## Original article

# Assessment of serum and cutaneous JAK3 in juvenile scleroderma

***Background:*** *Janus kinases (JAKs) family include JAK 1, 2, 3 and tyrosine kinase 2 play central role in cytokine and growth factor signaling and few studies suggest their possible contribution in the pathogenesis of scleroderma.*

***Objective****: To evaluate serum and cutaneous expression of JAK3 in pediatric patients with juvenile scleroderma (JSD) and their association with the disease severity.*

***Methods:*** *This was a pilot study that included 16 pediatric patients with JSD; they were 11 patients with juvenile systemic sclerosis (JSSc) and 5 patients with juvenile localized scleroderma (JLS). The patients were compared to 17 healthy controls. Disease severity was assessed using modified Rodnan skin score (mRSSc) and JSSc severity score (J4S) in JSSc patients while localized scleroderma damage index (LoSDI) was used in JLS patients. Serum and cutaneous expression of JAK3 were measured by enzyme linked immunosorbent assay.*

***Results:*** *The median (IQR) of serum JAK3 was significantly higher among JSSc patients as compared to that of controls [430 (320-520) versus 270 (180-385), p = 0.005], while comparable values were found among JLS and controls as well as JLS and JSSc patients (p>0.05). Cutaneous expression of JAK3 was comparable between all patients and controls (p>0.05). Serum and cutaneous expression of JAK 3 did not correlate significantly with mRSSc and J4S in JSSc patients and LoSDI in JLS patients (p>0.05).*

***Conclusion:*** *JAK3 seems to contribute to the pathogenesis of JSSc and further studies are needed to establish its role and the usefulness of using JAK 3 inhibitors in these patients.*

Keywords: JAK3, modified Rodnan skin score, Juvenile systemic sclerosis.

**Dalia H. El-Ghoneimy,[1] Fatema A. El-Saeed,[2] Marwa R. El-Najjar,[3] Naglaa S. Ahmad,[4] Eman Fahmy, Naglaa S. Osman,[5] Ghada A. Shousha.[1]**

*[1]Pediatric Allergy, Immunology and Rheumatology Unit, Children's Hospital, Ain Shams University, Cairo, [2]Department of Pediatrics, Specialized El-Salam Hospital, El-Shorouk, [3]Department of Clinical Pathology, Ain Shams University, [4]Department of Pathology, Ain Shams University, [5]Pediatric Allergy, Immunology and Rheumatology Unit, Children's Hospital, Sohag University. Assiut University.*

**Correspondence:** *Dalia H. El-Ghoneimy, MD, PhD. Professor of Pediatrics, Pediatric Allergy, Immunology and Rheumatology Unit, Children's Hospital, Ain Shams University, Cairo, Egypt*
*Email: dalia.elghoneimy@gmail.com*

*Received: February 2024*
*Revised: March 2024*
*Accepted: April 2024*

## INTRODUCTION

Janus kinases (JAK) are non-receptor tyrosine kinases (TYK) with central roles in cytokine and growth factor signaling. There are four known JAKs (JAK 1, 2, 3 and TYK2), which are members of the tyrosine kinase (TYK) family of protein kinases**.** Among JAKs, JAK3 is widely expressed in both immune cells and in intestinal epithelial cells

of both humans and mice.[1,2] JAK3 is activated only by cytokines whose receptors contain the common gamma chain (γc) subunit: interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15 and IL-21. Several ligands such as cytokines and growth factors have been reported to activate the JAK-signal transducer and activator of transcription (STAT) pathway, afterwards phosphorylation and dimerization of STATs take place.[3] The JAK-STAT signaling pathway is found to contribute to the pathogenesis of many inflammatory and autoimmune diseases.[4]

The etiology of scleroderma is believed to be a combination of genetic and environmental factors that can trigger immune dysregulation involving inflammation, autoimmunity, vasculopathy, and progressive fibro-proliferative changes.[5] **A**utoreactive T cells and autoantibodies produced by B cells plays a central role in systemic sclerosis (SSc) pathogenesis where T helper 1 (Th)1 pro-inflammatory cytokines predominate early in the disease while transition to the Th2 profibrotic cytokines occurs later. Similar Th phenotype exists in early and late stages of localized scleroderma. [6,7]

Macrophages are one of the main types of effector cells in SSc pathogenesis where they promote SSc fibrosis via various pro-fibrotic and pro-inflammatory cytokines. Among which, transforming growth factor β (TGFβ1), which can promote fibrosis by inducing the recruitment and proliferation of fibroblasts, and inducing their differentiation into myofibroblasts, followed by extracellular matrix (ECM) deposition. [8,9]

JAKs downstream TGF-β mediated profibrotic signaling and activation of the JAK/STAT pathway has been demonstrated to result in fibrosis. JAKs phosphorylate STAT proteins, initiating transcription of target genes, which includes profibrotic and proinflammatory genes.[10]

A previous study demonstrated over expression of JAK1, JAK2, JAK3, and STAT3 in skin of adult patients with SSc as compared with healthy controls.[11] Recently, JAK inhibitors demonstrated inhibition of the TGF-β mediated effects in skin sclerosis. [10]

Tofacitinib, a JAK 1 and 3 inhibitors, was used to treat LS and SSc with satisfactory improvement in skin sclerosis and lung fibrosis in bleomycin-induced SSc by inhibiting proinflammatory cytokine production from T and B cells and promoting regulatory balance. [12,13]

Juvenile scleroderma (JSD) although rare disease but is associated with significant morbidity and impairment of the quality of life and psychological disorders. Current treatment has limited effect on reversing skin and internal organ fibrosis which highlights the need for targeted therapy to improve the prognosis and outcome of JSD. Therefore, we aimed in this study to evaluate JAK3 level in serum and skin of children and adolescents with JSSc and JLS and its variation with the grade of disease severity.

## METHODS

**Study design and population:**

This is a cross-sectional pilot study which was conducted at the Pediatric Allergy, Immunology and Rheumatology Unit, Children's Hospital, Ain Shams University. The study included 16 pediatric patients with established diagnosis of juvenile scleroderma of whom 11 patients (68.7%) with JSSc and 5 patients (31.3 %) with JLS. Diagnosis was based on the *PRES/ACR/ EULAR* preliminary classification criteria for JSSc [14] and for JLS [15] after considering the inclusion and exclusion criteria as follow:

**Inclusion criteria:**

Both genders aged 1 to 17 years old.

**Exclusion criteria:**

Any patient with scleroderma mimickers including the following: Mixed connective tissue disease, juvenile dermatomyositis, primary immunodeficiency diseases and/or sepsis was excluded.

A group of 23 healthy subjects served as the control group of whom, 14 adult subjects (61%) were first degree relative of the patients and the remaining 9 subjects (39%) were unrelated; they were 4 adults and 5 children.

This study was conducted after approval of the research ethics committee of Ain Shams university hospitals, with approval number MS421/2021 and informed consent was obtained from the parents or caregivers of participants after explaining the aim and procedures of the study.

**Study methods:**

***A. Clinical evaluation:***

All the studied patients were subjected to detailed history taking and physical examination to determine the extent and severity of the skin manifestations, and the systemic involvement including cardiopulmonary, gastrointestinal, renal and central nervous systems. In addition, assessment of diseases activity and severity was done as follow:

**I-Juvenile systemic sclerosis:**

- **Modified Rodnan skin score (mRSS**) is a semiquantitative score, ranging from 0 (normal) to 3 (severe), used to evaluate the skin thickness in 17 different cutaneous sites (with a maximum

score of 51) to assess the degree of skin involvement in JSSc. [16]

- **The JSSc severity score (J4S)** was used to assess the overall disease status, included indices of 9 organ systems each scored on a scale of 0-4 with maximum score of 40.[17]

**II-Juvenile localized scleroderma:**

**The localized scleroderma skin damage index (LoSDI) of the localized scleroderma cutaneous assessment tool (LoSCAT)** was used as all patients with JLS had only skin damage, LoSDI is composed of skin scoring for dermal and subcutaneous/deep atrophy, dyspigmentation and skin thickness at the center with scores ranging between 0 (none) to 3 (marked) and physician's global assessment of damage (PGA-D) on a scale ranged between 0 (no damage) to a maximum of 100 (marked damage). Mild, moderate and severe damage corresponded with LoSDI scores of 0–10, 11–15 and 16 and over respectively, and with PGA-D scores of 0–18, 19–30 and 31 and over respectively. [18]

***B-Laboratory workup:***

- **Routine laboratory investigations:** complete blood picture (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum creatinine, urea, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase and creatine phosphokinase (CPK).
- **Autoantibodies measurement**: antinuclear antibodies (ANA) titer with immunofluorescence and anti-topoisomerase (anti-Scl70) autoantibodies.
- **Measurement of JAK3 in serum and skin expression for both patients and healthy controls** were performed using quantitative human JAK3 enzyme-linked immunosorbent assay (ELISA) Kit (Catalog No: SG-12798), Sino Gene Clon Co., Ltd, with detection range: 50 pg/ml-1600 pg/ml.

Serum samples were stored at <-70°C till processing. Samples with high hemolysis or much lipid were excluded. Repeated thawing and freezing were avoided.

Skin biopsy was punched out using a disposable, sterilizable punch of 3.5 mm sizes, non-facial lesions from arm or leg of diseased area. The area was anaesthetized, and the skin was stretched in a direction perpendicular to the resting skin lines. The punch was taken from two areas of affected skin (one kept in saline for JAK3 measurement and the other in formalin for histopathological examination). The resultant wound was left to heal by secondary intention.

- **Histopathological examination of skin biopsy** from all patients with assessment of the epidermal thickness, degree of inflammatory cellular infiltrate and the amount of collagen tissue.

**Statistical analysis:**

Statistical package for social science 25 was used where parametric data was described in the form of mean and standard deviation. Non-parametric data was described as median and interquartile range (IQR). Mann Whitney Test was used to assess the statistical significance of the difference of a non-parametric variable between two groups. Correlation analysis (using Spearman's rho method) to assess the strength of association between two quantitative variables. The Kruskal-Wallis test was used to assess the significance of the difference between more than two study group ordinal variables. Post Hoc Test was used for comparisons of all possible pairs of group means. The ROC Curve (receiver operating characteristic) was used to evaluate the sensitivity and specificity for JAK 3 that categorize cases into one of two groups. P value < 0.05 was considered significant.

## RESULTS

**Demographic data and disease characteristics of the studied groups:**

The eleven patients (68.7%) with JSSc included 8 females (72.7%) and 3 males (27.3%) whose ages ranged between 6 and 16 years with mean (SD) of 11.2 (3.3) years. The five patients (31.2%) with JLS were all females whose ages ranged between 7 and 14 years with mean (SD) of 10 (2.7) years. All patients with JSSc had diffuse cutaneous systemic sclerosis (dcSSc) except two cases (18.2%) with overlap syndrome (OS). Patients with JLS included 3 patients (60%) with morphea and two patients (40%) with linear scleroderma, present in either lower or upper extremities, following the Blaschko´s lines. Five JSSc patients (45.5%) had gastrointestinal involvement in the form of gastroesophageal reflux disease and/or constipation. Two patients (18%) had interstitial lung disease (ILD), however, pulmonary function test (PFT) revealed mild restrictive disease in ten patients (91%) with mean (SD) of forced vital capacity (FVC) was 76.6% (10%), and ranged between 76% and 85% and the mean (SD) of forced expiratory volume in one second (FEV1) was 76.5% (10.7%), and ranged between 72% and 90% and one patient had severe disease with FVC= 47.3% and FEV1= 48.3% in whom primary pulmonary hypertension (PAH) was a complication of ILD. Among these 11

patients with abnormal PFT, high resolution computed tomography (HRCT) scan of the chest was positive in only two patients (18.2%): One patient with mild restrictive PFT (FVC= 78, FEV1= 80), whose mRSS was 14 and J4S was 3, and the other one with the severe restrictive PFT whose mRSS was 41and J4S was 16. None of the studied patients had heart failure or arrhythmia but primary pulmonary arterial hypertension (PAH) was diagnosed in only one patient with dcSSc. All patients with JLS (100%) and 2 patients with JSSc (18%) did not suffer from any organ affection (table 1).

Routine laboratory investigations were normal in all patients with JLS and JSSc. Only one patient had mildly elevated CPK. Among patients with JSSc, immunological work up revealed positive ANA in 4 patients (36.4%). Anti- Scl70 was the only scleroderma related autoantibody done in 9 patients with JSSc and was negative in all patients.

Patients with JSSc had moderately severe skin disease where the median (IQR) mRSS was 18 (14-30) and ranged between 4-41. On the other hand, the overall disease severity was mild to moderate as assessed by J4S with a median (IQR) of 5.5 (3 - 6.5) and ranged between 1-16. With respect to patients with JLS, LoSDI has a median (IQR) of 7 (5 - 17) and ranged between 3 to 30.

Prednisolone was prescribed to all JSSc studied patients, at a dose ranged between 0.25 to 1 mg/kg/day (the latter is used in the two patients with ILD). Nine patients with JSSc (81.8%) were on mycophenolate mofetil (MMF). Rituximab and tocilizumab are given to two patients consecutively, both of moderately severe skin and lung involvement and dry polyarthritis. With respect to JLS, MMF was given to 2 patients (40%) and methotrexate (MTX) was used in one patient (20%).

**Serum level and cutaneous expression of JAK3 among the studied patients and healthy controls:**
Patients with JSSc had significantly higher serum JAK3 as compared to the control group, while comparable values were noted between JLS and healthy controls. Although serum levels of JAK3 were higher among patients with JSSc than those with JLS, the difference was non-significant. Also, cutaneous expression of JAK3 was comparable between patient groups and healthy controls (table 2). Two patients with JSSc had serum and cutaneous JAK 3 ≥ 500 pg/ml; one patient with OS, had the highest serum level and cutaneous expression of JAK3 (600 and 1400 pg/ml, respectively) with mRSS of 10 and J4S was one. Another patient with early dcSSc had serum and cutaneous JAK3 expression of 500 pg/ml with mRSS of 32 and J4S value of 3. Using the ROC curve, serum JAK3 levels > 400 mg/dl has 64 % sensitivity and 87 % specificity for JSSc ($p < 0.001$) (figure 1).

As compared to unrelated healthy subjects, healthy first degree relative of the studied patients had higher albeit non-significant median (IQR) serum JAK3 [210 (180 -290) versus 360 (220 - 400) respectively, p=0.09] and similarly median (IQR) cutaneous JAK 3 [200 (150 - 250) versus 250 (200 - 400) respectively, p=0.15]. Serum and cutaneous expression of JAK3 did not vary significantly between males and females of the studied sample as well as no effect of age is found.

**The relationship between serum and cutaneous expression of JAK 3 and severity of JSSc and JLS:**
Severity of skin and systemic involvement in JSSc measured by mRSS and J4S respectively, did not correlate significantly with either serum or cutaneous JAK3. Similarly, LoSDI measuring JLS damage had no significant correlation with either serum or cutaneous JAK3 (table 3).

**Variation of serum and cutaneous JAK3 expression with different grades of cellular infiltrate and skin fibrosis among JSSc patients:**
Serum and cutaneous expression of JAK3 were comparable among JSSc patients with different grades of cellular infiltrate and skin fibrosis demonstrated in skin biopsies from these patients (table 4). Similarly, patients with mild and moderate skin fibrosis have comparable mRSS and J4S ($p>0.05$).

**Table 1.** Demographic data and disease characteristics of the studied JSD patients and healthy controls

| | | Patients with JSS N (%) = 11 (68.7%) | Patients with JLS N (%) = 5 (31.2%) | Healthy controls (N=23) | | |
|---|---|---|---|---|---|---|
| | | | | | Relatives N (%): 14 (61%) | Non-relatives N (%): 9 (39%) |
| Age (years) | Mean (SD)<br>Range | 11.18 (3.28)<br>6 yrs -16 yrs | 10 (2.74)<br>7 yrs -14 yrs | Adult<br>N (%)<br>Mean (SD)<br>Range | 14 (61%)<br>36.4 (3.4)<br>31 - 40 | 4 (44.4%)<br>34.2 (3.78)<br>31 - 38 |
| | | | | Children<br>N (%)<br>Mean (SD)<br>Range | — | 5 (55.6)<br>10.25 (1.3)<br>8 - 13 |
| Gender | Female N (%)<br>Male N (%) | 8 (72.73%)<br>3 (27.27%) | 5 (100%)<br>0 (0%) | Adult<br>Female N (%)<br>Male N (%) | 12 (86%)<br>2 (14.3%) | 3 (75%)<br>1 (25%) |
| | | | | Children<br>Female N (%)<br>Male N (%) | | 5 (100%)<br>0 (0%) |
| Family history | Positive N (%) | 1 (9.1%) | 0 (0%) | 14 (61%) | | - |
| | Negative N (%) | 10 (90.9%) | | - | | 9 (39%) |
| Disease Duration | Median (IQR)<br>Range | 5 (0.83 - 7)<br>0.17 – 11 months | 3 (2 - 4)<br>1 -4 yrs | | | |
| Diagnosis Lag (Month) | Median (IQR)<br>Range | 4 (2 - 12)<br>1 – 48 months | 6 (5 - 12)<br>2 – 24 months | | | |
| Associated symptomatic organ affection N (%) | GIT | 5 (45.45%) | 0 (0%) | | | |
| | Respiratory | 4 (36.36%) | 0 (0%) | | | |
| PFT | FVC Mean (SD)<br>Range | 76.6 (10.1) %<br>47-85 | - | | | |
| | FEV1 Mean (SD)<br>Range | 76.5 (10.7) %<br>48-90.5 | - | | | |
| PAH | N (%) | 2 (18.2%) | - | | | |
| ILD | N (%) | 2 (18.2%) | - | | | |
| mRSS | Median (IQR)<br>Range | 18 (14 - 30)<br>4 – 41 | - | | | |
| J4S | Median (IQR)<br>Range | 5.5 (3 - 6.5)<br>1 – 16 | - | | | |
| LoSDI | Median (IQR)<br>Range | - | 7 (5 - 17)<br>3 – 30 | | | |

FVC: forced vital capacity; FEV: forced expiratory volume; GIT: gastro-intestinal tract; JLS: Juvenile localized scleroderma; JSSc: Juvenile systemic sclerosis; J4S: Juvenile systemic sclerosis severity score,; ILD: interstitial lung disease, IQR: Interquartile range, mRSS: modified Rodnan skin score, N: number; PAH: pulmonary arterial hypertension, PFT: pulmonary function test, SD: standard deviation

**Table 2.** Variation of serum and cutaneous JAK 3 expression between the studied patients and healthy controls

| | Controls N = 23 | JSSc N =11 | JLS N= 5 | Kruskal Wallis test | p-Value |
|---|---|---|---|---|---|
| Serum JAK3 (pg/ml)<br>Median (IQR)<br>Range | 270 (180 - 385)<br>155-520 | 430 (320 - 520)<br>220-600 | 300 (250 - 400)<br>165-425 | 8.01 | 0.02 |
| Cutaneous JAK3 (pg/ml)<br>Median (IQR)<br>Range | 220 (190 - 340)<br>110-420 | 340 (200 - 400)<br>150-1400 | 345 (250 - 350)<br>200-500 | 2.89 | 0.24 |
| **Post Hoc Test** | | | | | |
| Serum JAK3 | | Kruskal Wallis test | | P-Value | |
| Controls versus JLS | | -1.72 | | 0.76 | |
| Controls versus JSSc | | -11.70 | | 0.005 | |
| JLS versus JSSc | | 9.98 | | 0.10 | |

JAK 3: Janus kinase 3; JLS: Juvenile localized scleroderma; JSSc: Juvenile systemic sclerosis

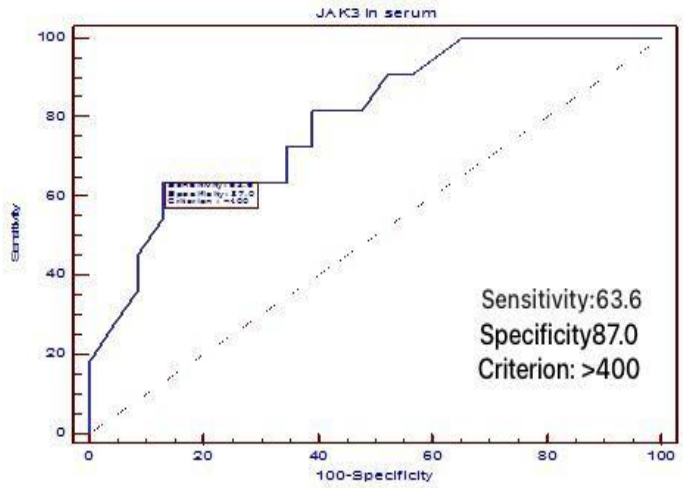


**Figure 1:** Receiver operating curve to determine the cut off serum level of JAK3 between JSSc patients and healthy controls.

At serum level of JAK3s > 400 mg/dl can discriminate between JSSc and healthy subjects with 63.6% sensitivity and 86.9% specificity, AUC: 0.79 and CI: 0.62 to 0.91 ($p < 0.001$)

**Table 3.** The relationship between serum and cutaneous JAK3 expression levels and JSSc severity scores

| JSSc group | | mRSS | J4S |
|---|---|---|---|
| Serum JAK3 (pg/ml) | Spearman' s rho | -0.48 | -0.28 |
| | p-Value | 0.13 | 0.40 |
| Cutaneous JAK3 (pg/ml) | Spearman' s rho | -0.37 | -0.43 |
| | p-Value | 0.26 | 0.19 |

JAK3: Janus kinase 3; JSSc: Juvenile systemic sclerosis; J4S: Juvenile systemic sclerosis severity score; mRSS: modified Rodnan skin score

**Table 4:** Variation of the JSSc severity scores between mild and moderate skin fibrosis

| | Fibrosis degree | | Mann-Whitney test | |
|---|---|---|---|---|
| | Mild | Moderate | | |
| | Median (IQR) | Median (IQR) | Z | p-Value |
| mRSS | 18 (14 - 28) | 28 (10 - 41) | 0.54 | 0.63 |
| J4S | 5.25 (3 - 6.25) | 6 (1 - 16) | 0.76 | 0.78 |

JSSc: Juvenile systemic sclerosis; J4S: Juvenile systemic sclerosis severity score; mRSS: modified Rodnan skin score

## DISCUSSION

The pathogenesis of JSD is complex and multifactorial where the immune system dysregulation with inflammation, vascular injury, and fibrosis contributes to skin thickening with associated comorbidities.[19,20] Several cytokines transduce their signaling via JAK/STAT pathways in various combinations (JAK1, JAK2, JAK3, tyrosine kinase-2/STAT1, 2, 3, 4, 5a, 5b, 6), among these cytokines are IL-6, TNF-α and TGFß1 which are upregulated in systemic sclerosis. Dysregulation of the JAK and STAT has been shown to contribute to fibrosing in scleroderma.[21] In this study, the significantly elevated serum levels of JAK3 among patients with JSSc as compared to those with JLS and healthy control add to the growing body of evidence favoring contribution of the constitutively activated JAK/STAT pathway in the pathogenesis of JSSc, However, cutaneous JAK3 expression was comparable among JSSc and JLD patients and controls, whether this related to the stage of the disease in the skin area punched for biopsy and the small sample size. The two patients with early onset JSSc in the edematous phase have the highest serum and cutaneous JAK3 expression which could support the transition of the dysregulated immune system through different phases in JSSc. In SSc, it has been indicated that the immune profile of early disease differs from that of late disease where inflammation predominates in the early disease and fibrosis defining later stages of disease. The Mononuclear

cell infiltrates in early lesions and the altered function of Th cells throughout the disease course, are thought to create a cytokine, chemokine, and growth factor profile that stimulates fibroblasts to promote fibrosis [6]; Notably, these findings are similar in LS, and tend to be difficult to distinguish by histopathology. [22]

A previous study that measured levels of phosphorylated (pY)-JAK1, JAK2, JAK3, and STAT3 in skin biopsies from SSc adult patients and healthy controls demonstrated differential expression of JAK/STAT in different stages of the disease and different sites of the skin where there were comparable expression levels in the epidermis between control subjects and patients. However, in the dermis, expression of pY-JAK1, pY-JAK2, pY-JAK3, and STAT3 were significantly elevated in SSc biopsies of interstitial cells compared with control biopsies. Also, patients with late-stage disease showed significantly increased expression of pY-JAK2 and pY-STAT3 compared with those with early-stage disease.[11]

Interestingly, the healthy controls who are first degree relatives of the studied patients had higher levels of serum and cutaneous JAK3 than that of the unrelated controls albeit non-significant, whether the first-degree relatives of JSD patients might have subclinical immune dysregulation, this needs more in-depth evaluation of the first-degree relatives of these patients. It has been reported that there is genetic contribution in SSc where first degree relatives of patients with SSc carried a 10–16-fold relative risk for disease and siblings a 10–27-fold risk.[23] Interestingly, monozygotic twins discordant for SSc were found to have a similar fibroblast gene expression pattern, suggesting a strong genetic predisposition to SSc at the molecular level in skin fibroblasts. [24] The ability of serum JAK 3 > 400 mg/dl to differentiate JSSc patients from healthy subjects with acceptable sensitivity and specificity of 64 % and 87 % respectively might help the early diagnosis of JSSc in its very early presentation or with isolated presentation of Raynaud's phenomenon in a similar way as using certain scleroderma specific autoantibodies where anti-centromere antibodies (ACA) and anti-Scl-70 antibodies were reported to be very useful in distinguishing patients with SSc from healthy controls, from patients with other connective tissue disease, and from unaffected family members.[25]

In the current study, we did not find significant relationship between serum and cutaneous JAK 3 expression and the clinical skin and overall disease scores in JSSc and JLS patients. Also, there was no significant correlation between the degree of the skin fibrosis and inflammatory cell infiltrates with the mRSS and systemic disease severity as assessed by J4S. The limited number of the patients confounds the interpretation of this finding and further assessment is needed as the clinical evaluation of the skin is generally reflecting the histopathological features of the skin in scleroderma. But in line with our finding, a previous study reported that there was no association of JAK/STAT pathway activation with the skin score in adult SSc patients.[11] On the other hand, another study reported that the skin symptoms significantly correlated with the pathological skin findings in SSc adult patients.[26]

The involvement of JAK/STAT family in the immunopathogenesis of scleroderma could be indirectly concluded from the response to JAK inhibitors (jakinibs) used in treatment of this disease. Different jakinibs have been tried in few studies and case reports of adult scleroderma and JSD with as well as murine models of SSc with variable outcomes. A previous study using JAK-2 inhibitor for treatment of the bleomycin-injected mice demonstrated in >70% reduction in dermal thickness compared to control bleomycin-injected mice treated with placebo. Increased dosage of the JAK-2 inhibitor nearly resolved the dermal thickening in bleomycin-injected mice. Additionally, the tight skin (TSK-1) mouse model of SSc was used for evaluation of the efficacy of jakinibs on models of later-stage disease. Similar to the bleomycin-injected mouse model, the TSK-1 mice treated with a JAK-2 inhibitor had >80% average reduction in dermal skin thickening compared to control, untreated TSK-1 mice.[27] A prospective study compared 10 patients with dcSSc treated with tofacitinib 5 mg twice daily to 12 dcSSc patients with similarly matched disease severity who received intensive conventional immunosuppressive therapies. The authors found that 90 % of the patients on tofacitinib had improvement with reduction of skin thickness, with 80% achieving the goal of a reduction in mRSS by >5 points and a greater than 25% improvement from baseline. A 60% response rate was reached by 3 months of therapy versus 16.7 % in the comparator group. Only one patient progressed in their disease on tofacitinib and another patient did not show significant improvement, Generally, the patients treated with tofacitinib improved faster compared to patients treated with cyclophosphamide or MMF in combination with steroids. [28]

An additional case of an HLA-B27 positive patient with dcSSc and ankylosing spondylitis treated with tofacitinib demonstrated clinical improvement in skin thickness as measured by the mRSS as well as symptom improvement of ankylosing spondylitis.[29] Also, a patient with refractory morphea of the legs that failed prednisone 40 mg daily and MTX achieved notable improvement on tofacitinib 10 mg daily for 3 months, both clinically and on skin biopsy.[30] Some other case reports demonstrated significant skin improvement in generalized morphea with onset of response one month after initiation of tofacitinib and maximal response was variable between 4 months to one or 2 years.[31,32]

On another scale, very few reports of the use of jakinibs in children with morphea or JSS; a 13-year-old female with JSSc, who failed prednisone, mycophenolic acid, and had an infusion reaction to rituximab, experienced notable increase in her joint mobility after 3 months of tofacitinib with significant reduction in her mRSS.[33] However, another 5-year-old child with pansclerotic morphea had no improvement with progression of the disease on ruxolitinib (selective JAK 1 and JAK 2 inhibitor) 10 mg twice daily after failing numerous prior therapies including prednisolone, MTX, tocilizumab and MMF. [34]

In conclusion, JAK 3 seems to contribute to the pathogenesis of JSSc and a serum level of > 400 pg/dl might serve as a biomarker of JSSc to allow early diagnosis in patients with mild skin changes or isolated RP. Patients with early JSSc may have higher JAK3 expression both in serum and skin. JAK 3 expression was non-significant in JLS patients with advanced skin disease. Further wider scale studies are needed to evaluate JAK3 expression in different stages of JSSc and JLS. Also, prospective studies are needed to demonstrate the possible changes in JAK 3 expression during the course of the disease and its relation to the activity scores and treatment given.

## AUTHORS CONTRIBUTION

**DHE:** study concept and design, recruitment of cases, analysis of the results and drafting the manuscript. **FAE:** recruitment of the cases, collection of the patients' data and share in drafting the manuscript. **MRE**: the laboratory workup with measurement of the JAK3 in skin and serum of the studied population, **NSA:** Histopathological examination of the skin biopsies, **EF:** Provision of 2 cases and their clinical data, **NS:** Provision of 2 cases and their clinical data, **GAS:** shared in analysis of the results and drafting the manuscript. All authors have read and approved the final manuscript.

## ACKNOWLEDGMENT

We would like to thank Dr. Shady Sherin Shokry, lecturer of Pediatric Surgery for taking the punched skin biopsy.

## CONFLICTS Of INTEREST

The authors declare no conflict of interest.

## REFERENCES

1. **Kerrigan SA, McInnes IB.** JAK Inhibitors in Rheumatology: Implications for paediatric syndromes? Curr Rheumatol Rep 2018; 20(12):8
2. **Dai J, Yang L, Addison G.** Current status in the discovery of covalent Janus kinase 3 (JAK3) inhibitors. Mini Rev Med Chem 2019; 19(18):1531-43.
3. **Morris R, Kershaw NJ, Babon JJ.** The molecular details of cytokine signaling via the JAK/STAT pathway. Protein Sci 2018; 27(12):1984-2009.
4. **Banerjee S, Biehl A, Gadina M, Hasni S, Schwartz DM.** JAK-STAT Signaling as a Target for Inflammatory and Autoimmune Diseases: Current and Future Prospects. Drugs 2017; 77(5):521-46.
5. **Zulian F, Tirelli F.** Treatment in Juvenile Scleroderma. Curr Rheumatol Rep 2020; 22(8):45.
6. **Baraut J, Michel L, Verrecchia F, Farge D.** Relationship between cytokine profiles and clinical outcomes in patients with systemic sclerosis. Autoimmun Rev 2010;10(2):65-73 .
7. **Kurzinski K, Torok KS.** Cytokine profiles in localized scleroderma and relationship to clinical features. Cytokine 2011; 55(2):157-64.
8. **Toledo DM, Pioli PA.** Macrophages in Systemic Sclerosis: Novel Insights and Therapeutic Implications. Curr Rheumatol Rep 2019; 21(7):31 .
9. **Fang D, Chen B, Lescoat A, Khanna D, Mu R**. Immune cell dysregulation as a mediator of fibrosis in systemic sclerosis. Nat Rev Rheumatol 2022;18(12):683-93 .
10. **Lescoat A, Lelong M, Jeljeli M, Piquet-Pellorce C, Morzadec C, Ballerie A, et al.** Combined anti-fibrotic and anti-inflammatory properties of JAK-inhibitors on macrophages in vitro and in vivo: perspectives for scleroderma-associated interstitial lung disease. Biochem Pharmacol 2020; 178:114103.
11. **Wang W, Bhattacharyya S, Marangoni RG, Carns M, Dennis-Aren K, Yeldandi A, et al.** The JAK/STAT pathway is activated in systemic sclerosis and is effectively targeted by tofacitinib. JSRD 2020; 5(1):40-50.

12. **McGaugh S, Kallis P, De Benedetto A, Thomas RM**. Janus kinase inhibitors for treatment of morphea and systemic sclerosis: A literature review. Dermatol Ther 2022; 35(6):e15437.
13. **Li SC, Zheng RJ.** Overview of Juvenile localized scleroderma and its management. World J Pediatr 2020; 16(1):5-18.
14. **Zulian F, Woo P, Athreya BH, Laxer RM, Medsger TA Jr, Lehman TJ, et al**. The Pediatric Rheumatology European Society/American College of Rheumatology/ European League against Rheumatism provisional classification criteria for juvenile systemic sclerosis. Arthritis Rheum 2007; 57(2):203-12.
15. **Laxer RM, Zulian F.** Localized scleroderma. Curr Opin Rheumatol 2006;18(6):606-13.
16. **Clements PJ, Lachenbruch PA, Ng SC, Simmons M, Sterz M, Furst DE.** Skin score. A semiquantitative measure of cutaneous involvement that improves prediction of prognosis in systemic sclerosis. Arthritis Rheum 1990; 33(8):1256-63
17. **La Torre F, Martini G, Russo R, Katsicas MM, Corona F, Calcagno G, et al.** A preliminary disease severity score for juvenile systemic sclerosis. Arthritis Rheum 2012; 64(12):4143-50.
18. **Kelsey CE, Torok KS.** The Localized Scleroderma Cutaneous Assessment Tool: responsiveness to change in a pediatric clinical population. J Am Acad Dermatol 2013; 69(2):214-20 .
19. **Mertens JS, Seyger MMB, Thurlings RM, Radstake TRDJ, de Jong EMGJ.** Morphea and eosinophilic fasciitis: an update. Am J Clin Dermatol. 2017;18(4):491-512).
20. **Pearson DR, Werth VP, Pappas-Taffer L.** Systemic sclerosis: Current concepts of skin and systemic manifestations. Clin Dermatol 2018; 36(4):459-74 .
21. **Tanaka Y.** Recent progress and perspective in JAK inhibitors for rheumatoid arthritis: from bench to bedside. J Biochem 2015; 158:173–9 .
22. **Torok KS, Stevens AM.** Juvenile systemic sclerosis. In: (Petty RE, Laxer RM, Lindsley CB, Wedderburn LR, Mellins ED, Fuhllbrigge RC (editors): Textbook of Pediatric Rheumatology. Elsevier, Philadelphia, 8th edition 2021; pp.377-400.
23. **Arnett FC, Cho M, Chatterjee S, Aguilar MB, Reveille JD, Mayes MD.** Familial occurrence frequencies and relative risks for systemic sclerosis (scleroderma) in three United States cohorts. Arthritis Rheum 2001; 44:1359–62.
24. **Zhou X, Tan FK, Xiong M, Arnett FC, Feghali-Bostwick CA.** Monozygotic twins clinically discordant for scleroderma show concordance for fibroblast gene expression profiles. Arthritis Rheum 2005; 52(10):3305–14.
25. **Ho KT, Reveille JD.** The clinical relevance of autoantibodies in scleroderma. Arthritis Res Ther 2003; 5(2):80–93.
26. **Ziemek J, Man A, Hinchcliff M , Varga J, Simms RW, Lafyatis R.** The relationship between skin symptoms and the scleroderma modification of the health assessment questionnaire, the modified Rodnan skin score, and skin pathology in patients with systemic sclerosis. Rheumatology (Oxford) 2016; 55(5):911-7.
27. **Dees C, Tomcik M, Palumbo-Zerr K, Distler A, Beyer C, Lang V, et al.** JAK-2 as a novel mediator of the profibrotic effects of transforming growth factor β in systemic sclerosis. Arthritis Rheum 2012; 64(9):3006-15.
28. **You H, Xu D, Hou Y, Zhou J, Wang Q, Li M, et al**. Tofacitinib as a possible treatment for skin thickening in diffuse cutaneous systemic sclerosis. Rheumatology (Oxford) 2021; 60(5):2472-7 .
29. **Kyriakou A, Parperis K, Nikiphorou E, Psarelis S.** Successful use of tofacitinib in the treatment of diffuse systemic sclerosis and axial spondyloarthritis: a case-based review. Rheumatol Int 2021; 41(3):671-5.
30. **Scheinberg M, Maluf F, Wagner J.** Steroid-resistant sarcoidosis treated with baricitinib. Ann Rheum Dis 2020; 79(9):1259-60 .
31. **Kim SR, Charos A, Damsky W, Heald P, Girardi M, King BA.** Treatment of generalized deep morphea and eosinophilic fasciitis with the Janus kinase inhibitor tofacitinib. JAAD Case Rep 2018; 4(5):443-5.
32. **Damsky W, Patel D, Garelli CJ, Garg M, Wang A, Dresser K, et al.** Jak Inhibition Prevents Bleomycin-Induced Fibrosis in Mice and Is Effective in Patients with Morphea. J Invest Dermatol 2020;140(7):1446-9 .
33. **Pin A, Tesser A, Pastore S, Moressa V, Valencic E, Arbo A, et al.** Biological and Clinical Changes in a Pediatric Series Treated with Off-Label JAK Inhibitors. Int J Mol Sci 2020; 21(20):7767 .
34. **Soh HJ, Samuel C, Heaton V, Renton WD, Cox A, Munro J.** Challenges in the diagnosis and treatment of disabling pansclerotic morphea of childhood: case-based review. Rheumatol Int 2019; 39(5):933-4