Egypt J Pediatr Allergy Immunol 2025; 23(2):51-61. DOI. 10.21608/ejpa.2025.429473.1092

Original article

Study of blood and renal Janus kinase 3 expression in pediatric systemic lupus erythematosus: a potential therapeutic target

Background: *Janus kinase 3 (JAK3) is a member of the JAK family which plays an important role in cytokine signal transduction. Dysregulation of JAK pathway has been linked to several autoimmune disorders including systemic lupus erythematosus (SLE). This study aimed to evaluate the expression of JAK3 among pediatric SLE (pSLE) patients.* ***Patients and Methods:*** *Forty-six children and adolescents with active SLE were recruited from the Pediatric Rheumatology Unit, Assiut University. All patients were subjected to clinico-laboratory evaluation as well as assessment of SLE status using SLE disease activity index (SLEDAI). Blood and renal JAK3 expression were assessed using quantitative polymerase chain reaction (q-PCR) and enzyme-linked immunosorbent assay (ELISA) in all patients as well as 20 age-and gender-matched healthy controls and in 26 patients with biopsy-proven lupus nephritis (LN) respectively.* ***Results:*** *The mean (SD) age of the studied patients was 12.2 (2.4) years, they were 34 females (74%) and 12 males (26%). Blood JAK3 expression both by ELISA and q-PCR were significantly higher among pSLE patients as compared to healthy controls ($p<0.000$). Both blood and renal JAK3 expression were significantly positively correlated with anti-double stranded-DNA and SLEDAI and were significantly negatively correlated with serum complement 3 ($p <0.000$). Renal JAK 3 expression was significantly positively correlated with 24-hour urinary proteins and urinary RBCs ($p<0.000$).* ***Conclusion:*** *The increased JAK 3 expression among pSLE patients and its strong association with validated parameters of SLE activity could make it a potential therapeutic target for pSLE patients especially those with LN.*

Keywords: Pediatric SLE, Janus kinase 3- Lupus nephritis- SLEDAI.

Dalia H. ElGhoneimy[1] Enas M. Sayed[2], Ahlam Badawy[3], Eman Radwan[4,5], Momtaz T. Allam[6], Naglaa S.Osman[2]

[1]Pediatric Allergy, Immunology and Rheumatology Unit, Children's Hospital, Ain Shams University, Cairo. [2]Pediatric Allergy, Immunology and Rheumatology Unit, [3]Pediatric Nephrology and Hemodialysis Unit, Children's Hospital, Assiut University, Assiut, [4]Department of Medical Biochemistry, Faculty of Medicine, Assiut University, [5]Department of Biochemistry, Sphinx University, New Assiut, Assiut, [6]Department of Radiology, Assiut University, Assiut, Egypt.

Correspondence:
Dalia H. ElGhoneimy
e-mail: *dalia.elghoneimy@gmail.com*
Received: August 2024
Revised: October 2024
Accepted: March 2025

INTRODUCTION

Janus kinases (JAKs) are non-receptor tyrosine kinase proteins constitutively associated with the intracellular domains of type I and type II cytokine receptors. The JAK family is composed of four members: JAK1, JAK2, JAK3, and TYK2 (tyrosine kinase 2)[1]. The Janus kinase-signal transducer and activator of transcription (JAK-STAT) is one of the

most important intracellular signaling pathways promoting various cell processes including proliferation, maturation, differentiation, activation, migration, and survival[2]. JAK3 activates multiple STAT proteins—principally STAT5 (downstream of interleukin (IL)-2R, IL-7R, IL-9R, and IL-15R), STAT6 (downstream of type I IL-4R), and STAT3 (downstream of IL-21R). The pathological consequences of dysregulated JAK3 signaling have been widely documented in many animal models of chronic inflammatory and autoimmune diseases as well as cancers. Moreover, the JAK-STAT signaling pathway has been found to contribute to the pathogenesis of many inflammatory and autoimmune diseases in humans[3,4]. Dysregulated innate and adaptive immunity, with subsequent excessive production of proinflammatory cytokines are cardinal mechanisms in the pathogenesis of pSLE. These pro-inflammatory cytokines, including IL-2, IL-4, tumor necrosis factor (TNF)-alpha, interferon (IFN)-alpha activate the JAK/STAT pathway creating a positive feedback loop that amplifies autoimmunity and inflammation.[5]

Little is known about the contribution of individual members of the JAK-STAT family to disease status and organ involvement in pSLE. In this study, we aimed to evaluate blood and renal JAK3 in pSLE patients and the relationship between JAK3 and clinico-laboratory parameters of SLE activity. Our ultimate objective is to shed light on JAK3 in pSLE as a potential therapeutic target.

METHODS

Study design:

This was an analytical cross-sectional study conducted at the Pediatric Rheumatology Unit, Children's Hospital, Assiut University. The study was approved by the Medical Ethics Committee, Faculty of Medicine, Assiut University (Approval number: 17200468). Informed consent was obtained from the caregivers of all participants, along with assent from older children and adolescents.

Study population:

Patient group:

Forty-six patients with an established diagnosis of pediatric systemic lupus erythematosus (pSLE), according to the Systemic Lupus International Collaborating Clinics (SLICC) Classification Criteria[6], were included. Patients with lupus nephritis (LN) were biopsy-proven according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification of LN[7]. Exclusion criteria include inactive SLE, end-stage renal disease, moderate or severe infection, associated renal vascular disorders such as secondary thrombotic thrombocytopenic purpura or hemolytic uremic syndrome.

Control group:

Twenty age- and sex-matched, apparently healthy children and adolescents served as healthy controls. They were recruited from the Pediatric Orthopedic and Ophthalmology Outpatient Clinics at Children's Hospital, Assiut University.

Study methods:

A-Clinical evaluation:

History taking included demographic data and review of symptoms affecting the constitutional, musculoskeletal, and other systems involved in SLE. General and systemic examinations were performed for each patient to determine the full clinical spectrum of SLE. Disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), with the following activity categories: mild activity: SLEDAI= 1-5, moderate activity: SLEDAI = 6-10, high activity: SLEDAI= 11-19, very high activity: SLEDAI ≥ 20[6,8].

B-Laboratory work up:

All patients underwent the following laboratory investigations:

Routine tests: Complete blood count (CBC), erythrocyte sedimentation rate (ESR), kidney and liver function tests, urinalysis, and 24-hour urinary protein.

Immunological tests: Serum antinuclear antibodies (ANA) titer, anti–double-stranded DNA (anti-dsDNA) titer, serum complement 3 and 4 (C3 and C4) levels, lupus anticoagulant (LA), and anticardiolipin antibodies (ACA) of IgM and IgG isotypes titers.

Sampling for JAK3 assay:

Blood samples: Five milliliters of blood were drawn and allowed to clot at room temperature, then centrifuged at 2000–3000 rpm for 20 minutes to obtain serum. The serum was aliquoted and stored at –80°C until ELISA measurement. Another five milliliters of blood were collected into ethylene diamine tetraacetic acid EDTA -containing tubes and used immediately for qPCR analysis.

Renal biopsy

Biopsies were performed on 28 patients using a Tru-Cut needle (Medplus, 18G, 10–15 cm depending on body build) under aseptic conditions. The procedure was guided by ultrasonography (Logiq P9 GE machine), targeting the lower renal cortex. Two biopsy cores were obtained and stored

at –80°C for JAK3 expression analysis by both qPCR and ELISA.

Quantitative polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assay (ELISA) for detection of JAK3 Levels in blood and renal tissue:

Blood JAK3: Assessed in all pSLE patients and healthy controls.

Renal JAK3: Assessed in pSLE patients with biopsy-proven LN: patients with LN class I served as controls for renal JAK3 by qPCR.

RNA Extraction and Real-Time PCR:

Total RNA was extracted using the RNeasy Mini Kit (Catalog No. 74104). RNA concentration and purity were determined using a NanoDrop spectrophotometer. Subsequently, 500 ng of RNA was reverse-transcribed into complementary DNA (cDNA) using the Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit (Catalog No. 4374966). cDNA was amplified using the Maxima SYBR Green qPCR Master Mix Kit (Catalog No. K0251) with the following primers for JAK3: (Forward primer: 5-CTACGCCCTCAACTATCTGGA-3 and Reverse primer: 5-TTCCGGGCAGAGACATTG-3). A two-step reaction protocol was used: Initial denaturation at 95 °C for 10 minutes followed by 40 amplification cycles of 95°C for 15 sec and 60 °C for 1 min using the Applied Biosystems 7500 Fast Real-time PCR machine (Applied Biosystems, Germany).

Gene expression in blood samples was expressed as fold-change relative to healthy controls. Gene expression in renal tissue samples was expressed as fold-change relative to LN class I samples (the least affected class, according to ISN/RPS classification). Gene expression was calculated using the 2^−ΔΔCT method, with GAPDH as the reference gene. GAPDH primers used: (Forward primer: 5-TTGAGGTCAATGAAGGGGTC-3 and reverse primer: 5-GAAGGTGAAGGTCGGAGTCA-3)

ELISA: JAK3 protein levels were determined and expressed as ng/mL in serum and ng/mg tissue weight in renal samples using the Human Tyrosine-Protein Kinase JAK3 ELISA Kit (Catalog No. SG-12798, SinoGeneClon Biotech Co., China), following the manufacturer's instructions.

Statistical analysis:

Data was analyzed using SPSS© Statistics version 21 (IBM© Corp., Armonk, NY, USA). Categorical variables were presented as number and percentage or ratio. Normality of numerical data distribution was examined using the Shapiro-Wilk test. Normally distributed numerical variables were presented as mean ± SD and intergroup differences were compared using the unpaired t test. Non-normally distributed numerical variables were presented as median and IQR and intergroup differences were compared using the Mann-Whitney test (for two-group comparison). Kruskal-Wallis test with adjusted Bonferroni correction test to compare three groups. The ROC Curve (receiver operating characteristic) was used to evaluate the sensitivity and specificity for JAK3. A probability (p) value < 0.05 was considered statistically significant. Correlations were tested using the Spearman rank correlation.

RESULTS

Demographic data and clinico-laboratory features of the studied patients with pSLE:

The studied population comprised 46 children and adolescents with pSLE, including 34 females (73.9%) and 12 males (26.1%). Their ages ranged between 8 and 16 years, with a mean (SD) of 12.2 (2.4) years. All patients were newly diagnosed and enrolled before the initiation of immunosuppressive treatment.

Anemia, cutaneous lupus, and musculoskeletal manifestations were the most common presenting features, affecting 97.8%, 82.6%, and 76% of patients, respectively. Twenty-nine patients (63%) had persistent urinary involvement, among whom 28 had biopsy-proven LN. Neuropsychiatric manifestations were observed in 7 patients (15.2%); of whom 6 patients (13%) had psychosis, and one patient had cerebral venous thrombosis complicated by venous stroke. Lupus-related serositis was observed in 3 patients (6.5%), including one with massive pericardial effusion .

All patients were positive for ANA and anti-dsDNA antibodies. Serum C3 and C4 were consumed in 41 patients (89.1%). Antiphospholipid antibodies were positive in 12 patients (26.1%); where 3 patients (6.5%) developed thrombosis—one had cerebral venous thrombosis, and two had deep vein thrombosis.

All patients had active SLE, with a median (IQR) SLEDAI score of 13 (10.3–18.8). Mild activity was seen in 3 patients (6.5%), moderate activity in 9 patients (19.6%), high activity in 23 (50%), and very high activity in 11 (23.9%).

Three patients (6.5%) died during the study. One was a child with Down syndrome who presented with SLE-related autoimmune hemolytic anemia (AHA) and thrombocytopenic purpura and died of intracranial hemorrhage. The second died of lupus

myocarditis complicated by acute congestive heart failure and cardiogenic shock. The third patient presented with clinico-laboratory features of LN and died of COVID-19 before doing renal biopsy (Table 1) .

The patients were compared to 20 healthy controls who were 9 females [45%] and 11 males [55%]), with a mean (SD) age of 9.9 (3.5) years (range: 6–16 years).

Clinical and histopathological characteristics of the studied patients with LN:

Clinical and laboratory features of LN were observed in 29 patients (63%), with generalized edema and hypertension being the presenting symptoms in 7 patients (24.1%). All patients (100%) had persistent hematuria and proteinuria, while serum creatinine and creatinine clearance remained within normal ranges for age and gender.

LN was confirmed by renal biopsy in 28 patients; proliferative LN (Class III and IV) was identified in 8 patients (28.6%), whereas non-proliferative LN (Class I, II, and V) was documented in 20 patients (71.4%) (Table 2) .

Blood JAK3 expression among the studied patients compared to healthy controls:

Patients with pSLE had significantly elevated blood JAK3 levels, as measured by both qPCR (mRNA) and ELISA (protein), compared to healthy controls ($p < 0.000$) (Table 3).

The blood JAK3 cutoff value distinguishing patients from controls was determined using the ROC curve: A blood JAK3 mRNA expression level of 1.35-fold-change (by qPCR) was an excellent discriminator between pSLE patients and healthy controls, with an AUC of 1.00, providing 100% sensitivity and specificity, and both positive predictive value (PPV) and negative predictive value (NPV) of 100% (Figure 1a). A serum JAK3 level of 2.95 ng/mL (by ELISA) also served as an excellent discriminator, with an AUC of 0.99, sensitivity of 97.8%, specificity of 100%, PPV of 100%, and NPV of 95.2% (Figure 1b).

Renal JAK3 expression among the studied LN patients:

Renal JAK3 expression was assessed in 26 pSLE patients with biopsy-proven LN (two patients had their renal biopsies performed just before enrollment). qPCR and ELISA were both performed on renal biopsy samples from 14 patients, while only one method was used in the remaining 12 patients due to technical difficulties in obtaining two core biopsies.

The median (IQR) renal JAK3 mRNA expression (fold-change) was 10.7 (2.5–15.4) and ranged between 1 and 18.8. Using ELISA, the median (IQR) renal JAK3 protein level among LN patients was 113.7 (2.4–142.8) ng/mg tissue, and between 1.1 and 165.1 ng/mg tissue.

Patients with proliferative LN (Class III and IV) and those with non-proliferative LN (Class II and V) had comparable renal JAK3 mRNA expression by qPCR. However, both groups showed significantly higher expression levels compared to those with LN Class I (control group). Also, renal JAK3 by ELISA showed no significant difference between patients with proliferative LN and those with non-proliferative LN (Table 4).

Concordance Between qPCR and ELISA in measuring blood and renal JAK3:

There was strong concordance between qPCR and ELISA techniques in measuring both blood and renal JAK3 levels. A highly significant positive correlation was observed between the two methods for blood JAK3 (Figure 2a) and renal JAK3 (Figure 2b). Additionally, there was a significant positive correlation between blood and renal JAK3 levels, whether assessed by qPCR (Figure 3a) or ELISA (Figure 3b) ($p = 0.000$) .

Blood JAK3 expression in patients with LN versus those without clinico-laboratory evidence of LN:

Blood JAK3 levels measured by ELISA were significantly higher in pSLE patients with biopsy-proven LN [14.1 (12.8–16.6)] compared to those without clinical or laboratory evidence of LN [10.3 (7.3–13.1)], ($p = 0.007$). On the other hand, blood JAK3 was comparable between the 2 groups when measured by qPCR ($p = 0.14$).

Correlation between JAK3 expression and laboratory features:

Blood JAK3 levels measured by ELISA showed a negative correlation with absolute lymphocyte count. Both blood and renal JAK3 levels (assessed by qPCR and ELISA) were negatively correlated with serum C3 and C4 levels and positively correlated with anti-dsDNA titers and SLEDAI. Both blood and renal JAK3 (by qPCR and ELISA) showed a significant positive correlation with 24-hour urinary protein and urinary RBC count (Table 5).

Table 1. Demographic data and clinico-laboratory features of the studied patients.

Clinical disease characteristics	No. of patients (%)	Laboratory parameters	No. of patients (%)
Age (years)		**Serum Anti-dsDNA (normal<20 IU/ml) Positive Anti-dsDNA**	46 (100%)
Mean ± SD	12.19 ±2.43	Median (IQR)	33 (25-60)
		Range	20-100
Gender -Female	34(73.9%)		
-Male	12(26.1%)	**Positive anti smith antibody**	14 (30.4%)
Family history of SLE	2(4.3%)	**Serum C3 (normal:79-152 mg/dl) Consumed C3**	44 (95.7%)
		Median (IQR)	60(30-70)
		Range	10-80
Cutaneous manifestations	38 (82.6%)	**Serum C4 (normal:15-45 mg/dl) Consumed C4**	42 (91.3%)
***Acute cutaneous lupus**		Median (IQR)	11 (6-13)
-Malar rash	33 (71.7%)	Range	2-25
***Chronic cutaneous lupus**			
-Discoid lupus	1(2.2%)	**Serum LA (normal <6.9 U/ml) Positive LA**	9 (19.6%)
***Others**		Median (IQR)	5(2.5-6)
-Oral painless ulcers	15 (32.6%)	Range	2-11
- Punctate erythema	7 (15.2%)		
- Raynaud's phenomena with photosensitivity, and livedo reticularis	1(2.2%)	**Serum ACA IgM (normal <6.9 U/ml) Positive ACA IgM (U/ml)**	7 (15.22%)
-Diffuse scalp alopecia	1(2.2%)	Median (IQR)	14 (10-22)
		Range	8-43
		Serum ACA IgG (normal< 9.9 U/ml) Positive ACA IgG (U/ml)	5 (10.87%)
		Median (IQR)	15 (12-15)
		Range	10-22
Musculoskeletal manifestations	35 (76%)	**Microscopic hematuria (RBCs > 5 cells/HPF)**	29 (63%)
-Polyarthritis	25 (54.3)	Median (IQR)	30 (20-40)
-Polyarthralgia	10(21.7%)	Range	10-100
Hematological manifestations	45(97.8%)	**24-hour urinary protein (normal< 150 mg/day)**	29 (63%)
-Anemia	45(97.8%)	Median (IQR)	950 (600-3500)
-Thrombocytopenic purpura	5 (10.9)	Range	300-4282
NP manifestations	7 (15.2%)	**Serum albumin (normal 3.5-5.5 g/dl)**	
-Psychosis	6 (13%)	Median (IQR)	3.7 (3.5-4)
-Convulsion	1(2.2%)	Range	1.9-4.5
-Stroke (venous)	1(2.2%)		
APS	12 (26%)	**HB level (mg/dl)** Median (IQR)	9 (7.7-10)
-Venous thrombotic events	3 (6.5%)	Range	4.0-12
LN	29 (63 %)	**WBCs count (cell/ul)**	
-Hypertension	9 (19.6%)	Median (IQR)	4.8 (3-6.6)
-Generalized edema	7 (15.2%)	Range	1-14
Cardiac manifestations	3 (6.5%)	**Lymphocyte count (cell/ul)**	
-Pericardial effusion	1(2.2%)	Median (IQR)	1000 (777-1200))
-Myocarditis	2 (4.3%)	Range	300-4000
Pulmonary manifestations	2 (4.3%)	Mild lymphopenia	19 (43.2%)
-Pleurisy	2 (4.3%)	Moderate lymphopenia	23 (52.3%)
-Pleural effusion	1(2.2%)	Severe lymphopenia	2 (4.5%)
SLEDAI		**Platelet count (cell/ul)**	
Median(IQR)	13(10.3-18.8)	Median (IQR)	191.5 (100-257))
Range	5-32	Range	19-450
Mortality	3(6.5%)	**Coombs positive AHA**	29 (63%)

No: number, %: percentage, SD: standard deviation, SLE: Systemic Lupus Erythematosus, NP: neuropsychiatric, APS: antiphospholipid syndrome, LN: lupus nephritis, Anti-dsDNA: anti double stranded DNA,IQR: interquartile range ab: antibody, C3: complement 3, C4: complement 4, LA: lupus anti-coagulant, ACA: anti-cardiolipin antibodies, Ig: immunoglobulins, CBC; Complete blood count, HB: hemoglobin, AHA: autoimmune hemolytic anemia, Mild lymphopenia (Absolute lymphocytic count (ALC) 1000-1500 cell/ul), Moderate lymphopenia (ALC 500-1000 cell/ul), Severe lymphopenia (ALC < 500 cell/ul).

Table 2. Urinary abnormalities in patients with LN in relation to their renal biopsy class.

	Renal biopsy class by ISN/RPS				
	Class I	**Class II**	**Class III**	**Class IV**	**Class V**
No of patients	9	5	3	5	6
Urinary hematuria (cell/HPF)					
Median (IQR)	20 (15-20)	28 (25-30)	30 (25-30)	40 (30-45)	55 (40-80)
Range	10-25	25-30	25-30	30-50	20-100
Sterile pyuria					
No of patients	2	0	1	4	2
24h urinary protein (mg/day)					
No of patients	9	5	3	5	6
Median (IQR)	600(500-600)	700(700-800)	1000(600-1534)	3400(3045-3500)	3900(3600- 4200)
Range	300-950	700-850	600-1534	3000-3580	3456-4282

No: number, HPF: high power field, IQR: interquartile range, ISN/RPS: International Society of Nephrology/Renal Pathology Society.

Table 3. Blood JAK3 expression among the studied patients compared to healthy controls.

	Patients (No.= 46)	**Controls (No.= 40)**	**P-value**
Serum JAK3 mRNA (qPCR) (fold-change)		(No.=20)	
Median (IQR)	11.1(7.3-18.2)	0.9(0.9-1)	**0.000***
Range	1.5-50	0.7-1.3	
Serum JAK3 protein level (ELISA) (ng/ml)		(No.=20)	**0.000***
Median (IQR)	13.2(10-16.6)	1.0(0.5-1.5)	
Range	1.5-30.7	0.1-2.9	

No: number, JAK: Janus kinase, IQR: inter quartile range.

Table 4. Variation of renal JAK3 levels (PCR and ELISA) among patients with proliferative LN and non-proliferative LN patients.

	Proliferative LN (LN III, IV)	**Non-proliferative LN (LN II, V)**	**Control (LN I)**	**P-value**
Renal JAK3 (qPCR) (Fold-change)	**(No=5)**	**(No=11)**	**(No=5)**	
Median (IQR)	10.7(10.1-12.5)	15.7 (2.6-17.3)	0.8 (0.8-1.0)	**0.007***
Range	8.8-12.5	1.0-18.8	0.7-1.2	
Renal JAK3 (ELISA) (ng/mg)	**(No=6)**	**(LN class I, II, V) (No=14)**		0.14
Median (IQR)	130.1(120.3-137.7)	3.0 (1.9-154.0)		
Range	115.6-147.8	1.1-165.1		

JAK: Janus kinase, IQR: inter quartile range, ELISA: enzyme linked immune-sorbent assay, qPCR: quantitative polymerase chain reaction.

Table 5. The relationship between JAK3 expression levels and different parameters in the studied patients.

	Blood JAK3 level				**Renal JAK3 level**			
	mRNA (fold-change)		**Protein (ng/ml)**		**mRNA (fold-change)**		**Protein (ng/ml)**	
	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value
HB (gm/dl)	0.15	0.32	0.18	0.24	0.25	0.29	0.27	0.25
PLT	0.08	0.6	0.18	0.24	0.24	0.32	0.08	0.72
ALC	-0.22	0.14	-0.35	**0.016***	-0.16	0.52	-0.14	0.55
Serum C3 (mg/dl)	-0.68	**0.000***	-0.62	**0.000***	-0.93	**0.000***	-0.84	**0.000***
Serum C4 (mg/dl)	-0.69	**0.000***	-0.68	**0.000***	-0.69	**0.001***	-0.85	**0.000***
Anti-dsDNA titer	0.82	**0.000***	0.82	**0.000***	0.78	**0.000***	0.82	**0.000***
SLEDAI	0.84	**0.000***	0.88	**0.000***	0.82	**0.000***	0.86	**0.000***
24-hs urinary protein	0.49	**0.001***	0.53	**0.000***	0.87	**0.000***	0.90	**0.000***
Urinary RBCs	0.36	**0.02***	0.45	**0.002***	0.74	**0.000***	0.77	**0.000***

JAK: Janus kinase, HB: hemoglobin, PLT: platelet, ALC: absolute lymphocytic count, C: complement, Anti-dsDNA: anti-double stranded DNA, SLEDAI: systemic lupus erythematosus disease activity index, hs: hours, RBCs: red blood cells.

(a) **(b)**

Figure 1. ROC curve to define the best cut off value of blood JAK3 expression measured by qPCR (a) and ELISA (b).

(a) **(b)**

Figure 2. A significant positive correlation exists between both ELISA and qPCR values of blood JAK3 (a) and renal JAK3(b).

(a) **(b)**

Figure 3. A significant positive correlation exists between blood and renal JAK3 levels measured by both qPCR (a) and ELISA (b).

DISCUSSION

In the current study, we evaluated blood and renal JAK3 levels using qPCR and ELISA among pSLE patients, we found that blood JAK3 levels, measured by both methods, were significantly higher in pSLE patients compared to healthy controls ($p < 0.001$). This finding was greatly expected, given the established role of the JAK-STAT pathway in regulating pro-inflammatory cytokine gene expression, thereby amplifying the chronic inflammation and autoimmunity that characterize SLE. Interestingly, the significant difference in blood JAK3 levels between patients and healthy controls enabled us to determine a cut-off value for JAK3 in pSLE: 1.35-fold change by qPCR, with 100% sensitivity and 100% specificity, and 2.94 ng/ml by ELISA, with 97.8% sensitivity and 100% specificity.

In this study, renal JAK3 expression by both ELISA and qPCR were comparable between patients with proliferative LN and those with non-proliferative LN. The absence of significant differences in blood and renal JAK3 levels between patients with proliferative and non-proliferative LN

should be interpreted cautiously, in the face of the small sample size .

Several previous studies on murine lupus models demonstrated that tofacitinib (a JAK1 and JAK3 inhibitor) effectively suppressed effector functions and impaired the survival of inflammatory cells in the kidneys, leading to improved kidney function. These results support the potential use of tofacitinib as a therapy for LN, particularly in refractory cases[9-11]. A randomized, double-blind clinical trial evaluated the safety and efficacy of filgotinib (a JAK1 inhibitor) in adult patients with LN Class V and demonstrated a significant reduction in 24-hour urinary protein and SLEDAI, with sustained improvement throughout the trial[12]. Additionally, baricitinib (a JAK1/2 inhibitor) use in murine lupus with renal involvement was associated with a marked reduction in renal inflammation and restoration of structural protein expression in podocytes[13]. Its use in adult SLE patients has also resulted in improvement of proteinuria in active LN[14].

We used both qPCR and ELISA in this study to measure blood and renal JAK3 levels, aiming to evaluate the sensitivity, specificity of either method and determine the degree of concordance between the 2 methods. The study demonstrated that blood and renal JAK3 levels measured by both qPCR and ELISA were significantly correlated. Therefore, either method could be reliably used in future research. However, ELISA may be more practical due to its simplicity and lower cost compared to qPCR. Additionally, our findings revealed that JAK3 values in both blood and renal tissues, measured by both techniques, showed comparable sensitivity and specificity, and were positively correlated with one another. Moreover, they support the notion that systemic inflammation in pSLE is reflected in in-situ renal inflammation among patients with LN.

To our knowledge, no previous studies had evaluated JAK3 expression in SLE. However, two studies assessed JAK2 expression in adult SLE and reported significantly elevated serum JAK2 levels compared to healthy controls[15,16].

In our study, blood JAK3 levels measured by ELISA, but not qPCR, were significantly higher in patients with LN compared to those without clinical or laboratory evidence of LN ($p = 0.007$). This finding suggests that ELISA may be more sensitive than qPCR in detecting JAK3 differences between LN and non-LN patients. Sensitivity is a crucial parameter when using a test to identify a serious but treatable disorder[17]. Considering the positive correlation between blood and renal JAK3 levels and markers of LN flare, including 24-hour urinary protein and urinary RBCs ($p < 0.001$), these findings support the active role of JAK3 in LN. Furthermore, blood JAK3 levels were significantly correlated with key immunological markers of SLE (serum C3 and C4 levels, and anti-dsDNA titers), further supporting a significant role for JAK3 in the immunopathogenesis and disease flare of pSLE.

A previous study demonstrated that baricitinib use led to increased serum C3, reduced anti-dsDNA, anti-Smith, and anti-RNP titers, and decreased levels of pro-inflammatory cytokines (IFN-α, TNF-α, IL-12, IL-17A, IL-1β), ultimately improving survival rates[18].

In human trials, ruxolitinib (a JAK1/2 inhibitor) was found to inhibit the production of extractable nuclear antigens and anti-dsDNA antibodies in adult SLE patients[19]. Additionally, tofacitinib, a JAK1/3 inhibitor, showed a positive effect on cardiometabolic markers (e.g., HDL and cholesterol levels) in adult SLE patients without causing unexpected serious adverse events, disease worsening, or thromboembolism. Tofacitinib also reduced the expression of IFN-response genes and decreased levels of low-density granulocytes (LDGs) and neutrophil extracellular traps (NETs)[20]. LDGs, a subset of proinflammatory neutrophils, are increased in SLE patients and release large amounts of NETs. NETs contribute to vascular damage, thrombosis, and act as sources of autoantigens, promoting autoimmunity and SLE pathogenesis[21].

Our study also found a significant positive correlation between blood JAK3 levels and SLEDAI. Two recent studies reported a similar association between serum JAK2 and SLEDAI in adult SLE[15,22]. The use of JAK inhibitors (Jakinibs) has been associated with improvements in SLE activity scores, indirectly supporting the role of JAKs in SLE flares.

Regarding JAK3 inhibition, tofacitinib use in adult SLE patients was associated with significant improvement in SLEDAI, BILAG, Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI)[23], and refractory alopecia[24]. Baricitinib was shown to improve SLEDAI (especially in remission of arthritis and mucocutaneous rash) and BILAG in adults with refractory SLE[25–27]. Two case reports also supported the beneficial effects of baricitinib in refractory subacute cutaneous lupus and scarring alopecia, with significant reductions in CLASI[28,29].

STUDY LIMITATIONS

This study had some limitations, including a small sample size and consequently limited numbers in subgroups, as well as its cross-sectional design, which precluded assessing longitudinal changes in JAK3 expression over the disease course.

CONCLUSION

JAK3 appears to play a significant role in SLE immunopathogenesis and disease flare. LN is specifically associated with increased JAK3 expression, which shows a strong positive correlation with indicators of renal flare. ELISA proved to be as reliable as qPCR for JAK3 assessment and showed greater sensitivity, making it a preferred method due to its lower cost and technical simplicity. These preliminary findings highlight the need for wider scale, longitudinal studies to extensively evaluate the role of JAK3 in different states of SLE , especially in severe or refractory pediatric cases, and therefore considering its value as a therapeutic target in pSLE.

AUTHORS CONTRIBUTION

DHE: study concept and design, analysis and interpretation of the results and share in writing of the manuscript. **EMS:** perform the enrollment and assessment of the patients, share in analysis and interpretation of the results, and wrote the manuscript draft, **NSO:** share in assessment of the patients; in analysis and interpretation of the results, and in writing of the manuscript, **AB:** share in analysis of the results and in manuscript draft, **ER:** perform the laboratory assessment of blood and renal JAK 3 expression, share in analysis and interpretation of the results and in writing the manuscript, **MTA:** perform the ultrasound guided-renal biopsy and wrote the relevant part. All authors revised and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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