK2* and *PTPRC* relative expression levels, SLE and AS patient groups had higher levels of *JAK2* and lower levels of *PTPRC* compared with controls with statistically significant differences between the three studied groups with *P*-value less than 0.001 (Table 1).

Regarding correlation between *JAK2*, *PTPRC*, and different parameters in the three studied groups, a significant positive correlation existed between *JAK2* and different clinical and laboratory parameters in the SLE patient group, including SLEDAI ($r = 0.517$, $P = 0.002$), CRP ($r = 0.693$, $P < 0.001$), ESR ($r = 0.435$, $P = 0.013$), and protein–creatinine ratio ($r = 0.470$, $P = 0.007$) with also positive correlation but not reaching significance with HB level, platelet count, serum creatinine, serum urea, and C3. *PTPRC* had a significant negative association with several parameters in the SLE group including SLEDAI ($r = -0.621$, $P < 0.001$), HB ($r = -0.449$, $P = 0.010$), CRP ($r = -0.543$, $P = 0.001$), ESR ($r = -0.35$, $P = 0.049$), protein–creatinine ratio ($r = -0.483$, $P = 0.005$), and serum creatinine ($r = -0.423$, $P = 0.016$) with also a negative correlation with platelets, serum urea, C3, and C4 not reaching significance. There was a strong significant positive correlation between *JAK2* and different clinical and laboratory parameters in the AS patient group, including CRP ($r = 0.351$, $P = 0.049$), ESR ($r = 0.399$, $P = 0.024$), BASDAI ($r = 0.378$, $P = 0.033$), BASFI ($r = 0.446$, $P = 0.010$), BASMI ($r = 0.495$, $P = 0.004$), with positive correlation with HB level, platelet count, serum creatinine, and serum urea not reaching significance. However, vitamin D had a significant negative correlation with *JAK2* ($r = -0.446$, $P = 0.011$), indicating that patients with lower vitamin D levels had higher expression levels of *JAK2* and higher disease activity scores proving the strong impact of *JAK2* expression levels on AS disease activity and progression. *PTPRC* levels had a significant negative correlation with several parameters in AS group including CRP ($r = -0.363$, $P = 0.041$), ESR ($r = -0.413$, $P = 0.019$), BASDAI ($r = -0.459$, $P = 0.008$), BASMI ($r = -0.372$, $P = 0.036$) with also negative correlation not reaching significance with platelet count, serum creatinine, serum urea, and BASFI. However, vitamin D had a positive correlation with *PTPRC* levels (Table 2).

ROC analysis of *JAK2* and *PTPRC* relative expression levels: regarding the predictive performance of *JAK2* and *PTPRC* to predict active disease in both SLE and AS, cutoff values of *JAK2* and *PTPRC* were determined. ROC analysis for *JAK2* and *PTPRC* in SLE showed an area under a curve (AUC) of 0.884 and 0.903, respectively. At a cutoff point of (>13.57 and ≤ 0.076), the sensitivity of *JAK2* and *PTPRC* relative expression level as a predictor of SLE is (0.89% and77.78%, the specificity is 82.61% and78.26%, the positive predictive value is 66.7% and 58.3%, and the negative predictive value is 95% and 90%, respectively. ROC analysis for *JAK2* and *PTPRC* in AS has shown an AUC of 0.779 and 0.770, respectively. At a cutoff point of (>11.67 and ≤ 0.033), the sensitivity of *JAK2* and *PTPRC* relative expression levels as a predictor of AS is 75.0% and 81.25%, the specificity is 75.0% and 68.75%, the positive predictive value is 75.0% and 72.2%, and the negative predictive value is 75.0% and 78.6% (Table 3 and Fig. 1).

Univariate and multivariate logistic regression analyses using univariate logistic regression, it may be possible to use upregulation of *JAK2* and downregulation of *PTPRC* as well as the disease duration as helpful predictors of SLE and AS disease activity and progression (Table 4).

4. Discussion

Systemic lupus erythematosus is an immunological disease that has a female predominance and affects the connective tissues of the body Teng and colleagues [21]. AS is a common inflammatory disease starting with pain, stiffness, progressing to joint ankylosis, and affecting mainly joints of the axial skeleton Saadi and colleagues [22]. Therefore, early detection of the two disorders is essential for an effective treatment that will improve their

Table 1. Comparison between the three studied groups regarding different parameters

	Group I (n = 32)	Group II (n = 32)	Group III (n = 32)	P
Sex				
Male	8 (25.0%)	14 (43.8)	13 (40.6)	0.248
Female	4 (75.0%)	18 (56.3)	19 (59.4)	
Age (years)	38.53 ± 8.44	37.16 ± 7.89	35.50 ± 9.23	0.368
Disease duration (y)	5.53 ± 5.18	9.87 ± 4.40		<0.001*
SLEDAI	8.06 ± 4.20			
BASDAI		4.76 ± 1.25		
BASFI		4.66 ± 1.49		
BASMI		4.13 ± 1.02		
HB (g/dL)	9.67 ± 2.41	12.45 ± 0.99	12.90 ± 0.80	<0.001*
WBCs (10^3/cm)				
Normal	12(37.5%)	32(100.0%)	32(100.0%)	
Mild leukopenia	18(56.3%)	0(0.0%)	0(0.0%)	
Leukopenia	2(6.3%)	0(0.0%)	0(0.0%)	^{MC}P <0.001*
Platelets (10^3/cm)	146.38 ± 65.48	224.73 ± 60.34	232.13 ± 58.65	<0.001*
CRP (mg/L)	51.04 ± 21.57	34.04 ± 26.85	3.25 ± 0.55	<0.001*
ESR (mm/hr.)	51.38 ± 33.65	29.84 ± 13.59	4.11 ± 0.55	<0.001*
Serum creatinine (mg/dL)	1.29 ± 0.60	0.92 ± 0.16	0.91 ± 0.17	<0.001*
Serum urea (mg/dL)	56.03 ± 34.20	31.19 ± 6.23	31.50 ± 6.06	0.020*
Protein creatinine ratio	0.78 ± 0.90		0.1 ± 0.09	<0.001*
C3 (mg/dL)	86.50 ± 32.62		120 ± 35	
C4 (mg/dL)	16.25 ± 9.02		25 ± 8	
ANA (AU/mL)				
Normal	2(6.3%)		32(100%)	
Positive	30(93.8%)			
HLA B27				
Normal		8(25%)	32(100%)	
Positive		24(75%)		
Vitamin D (nmol/l)		16.90 ± 4.70	33.43 ± 3.62	<0.001*
JAK2				
Min–max	7.68–25.22	3.14–40.41	0.65–2.32	<0.001
Mean ± SD	13.60 ± 4.13	12.76 ± 7.64	1.30 ± 0.51	
Median (IQR)	13.29(11.03–15.22)	12.42(7.69–15.35)	1.0 (1.0–1.65)	
PTPRC				
Minmax	0.01–1.46	0.01–5.62	0.56–3.20	<0.001
Mean ± S.	0.28 ± 0.41	0.26 ± 1.0	1.15 ± 0.64	
Median (IQR)	0.10(0.07–0.30)	0.03(0.02–0.07)	1.0 (0.88–1.06)	

Group I = systemic lupus erythematosus, group II = ankylosing spondylitis, group III = controls.
ANA, antinuclear antibody; BASDAI, Bath AS Disease Activity Index; BASFI, Bath AS functional index; BASMI, Bath AS metrology index; C3, complement 3; C4, complement 4; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HB, hemoglobin; HLA B27, human leukocyte antigen B27; n, number; *P*-value, probability value.
Statistically significant at *P* less than or equal to 0:05.

prognosis. Consequently, accurate biomarkers are urgently required for the early diagnosis of AS and SLE Lee and colleagues [23].

The *PTPRC* gene produces a protein tyrosine phosphatase that suppresses *JAK* kinases, adversely controls cytokine receptor signaling, and regulates T and B cell antigen receptor signaling. The Janus kinase family, of which the *JAK2* gene is a part, produces non-receptor *PTKs*, which are essential for immune cell differentiation, proliferation, and survival. Protein tyrosine kinases are also crucial components of the JAK-/STAT-signaling cascade Qian and colleagues [6], which has a crucial role in autoimmune disorders and aids in the immune system's defense against infections and tumor cells. These cytokines support lymphoid cell development and maturation in addition to T and NK cell differentiation and homeostasis Cornez and colleagues [24].

In this work, the expression levels of *JAK2* and *PTPRC* mRNA were compared in AS and SLE patients with healthy matched age and sex controls. In the meanwhile, we investigated the link between the expression levels of these gene mRNA and clinical symptoms in AS and SLE patients and assessed whether the gene mRNA expression had an impact on the SLEDAI or BASDAI.

This was the first study, as far as we are aware, to link both mRNA (*JAK2* and *PTPRC*) with AS diagnosis and activity in addition to SLE in our country with their genetic properties.

Table 2. Correlation between (JAK2, PTPRC) and different parameters in each group

	Group 1				Group 2			
	JAK2		PTPRC		JAK2		PTPRC	
	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
SLEDAI	0.517	0.002*	−0.621	<0.001*	–	–	–	–
HB	0.161	0.379	−0.449	0.010*	0.113	0.539	0.025	0.893
Platelet	0.075	0.683	−0.301	0.094	0.304	0.091	−0.019	0.917
CRP	0.693	<0.001*	−0.543	0.001*	0.351	0.049*	−0.363	0.041*
ESR	0.435	0.013*	−0.350	0.049*	0.399	0.024*	−0.413	0.019*
Protein creatinine ratio	0.470	0.007*	−0.483	0.005*	–	–	–	–
Serum creatinine	0.094	0.608	−0.423	0.016*	0.013	0.944	0.245	0.176
Serum urea	0.163	0.374	0.076	0.679	0.219	0.230	−0.100	0.587
C3	0.001	0.997	−0.256	0.158	–	–	–	–
C4	−0.015	0.936	−0.163	0.374	–	–	–	–
BASDAI	–	–	–	–	0.378	0.033*	−0.459	0.008*
BASFI	–	–	–	–	0.446	0.010*	−0.255	0.160
BASMI	–	–	–	–	0.495	0.004*	−0.372	0.036*
Vitamin D	–	–	–	–	−0.446	0.011*	0.117	0.522

ANA, antinuclear antibody; BASDAI, Bath AS Disease Activity Index; BASFI, Bath AS functional index; BASMI, Bath AS metrology index; C3, complement 3; C4, complement 4; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HB, hemoglobin; *JAK2*, Janus kinase; PLTs, platelets; *PTPRC*, protein tyrosine phosphatase.

Table 3. Prognostic performance for JAK2 and PTPRC to predict active disease in AS and SLE

	AUC	*P*	95% C. I	Cut off	Sensitivity	Specificity	PPV	NPV
JAK2 (SLE)	0.884	0.001	0.764–1.0	>13.57	88.89	82.61	66.7	95.0
PTPRC (SLE)	0.903	<0.001	0.801–1.0	≤0.076	77.78	78.26	58.3	90.0
JAK2 (AS)	0.779	0.007	0.614–0.944	>11.67	75.0	75.0	75.0	75.0
PTPRC (AS)	0.770	0.009	0.590–0.949	≤0.033	81.25	68.75	72.2	78.6

AS, ankylosing spondylitis; AUC, area under a curve; CI, confidence intervals; *JAK2*, Janus kinase; NPV, negative predictive value; PPV, positive predictive value; *PTPRC*, protein tyrosine phosphatase; *P*-value, probability value; SLE, systemic lupus erythematosus. Statistically significant at P less than or equal to 0:05.

This study showed matching in age and gender in the three groups. According to a study by Kocyigit and Akyol published in 2018 that was consistent with our findings, vitamin D levels in patients with AS were considerably lower when compared with the control group. Reduced vitamin D levels cause inhibition of immune cell activity by reduction of the production of pro-inflammatory cytokines; it also strengthens the anti-inflammatory response Kocyigit and Akyol [25]. This issue may illustrate how low vitamin D levels affect the occurrence and activity of the disease. This is consistent with several studies like the study by Cai and colleagues [26] which found that vitamin D contributes to AS through its role in immune function, suggesting the benefit of vitamin D supplementation for this particular group of sick people.

In our study, the expression levels of *PTPRC* and *JAK2* mRNA were statistically different in both AS and SLE when compared with the control group. There was an increase in mRNA expression levels of *JAK2* and a decrease in mRNA expression levels of *PTPRC* in SLE patients compared with the control group. and this matched with Qian and colleagues [6] who reported that *JAK2* expression at the median level was higher in SLE patients than in controls, while the PTPRC expression at the median level was lower in the SLE group of patients than in controls and explained that some cytokines, such as IL-6, IL-12, and IFN-γ, correlated particularly with JAK2. The expression levels of these cytokines are increased significantly in SLE patients compared with the levels in healthy controls, similar to the JAK2 expression pattern. These cytokines share in JAK/STAT signaling by stimulating the type I interferon-signaling pathway. The IFN signaling route, which is upstream of the JAK/STAT signaling system, has been linked to SLE, and IFN- has been identified as a critical part of this pathway. High levels of JAK2 expression were found in the TH17 cells of MS patients, indicating that JAK2 was crucial for the differentiation of these cells Asadzadeh-Aghdaei and colleagues [27]. A previous study revealed that PTPRC suppresses JAK kinases and that damaged CD45 leading to the activation of JAKs and STAT proteins. According to the aforementioned arguments, we can conclude that the decreased PTPRC expression may lead to increased

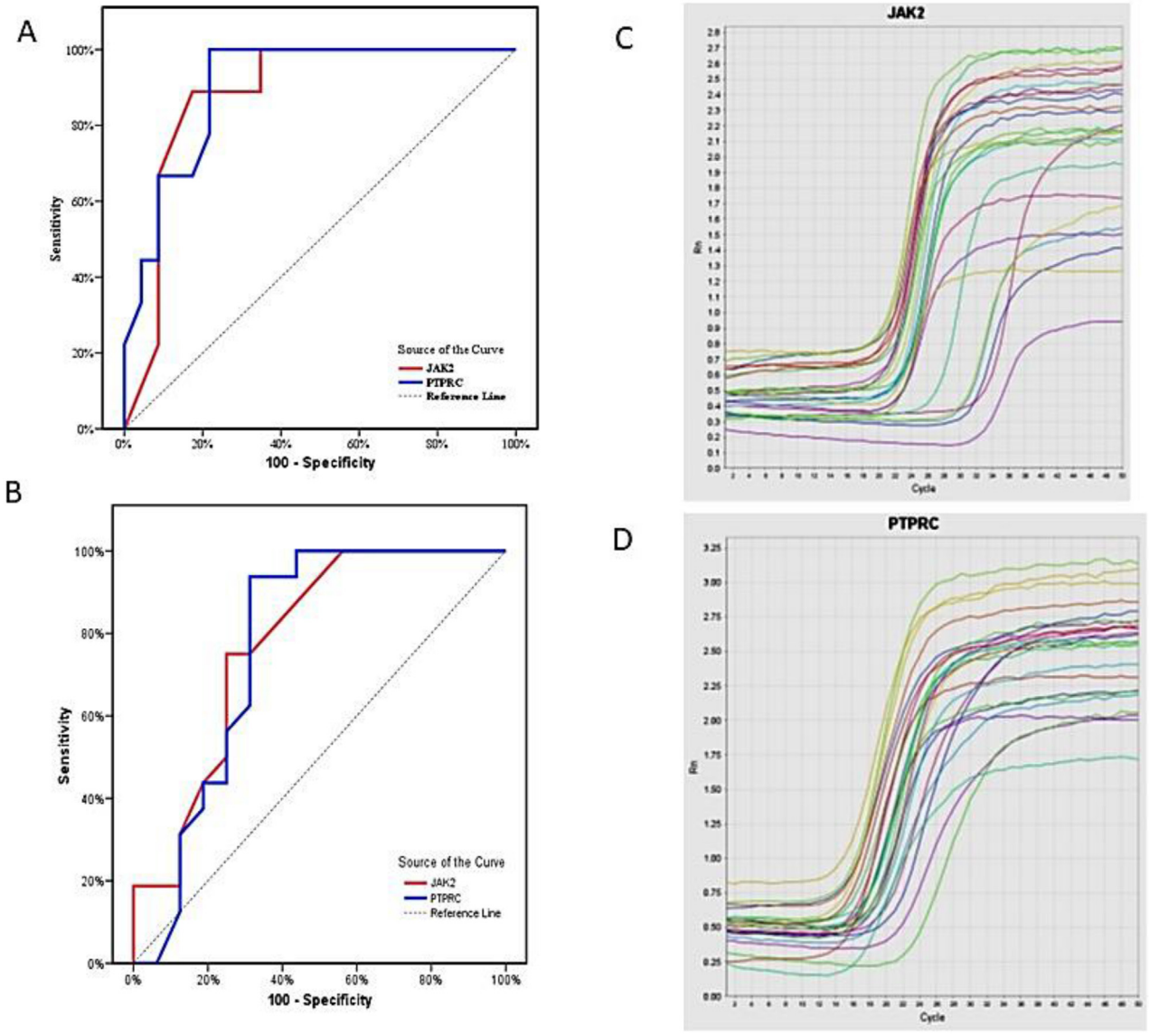

Fig. 1. (A) ROC curve for JAK2 and PTPRC to predict active diseases in group I.(B) ROC curve for JAK2 and PTPRC to predict active diseases in group II. (C) Amplification curve for JAK2. (D) Amplification curve for PTPRC.

Table 4. Univariate and multivariate logistic regression analyses for the parameters affecting active disease in AS and SLE

	Univariate		#Multivariate	
	P	OR (LL – UL 95%C. I)	*P*	OR (LL – UL 95%C. I)
JAK2 (SLE)	**0.037***	1.310(1.016–1.689)	0.032*	1.738(1.050–2.878)
PTPRC (SLE)	**0.022***	0.0(0.0–0.007)	0.016*	0.0(0.0–0.0)
Disease duration (SLE)	**0.891**	0.989(0.848–1.154)	–	–
JAK2(AS)	**0.014***	1.238(1.044–1.468)	0.449	1.092(0.869–1.372)
PTPRC (AS)	**0.028***	0.0(0.0–0.0)	0.021*	0.0(0.0–0.0)
Disease duration (AS)	**0.466**	1.063(0.902–1.253)		

AS, ankylosing spondylitis; CI, confidence interval; *JAK2*, Janus kinase; LL, lower limit; OR, odds ratio; *PTPRC*, protein tyrosine phosphatase; SLE, systemic lupus erythematosus; UL, upper limit.
Statistically significant at P less than or equal to 0:05.

expression of JAK kinases, which likely leads to the development of SLE through JAK/STAT signaling Qian and colleagues [6].

JAK2 mRNA expression levels had a significant positive correlation with SLEDAI and its components, protein–creatinine ratio, ESR, and CRP. However, PTPRC expression levels had a significant negative correlation with SLEDAI, inflammatory markers including ESR and CRP and protein–creatinine ratio.

This comes in accordance with Feng and colleagues [28] who reported a strong positive association between *JAK2* and SLEDAI and a significant negative association between *PTPRC* expression levels and SLEDAI. This matched with Kotyla and colleagues who stated that IL-6 is elevated in SLE patients and correlated with disease activity. This may be a theoretical justification for the use of biologics to target IL-6 or its receptor or the injection of JAK inhibitors to target JAK molecules linked to the IL-6 receptor as a therapeutic strategy for SLE patients [29].

However, the study by Qian and colleagues [6] was against our results as they compared the mRNA expression levels of the two genes with disease activity and found no statistically significant evidence that the SLEDAI score would impact the mRNA expression of *JAK2* and *PTPRC*.

Our study showed that there were significant statistical differences between the expression levels of the two mentioned genes in AS and controls. There was an increase in mRNA expression levels of *JAK2* and decreased mRNA expression levels of *PTPRC* in the AS group when compared with the controls. It has been reported that haplotype rs7857730-CGT/rs1536798/rs10119004 in the JAK2 locus is associated with the susceptibility of AS in the Chinese population Asadzadeh-Aghdaei and colleagues [27].

JAK2 mRNA expression levels had a significant positive correlation with BASDAI, BASMI, and BASFI. However, *PTPRC* expression levels had a significant negative correlation with the components of AS disease activity including BASDAI, BASMI, and BASFI. This means that *JAK2* and *PTPRC* could be used as new potential diagnostic and prognostic factors in both SLE and AS correlating with disease activity and severity scores.

This agreed with Chen and colleagues [30] who stated that numerous studies had shown a link between mRNA genes and inflammatory bowel disease and because it is related to AS clinically and the two conditions may have a strong genetic association, *JAK2* and *PTPRC* may have a possible role in the pathogenesis of AS. This possibility was supported by our research, which found that *JAK2* and *PTPRC* were prospective genes associated with AS.

In our study, we concluded that *JAK2* and *PTPRC* mRNAs could diagnose SLE and AS patients, because there was a difference in the expression profile of both genes between SLE, AS patients, and controls. ROC curve analysis to prove the diagnostic potential of both *JAK2* and *PTPRC* mRNAs with an evident diagnostic accuracy (AUC is 0.884 for JAK2 and 0.903 for *PTPRC* in the SLE group; AUC is 0.779 for *JAK2* and 0.770 for *PTPRC* in the AS group, respectively) with a cutoff point of 13.57 and 0.076 for *JAK2* and *PTPRC*, respectively in SLE and 11.67and 0.033 for *JAK2* and PTPRC, respectively in AS, making them convenient biomarkers for the diagnosis of SLE and AS.

In addition, we indicated that upregulation of *JAK2* along with downregulation of PTPRC and long disease duration were evaluated as the only predictors of SLE and AS activities by univariate logistic regression.

In the international literature, there was practically no information available regarding the biological effects of *JAK2* and *PTPRC* polymorphisms. For an in-depth understanding of the links between these SNPs and AS, we think it is crucial to consider how they influence the way the two genes function. Therefore, functional analysis of the SNPs must be taken into account in future research.

To our best knowledge, this was the first study relating the genetic makeup of both mRNA (*JAK2* and *PTPRC*) with AS diagnosis and activity as well as SLE in our country.

4.1. Conclusion

In conclusion, altered expression levels of *JAK2* and *PTPRC* genes in both AS and SLE suggest their potential role in disease pathogenesis. Also, their correlation with the severity of the two diseases provides a promising molecular diagnostic tool for them.

4.2. Limitations of the study

There are some obstacles to this study. First, the number of patients involved was limited. Second, to validate the diagnostic and prognostic burden of both *JAK2* and *PTPRC* in AS and SLE, more large-scale prospective studies are mandated. Finally, future studies should ideally focus on the hypothesized therapeutic effects rather than the diagnostic implications of these putative biomarkers.

Conflicts of interest

No conflicts of interest.

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